Guidelines for Harmonizing Ocean Surface Microplastic Monitoring Methods

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The suggested citation for this document is:

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Acknowledgements

The contributions and cooperation of the following laboratories were extremely important and essential to the preparation of these Guidelines, in analyzing standard microplastic samples prepared for experimental analysis, and reporting the results of their analyses to clarify the differences among the various analytical methods and practices.

Association Oceaneeye, Chulalongkorn University, Ehime University, Korea Institute of Ocean Science and Technology, Kyushu University, Norwegian Institute for Water Research, National Marine Environmental Monitoring Center, MEE, National Oceanic and Atmospheric Administration, Pacific Institute of Geography, Tokyo University of Marine Science and Technology, University of Cadiz, and Vancouver Aquarium Marine Science Centre.

Special thanks are noted to Marie Delorenzo, Nina T. Buenaventura, Peter S. Ross, Stephen Chastain, Voranop Viyakarn, and Weiwei Zhang for their contributions to the inter-laboratory comparison in 2017 (ILC2017).
Preface:

This document presents the first version (1.0) of 'Guidelines for Harmonizing Ocean Surface Microplastic Monitoring Methods' (herein after referred to as the Guidelines) to propose ways of harmonizing methodologies for monitoring microplastic densities at the ocean surface using net sampling to deliver comparable results.

Specifically, the Guidelines indicates the rationale for various net sampling methods, sample handling and analysis procedures, reporting requirements, and other matters necessary or desirable for harmonization.

Preparation of the Guidelines were based on the output of the international workshop held in 2015 as a follow-up to the 'G7 Action Plan to Combat Marine Litter' agreed on in the G7 Elmau Summit 2015, where it was indicated that Japan would lead the harmonization efforts for microplastic monitoring methods.

The Guidelines were developed on the basis of opinions and recommendations compiled at international meetings of microplastic monitoring experts and the results of dedicated in situ and laboratory experiments newly conducted toward harmonization, as well as existing findings collected and summarized from published microplastic monitoring survey reports, guidelines, and manuals.

At present, several sets of guidelines and other documents are being developed by GESAMP and other international organizations because estimating the abundance and/or distribution of microplastics in water bodies has become important internationally. The Guidelines presented here were designed to supplement and complement such documents, and to propose detailed methodologies focusing on net sampling and analysis aimed at producing horizontal distribution maps of microplastics at the ocean surface.

Many studies are expected to be carried out involving microplastic monitoring at the ocean surface. Application of the harmonized methods proposed in the Guidelines could help these efforts generate results in a comparable manner, enabling researchers to analyze, consolidate and integrate the results on a wider scale. Through such applications, we strongly believe that our understanding of the abundance of microplastics in the ocean will improve, based on shared and integrated monitoring results, and that this will promote higher level analysis of microplastic issues and application to policy development.

The first version of the Guidelines is a working document and will be updated and improved in the near future based on additional research and feedback from users.
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List of Acronyms and Abbreviations

ILC2017: Inter Laboratory Comparison 2017
CMSM2018: Comparison of Microplastic Sampling Methods 2018
FTIR: Fourier Transform Infrared Spectroscopy.
CPR: Continuous Plankton Recorder
SOP: Standard Operational Procedure
EC: European Commission
GESAMP: The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
MOEJ: Ministry of the Environment, JAPAN
NOAA: National Oceanic and Atmospheric Administration
1. Introduction

1.1 Background and purpose

Marine litter, including microplastics, is now a global challenge. In particular, pollution of the marine environment by microplastics has been recognized as a serious international issue over the past decade: microplastics are likely to affect marine ecosystems and are extremely difficult to recover. Determining the current status of distribution and quantity of microplastics in the ocean is an urgent task. It is important for policy planning promotion to be based on concrete scientific knowledge while getting a head start with preventive measures against plastic litter in the ocean.

In response to the growing interest surrounding microplastics in the ocean, microplastic monitoring (sampling and laboratory analysis) has been carried out by many institutions around the world using various methods, and accordingly findings are gradually accumulating. It is expected that more monitoring will be conducted in the future, but as different sampling and analytical methods are used, depending on the purpose of the surveys of each country and research institution, there is a lack of comparability among currently available data. There is also speculation that research will be carried out under limited resource availability, technical capacity or institutional arrangements, or that monitoring will be conducted using the latest equipment that is not yet globally common, thus, further hampering researchers’ ability to build comparisons.

Inability to compare data obtained by different monitoring methods may pose an obstacle to research determining the global distribution and fate of microplastics in the ocean. Hence, standardization and harmonization of monitoring methods for marine litter, including microplastics, are recognized as important tasks/activities.

At the G7 Elmau Summit in 2015, marine litter, especially plastic litter, was acknowledged as a global challenge due to its effects on ocean and coastal ecosystems, its direct impacts on ecosystems, and potential impacts on human health. The communiqué adopted at the G7 Toyama Environment Minister’s Meeting in 2016 states its commitment to implementing five priority measures including standardization and harmonization of monitoring methodologies for marine litter. Based on shared recognition of these issues, various activities have been set in motion such as development of guidelines for monitoring, analysis and evaluation by GESAMP and other organizations. At the expert workshop in Berlin, November 2015 following the Elmau Summit, it was agreed that Japan would play a leading role in standardizing and harmonizing the monitoring methodologies for ocean microplastics.

To remedy the situation, the Ministry of the Environment of Japan (hereinafter the MOEJ) has been advancing efforts to ascertain the actual state of marine pollution by encouraging to horizontal distribution mapping of microplastic densities at the ocean surface worldwide. Aimed at harmonizing ocean surface layer microplastic sampling and analytical methods, the Guidelines were developed based on the results of two projects implemented by scientists which were supported by the MOEJ, as shown below (Fig. 1-1). In addition, a comparative study of the research being undertaken around the world was conducted. For examining analytical methods, an inter-laboratory comparison was
conducted by 12 laboratories in 10 countries (Canada, Norway, China, Russia, Korea, Spain, Switzerland, Thailand, USA and Japan) in 2017 (Hereinafter "ILC2017") to cross-check standard samples containing a predetermined amount of non-plastic material and a predetermined quantity of plastic particles using various analytical methods (see Isobe et al. (submitted). For examination of sampling methods, a comparison of microplastic sampling methods was conducted in FY2018 (hereinafter "CMSM2018"), by sampling microplastics in the sea surface of Tokyo Bay in various ways. Based on an analysis of differences in the results from various analytical and sampling methods obtained in these projects, recommendations for harmonization, as well as points to be noted when understanding monitoring results were summarized.

The Guidelines were prepared with the view of enabling practitioners of ocean surface layer microplastic monitoring to design their monitoring protocols and interpret their results to enable their results to be compared with other monitoring results.
Purposes of the Guidelines:

- To focus on determining the actual state of "microplastics in ocean surface layer"* rather than other forms of marine pollution caused by plastic litter.
- To provide recommendations for harmonizing sampling and analytical methods to enable comparison of the results obtained in ongoing studies and the many studies expected to be implemented worldwide in the future.
- To give consideration to studies carried out under various constraints, such as restrictive human or financial resources.

* Microplastic monitoring surveys have been carried out for many different purposes (Rochman et al., 2017) such as to evaluate diverse media or the effects of microplastic emission controls. Among these various research objectives, the Guidelines aim specifically at developing horizontal distribution maps of microplastics at the ocean surface.

**Fig. 1-1. Guidelines development process.**
1.2 Scope of the Guidelines

<Target readers>

- The main target readers of the Guidelines are practitioners and analysts who conduct oceanographic surveys of microplastics, and those who intend to analyze and evaluate the state of actual pollution by using survey results of their own and/or others from various areas in the world. Consideration has been given to some studies carried out under various constraints, such as restrictive human and financial resources.
- The Guidelines are not intended to present standards, but rather they have been prepared in the expectation that they will be helpful in choosing harmonized methods that would derive comparable results.

<Subject and monitoring methods>

- The subject matter of the Guidelines is microplastics at the ocean surface and their aim is to harmonize net sampling in the field and analytical methods in laboratories.
- Plastic particles with a size of less than 5 mm are treated as microplastics in the Guidelines, similarly to their definition in GESAMP (2019) and to the definition used in international organizations and many research projects that have been implemented in various countries around the world.
- Ascertaining microplastic presence inside living organisms is important to investigating the impact of microplastics on living organisms, but it is beyond the scope of the Guidelines.
- Although the scope of the Guidelines is microplastics at the ocean surface, as shown in Table 1-1, the sampling and laboratory analytical methods are considered applicable to surface water in both marine and freshwater environments. They can also be partially applicable to water columns and sediments of both seawater and freshwater.
Table 1-1. Microplastic sampling and analytical methods within the scope of the Guidelines.

<table>
<thead>
<tr>
<th>Category</th>
<th>Field Sampling</th>
<th>Laboratory analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface water</td>
<td>○*</td>
<td>○</td>
</tr>
<tr>
<td>Other methods</td>
<td>×</td>
<td>△</td>
</tr>
<tr>
<td>Water column</td>
<td>×</td>
<td>△</td>
</tr>
<tr>
<td>Sediments</td>
<td>×</td>
<td>△</td>
</tr>
</tbody>
</table>

Legend ○: Within the scope △: Partially referable ×: Not within the scope of the Guidelines

*...The Guidelines are for marine surveys. It should be noted there would be more clogging and vertical mixing in fresh water.

[Why focus on ocean surface net sampling?]

Presently, there are numerous microplastic particles in the ocean surface around the world. They are impacting invertebrates, fish, birds and other organisms living in or on the ocean surface. Plastic particles with a size of less than 5 mm are treated as microplastics in the Guidelines. Here, plastic particles size is Feret's diameter* that is generally defined as the distance between the two parallel planes restricting the object perpendicular to that direction. Among those measured Feret’s diameter values, such that the area of the rectangle enclosing the particle outline becomes a minimum is called “Minimum Feret’s diameter” and the dimension perpendicular to it is called “Maximum Feret’s diameter” (Pabst et al., 2017).

At the ocean surface, it is common to collect samples using nets. Net sampling is thought to have the following advantages:

- A large mass of water can be efficiently filtered.
- Nets can be deployed easily, compared to pumps, CPR (continuous plankton recorders), etc.,
- Abundant knowledge on surface net use and collection methods is available from plankton research.
- Proportionally more surveys using nets to sample microplastics have been conducted, so using nets facilitates comparison with the accumulated data.

* Feret’s diameter is shown in the figure below.
1.3 Composition

The Guidelines are divided into five chapters. Table 1-2 gives an outline of these. Each chapter is divided into sections, and the main content in each section is summarized as keynotes.

Each set of keynotes is highlighted in a box and provides the following information:

- Introduction of commonly used methods and parameters.
- Related results from projects (ILC2017, CMSM2018) and the results of literature reviews conducted for preparing the Guidelines.
- Recommendations based on the above information.

Further comments pertaining to keynotes are provided under the box.

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<tr>
<td>5. Conclusions</td>
<td>Summary of the Guidelines, items that require further consideration, etc.</td>
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</tbody>
</table>
2. Sampling methods

2.1 Outline

- Microplastics floating at the ocean surface have been collected by towing a net in many past investigations, according to the procedure illustrated below (Fig.2-1).

Fig.2-1. General flow of microplastic collection using a net.
Reviews of previous research identified differences in the type and size of mesh used across studies. A Manta net or Neuston net are most commonly used, and recommended in sampling guidelines, although differences between net mesh openings and towing methods have been observed between studies.

For this reason, prior to preparation of the Guidelines, a survey to collect microplastics from the ocean was conducted (hereinafter referred to as "CMSM2018") to investigate the effects of the following factors on sampling results: 1) differences between Neuston nets and Manta nets (§2.3.1), 2) differences in mesh openings (§2.3.2), 3) tow duration (§2.4.1), and 4) differences in tow position (i.e., towing at the stern) (§2.4.5).

The average “density” of microplastic particles (i.e., quantity per volume seawater) at the ocean surface observed in CMSM2018 was 3.0/m³, with a maximum density of about 15.0/m³ and minimum density of about 0.3/m³.

Conditions for harmonization were determined based on a comparison between the results of the CMSM2018 field survey where different net types with different mesh openings were towed at the same time in the same sea area. Specifically, two different nets were simultaneously set at port and starboard of the same survey vessel.

To examine the accuracy of the comparison, 13 test runs were conducted using two Neuston nets of the same design towed at port and starboard. In all but one tow, there was no significant difference between tow position at port and starboard of the vessel (§2.4.5).

The metadata necessary to enable comparison of the survey results were examined based on the environmental data acquired during the field survey conducted in CMSM2018.

Recommendations based on our literature review and field study (CMSM2018), are presented in detail in the following section.
2.2 Sea conditions

**Keynotes**

- Previous studies and available guidelines have noted that collection of microplastics at the ocean surface should be conducted under mild sea conditions whenever possible.

- In CMSM2018, it was observed that the density of microplastics in the same survey area changed by about one order within several hours, as sea conditions, including wind speed and wave height, changed.

- It is desirable to collect samples when sea conditions are as calm as possible. As this might not be practical in areas where it is always windy, metadata such as wind speeds and significant wave heights should be recorded to allow comparisons with other survey results (for more details, please refer to the §2.5, Recording metadata and §4, Reporting).

- It is desirable to avoid unfavorable timing and conditions for sampling, such as high concentrations of natural particles or organisms, i.e. algae and high plankton bloom.

**Explanatory Notes**

- In general, wind speed and wave heights are known to influence the degree of vertical mixing of the ocean surface layer and affect the amount of microplastics collected. According to recent guideline, microplastic surveys should be conducted in conditions where wave heights are under 0.5 meters and Beaufort wind force scale under 3 (GESAMP, 2019).

- In CMSM2018, it was observed that the quantity of microplastics at the ocean surface greatly decreased in situations where both wind speed and wave height increased during sampling (Fig. 2-2). This was probably due to enhanced mixing of the ocean surface layer caused by changes in the sea conditions and the dispersion of microplastics to a certain depth (Reisser et al., 2015).

- Care should be taken when sampling in sea areas near land following rainfall, as it has been reported that the density and composition of microplastic particles at the ocean surface can be influenced as a result of microplastic particle input from rivers (Kang et al., 2015, Lima et al., 2015, etc.).

- In CMSM2018, surveys conducted in the coastal area of Tokyo Bay showed an increased density of microplastics. This observation was thought to have been caused by the input from nearby rivers.

- In addition, in CMSM2018 a significant decrease in the amount of microplastics was observed when a large amount of jellyfish were caught in the same net. Clogging of the net by plankton, algae, jellyfish, floating seaweed, etc., affects survey results, so it is preferable to avoid collecting samples at times when they are expected to be observed in mass.

- To obtain mutually comparable results, situations with strong winds and/or waves, or in which
plankton are highly abundant should be avoided. Surveys need to be conducted when sea conditions are as mild as possible.

- Tidal currents and/or river inflows should be monitored and collecting samples under moderate to average sea conditions is desirable for comparison control.
- Several studies (Kukulka et al., 2012; Kooi et al., 2016. etc.) are underway proposing a method for estimating the vertical distribution of microplastics in the water column to correct ocean surface microplastic density depending on sea conditions. Recording wind speed and wave height during sampling will allow researchers to estimate the vertical distribution of microplastics in the water column and some studies have adopted these methods (Isobe et al., 2015; Suaria et al., 2016. etc.).

※Wind speed, wave height and density of microplastics are plotted at towing commencement times.

**Fig.2-2. Example of temporal changes in wind direction and wave height (a) and density of microplastics (b) measured in CMSM 2018.**
2.3 Sampling equipment

- To collect microplastic particles floating at the surface sea surface, most researchers use nets that can efficiently filter a large mass of water (Neuston or Manta nets).

- Generally, except under specific sea conditions, the quantity of microplastics per cubic meter of the ocean surface is low. Even in Tokyo Bay, where the concentration of plastic particles is expected to be relatively high, the density is only 1 to 10/ m$^3$ (Isobe et al., 2016.). Therefore, it is necessary to sample a large amount of seawater to capture a sufficient quantity of microplastics to accurately estimate microplastic abundance. Towing the net for a set duration to account for the influence of water masses with aggregated floating matter, including current rips, is also effective.

- Since Neuston nets and Manta nets have been widely used in plankton surveys, knowledge has accumulated on trawling methods that can facilitate their introduction for anyone wanting to start or expand investigations of microplastics in the future. Also, their use makes it easier for the data obtained to be compared with data accumulated in the past.

- When net sampling, particles smaller than the mesh openings escape through the net. Therefore, when collecting smaller particles, it would be more effective to sample the water using bottles, buckets, pumps, etc., and filter the water on the vessel, or collect the ocean surface water using a mesh screen sampler.

- It should also be noted that the results obtained using other sampling equipment may not be directly compared to results obtained by net sampling because the differences in the sampled layer and collected water volume are extremely large. To compare the results of such surveys, further discussion on harmonization is needed.

- Recently, unique devices for sampling have been proposed, for example, a series of sieves with different mesh openings installed within the cod end of Manta net to fractionate plastic particles by size whilst towing (Syakti et al., 2018).

- The following section highlights points to be noted regarding equipment to be used for surveys by net towing.
2.3.1 Net types

Keynotes

- Neuston nets or Manta nets are most commonly used for sampling at the ocean surface.
- Each type of net has its own features:
  - (1) Neuston nets can capture the ocean surface layer even in wavy conditions, but it is difficult to estimate the volume of water filtered accurately because the net’s immersion depth changes constantly.
  - (2) Manta nets can maintain a constant immersion depth under the sea surface and thus filtered water volume can be estimated fairly accurately providing there are no waves on the sea surface. If the wave height exceeds a certain level, the net tends to jump and skip on the water surface.

- In CMSM2018, the quantity of particles per unit of filtered water volume was compared for particles larger than 1 mm and less than 5 mm in their maximum Feret’s diameter (longest diameter) sampled by simultaneous towing using a Manta net and a Neuston net in the same area. The results showed the quantity of particles caught by the Manta net tended to be slightly larger than by the Neuston net, although not statistically different. This tendency was thought to be caused by differences in net immersion depth.

- Assuming that either a Neuston net or Manta net will be selected based on the respective advantages and limitations of each (to suit the purpose of the survey and conditions in the target sea area optimally), it is necessary to observe weather and sea conditions at the time of sampling along with net immersion depth.
- It is desirable to perform comparisons of particles in the size range 1 – 5 mm. This is because data obtained for particles < 1 mm, are regarded as underestimated for both nets. This is
related to sampling as well as analytical processing in the laboratory, which is thought to have lowered the accuracy of analysis (see §3.3).

Explanatory Notes

- Neuston nets with side length of about 45 to 100 cm, or Manta nets with width of 60 to 100 cm and height of about 15 to 40 cm are most commonly used to collect microplastics from the ocean surface. Both net types were developed and designed to collect plankton, etc., floating in the surface layer.

- The Neuston net used in CMSM2018 (JMA Neuston net, RIGO Co., Ltd., No.5552) had a square net mouth width and height of 75 cm each, and a net with 0.35 mm mesh openings. When towing the Neuston net, immersion depth was set to 1/2 of the height (37.5 cm). The Manta net (Manta net System, Ocean Instruments, Inc., OI-100) had a rectangular net mouth 100 cm wide and 20 cm in height, and a net with 0.24 mm mesh openings. When towing the Manta net, it was submerged to the upper end of the net mouth.

- A comparison between a Manta net and a Neuston net was conducted by simultaneously towing the nets during CMSM2018. The results were compared in terms of quantity of collected plastic particles (>1 mm) per unit filtered water volume for particles. The Manta net tended to have densities of microplastics which were slightly higher than those of the Neuston net although there was no statistical difference between the two.

- The Manta net is thought to have contained slightly higher quantities as it collects the very surface of the water, where a high density of plastic particles is likely to occur.

- Similarly, Eriksen et al., 2017 reported that although there was no statistical difference in the quantity of plastic particles collected with a Manta net (net immersion depth: 16 cm) and an AVANI net (elongated rectangular Neuston net with an aspect ratio of about 5: 1 and net immersion depth of 30 to 60 cm), there was a statistically significant difference in weight of particles. This difference is speculated to have arisen from a difference in collection layer and a tendency for plastics at relatively high densities to float slightly below the surface layer, such that as a result the AVANI net would catch more particles in high density areas than the Manta net in sea areas where there were many such particles.

- Also, when a Neuston net and Manta net were compared in CMSM2018, the wind was relatively strong (5 to 6 m/s), and vertical mixing of the sea surface relatively high. In the case of moderate sea conditions, the quantity of particles collected per unit of filtered water volume may be larger for the Manta net, which filters only water closer to the surface of the ocean. Further study on the effect on collection results under moderate sea conditions is required.

- For nets used for quantitative collection, the net opening ratio (ratio of the total area of the net’s mesh openings to the area of the net’s mouth opening) needs to be 5 or more when using a net with mesh openings of 0.3 mm or more (Tranter & Smith, 1968), and preferably 9 or more when using a net with smaller mesh openings (Saito, 2018).
For net sampling of microplastics at the ocean surface, conducting sampling under conditions that avoid clogging and inhibition of filtering is recommended, in addition to confirming the net opening ratio of the net to be used.

### Table 2-1 Advantages and disadvantages of different nets for collecting floating microplastics identified by CMSM2018.

<table>
<thead>
<tr>
<th></th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manta net</td>
<td>Remains in surface water except in rough water.</td>
<td>Tends to jump and skip on rough water.</td>
</tr>
<tr>
<td>Neuston net</td>
<td>Operates in relatively rough water.</td>
<td>Needs some efforts to maintain the stable net immersion depth.</td>
</tr>
</tbody>
</table>
2.3.2 Mesh openings

**Keynotes**

- Past surveys generally used mesh openings of about 0.3 mm. Reasons for this choice include ability to filter amount of seawater, suitability for sea conditions and plankton abundance. Nets with mesh openings of 0.2 mm or 0.1 mm have also been used.

- Two kinds of Neuston nets with the same design but with different mesh openings, 0.35 mm and 0.1 mm, were employed in CMSM2018. Both nets were towed simultaneously. There was no significant difference in the quantity of particles > 1 mm in size. However, for particles < 1 mm, the quantity of particles collected with a net with mesh openings of 0.10 mm was about four times larger compared to those collected with a net with 0.35 mm mesh openings (Table 2-1).

- Microplastic particles which are similar in size to the mesh openings may be under-sampled if their shortest length is smaller than the mesh openings. Additionally, a significant decrease in precision was observed in ILC2017 on microplastic analysis for particles less than 1 mm in maximum Feret’s diameter. Given these, it is advisable to measure and report particles <1 mm separately from particles 1 mm - 5 mm.

- For the purpose of comparing floating microplastic pollution of various sea areas, or from a broader, global perspective, the use of the most common mesh opening (0.3 mm) is considered desirable.

- On the other hand, monitoring using a net with finer mesh openings would be useful because data on smaller particles are essential for elucidating the behavior of microplastics in the ocean as well as the effect of uptake of by organisms.

- Obtaining data related to smaller mesh openings would be beneficial to obtaining information (providing a coefficient to convert between sizes) on smaller particles, although this can be influenced by sampling location, size distribution and the accuracy of analysis of smaller particles.
Explanatory Notes

- In CMSM2018, the quantity of particles with longest length <1 mm was two to five times greater in nets with smaller mesh openings (0.1 mm) (Table 2-2).
- There were fewer particles with shortest length less than 0.5 mm when using a net with mesh openings of 0.35 mm (Fig. 2-3). It is conceivable that some that particles may pass through a net with mesh openings of 0.35 mm and not be collected.
- The net with mesh openings of 0.35 mm used in CMSM2018 had rectangular openings with side length of 0.35 mm separated by mesh thread through which sea water passed. Assuming the particles and the mesh did not distort, particles with a shortest length of 0.49 mm or less, which is the diagonal length of the mesh openings, could pass through the screen.
- Using a net with mesh openings of 0.1 mm or 0.2 mm enables collection of small particles which could be under-sampled when using one with larger openings of 0.3 mm. Considering the possibility of clogging, however, the sampling time may have to be limited (for example, 5 minutes), and problems may arise from the viewpoint of securing the required amount of filtered water.
- Also, in the laboratory analysis, as described in Chapter 3, the accuracy of separating microplastic particles of < 1 mm decreases. Thus, in measuring microplastics collected using a net with mesh openings of about 0.3 mm, it is advisable that the results for particles 1-5 mm be reportedly separately from those of particles of < 1 mm in size.
- Therefore, from the viewpoint of harmonizing monitoring methods, using a net with mesh openings of about 0.3 mm is recommended as it is currently most commonly used. It should be noted, however, that even if the longest length is sufficiently greater than 1 mm, particles with a sufficiently short shortest length (fibrous particles) may pass through the net.
- Thus, it should be kept in mind that results for particles which are almost the same size as the mesh openings, and those with a much shorter shortest length may be underestimated when comparing the results collected by nets with different mesh openings.
Table 2-2. Comparison of mesh openings (0.35 mm vs. 0.1 mm), quantities of particles obtained in simultaneous sampling cases, and their ratio.

<table>
<thead>
<tr>
<th>Sampling No.</th>
<th>Mesh openings (mm)</th>
<th>Quantity of particles (items/sample)</th>
<th>d &lt; 1.0 mm</th>
<th>1.0 – d &lt; 5.0 mm</th>
<th>Total (d &lt; 5.0 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Quantity:</td>
<td>Ratio</td>
<td>Quantity:</td>
</tr>
<tr>
<td>No.1</td>
<td>0.35</td>
<td>146</td>
<td>1.98</td>
<td>0.35</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>289</td>
<td></td>
<td></td>
<td>217</td>
</tr>
<tr>
<td>No.2</td>
<td>0.35</td>
<td>105</td>
<td>4.46</td>
<td>0.35</td>
<td>154</td>
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<td></td>
<td>0.10</td>
<td>468</td>
<td></td>
<td></td>
<td>227</td>
</tr>
<tr>
<td>No.3</td>
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<td>116</td>
<td>4.78</td>
<td>0.35</td>
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<tr>
<td></td>
<td>0.10</td>
<td>555</td>
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<tr>
<td>Average</td>
<td>0.35</td>
<td>122</td>
<td>3.57</td>
<td>0.35</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>457</td>
<td></td>
<td></td>
<td>219</td>
</tr>
</tbody>
</table>

Notes: 1) 'd' is maximum Feret’s diameter; 2) 'Ratio' refers to the ratio of the quantity obtained with '0.10 mm' to that obtained with '0.35 mm'; 3) For particles of < 1 mm, final results are regarded as underestimated for both nets, due to discrepancies arising during analytical processing in the laboratory.

![Size distribution of plastic particles collected using nets with mesh openings of 0.3 mm and 0.1 mm at the same time in the same area.](image)

Notes: The X axis plots the longest length (maximum Feret’s diameter) and the color of the bars indicates the shortest length (minimum Feret’s diameter).

Fig.2-3. Size distribution of plastic particles collected using nets with mesh openings of 0.3 mm and 0.1 mm at the same time in the same area.
• “Mesh openings” as used in these Guideline is expressed as the side length of a quadrangle separated by mesh thread and through which sea water passes (① in figure on right), but in some cases the length of the diagonal line (② in figure on right) is used as the mesh opening. The researcher should confirm which mesh opening is meant and record the mesh opening used for the survey.

• The Manta net used in the study was 0.33 mm in diagonal line length (②), but it had a side length (①) of 0.24 mm.

• The results of the study indicate that if the mesh openings of the net used are between 0.1 to 0.35 mm, the results are considered comparable regardless of the definition of the mesh opening (side length or diagonal) for particles larger than 1 mm.
2.4 Tow parameters

2.4.1 Tow duration

**Keynotes**

- Tow duration has most commonly been set at about 10 to 30 minutes in past surveys. This usually depends on the amount of sampled particles required for analysis and the abundance of plankton or floating matter at the ocean surface.

- Earlier studies have reported that dispersion in collected results of microplastics increased in locations where the density distribution of the plastic at the ocean surface was higher on average compared to locations with lower plastic distribution density on average (Van del Hal et al., 2017).

- In CMSM2018, quantities of microplastic particles were assessed using simultaneous net sampling in the same sea section to assess tow duration. Two different comparisons were conducted 1) 20 minutes towing at port side and two consecutive runs of 10 minutes towing at starboard; and 2) 10 minutes towing at portside and two consecutive runs of 5 minutes towing at starboard.

- There was no significant difference in quantity of particles sampled between any tows of 20 minutes, 10 minutes, or 5 minutes in duration (Fig. 2-4). However, when particles density was relatively high in the ocean (~10 pieces/m$^3$) there were discrepancies between the first and second run of consecutive tows (both 5 and 10 minutes). There was also large variation between port and starboard results. These findings are similar to those of the above-mentioned study by Van del Hal et al. (2017).

- It is recommended to ensure appropriate volume of sampled water to reduce the influence of heterogenous microplastic distributions. For example, by setting the tow duration at approximately 20 minutes, as seen in many of the previous studies. Appropriate volume could be adjusted depending on the microplastic density in ocean.

- Also, it is desirable to sweep at least 1000 m$^2$ of ocean surface. This is equivalent to 200-500 m$^3$ of filtered water when towed with a typical net. However, this may not always be applicable depending on sampling conditions. For example, high densities of floating material may affect applicability. If clogged, one net could be replaced with a second one to sweep the required area of ocean surface when combined.
Explanatory Notes

- In many earlier studies, net towing was conducted for 10 to 30 minutes. At the 1st and 2nd International Expert Meetings, 20 minutes was recommended for tow duration to cover the area of trawling required to obtain representative values in the sea area.

- In CMSM2018, the quantity of microplastic particles was compared between those collected by towing at port side for 20 minutes or 10 minutes, and in the same sea area and at the same time, with the net at starboard exchanged in the middle of the trip to make two 10 minutes tows or two 5 minutes tows. The results showed no significant difference observed in the quantity of plastic particles of larger than 1 mm and less than 5 mm due to tow duration, but dispersion has been observed when densities of particles at the ocean surface are relatively high in the survey area.

- Dispersion in the results may have been due to coincidental sampling of patchy high-density water masses, for example, from prominently uneven distributions of particles that are formed when microplastic densities at the ocean surface are high.

- To capture representative values in the sea area, it is necessary to reduce the influence of such high-density water masses and obtain a leveled result.

- Regarding tow duration, many guidelines state that 15 to 30 minutes is appropriate (Lippiatt et al, 2013; EC, 2013; GESAMP, 2015; etc.). Considering the uneven distribution of microplastics as mentioned above, trawling for shorter durations may be inappropriate from the viewpoint of obtaining values representative of the sea area.

- Consequently, it is deemed desirable to set tow duration at about 20 minutes, within a range that does not cause clogging of the mesh due to plankton or floating matter.
Fig. 2-4. Comparison of microplastic particle densities at different tow durations.
### 2.4.2 Vessel speed

**Keynotes**

- Vessel speeds at the time of towing were reported as approximately 1 to 3 knots in earlier surveys.

- Tow runs were conducted with a vessel speed of 1 to 3 knots as speed against water (normally referred as log speed) during CMSM2018. Towing at 2 knots (about 1 m/sec) for 20 minutes with a net 75 cm in width resulted in approximately 1,200 m of tow distance, and samples collected from about 1000 m² of sea surface area or approximately 350 m³ of sea water volume.

- Regarding vessel speeds for towing, if the speed is too fast, the inflow at the net mouth becomes turbulent and the filtering efficiency may sometimes decrease (Ogi, 1991; GESAMP, 2016). It is thought that the towing vessel speed should be set at 1 to 3 knots, although this depends on the type of equipment and vessels.

### 2.4.3 Sweep area and filtered water volume

**Keynotes**

- Microplastics observed at the ocean surface are often reported as quantity of particles or weight per unit area (l/m², /km²) and/or as quantity of particles per unit water volume (l/m³). Therefore, it is necessary to obtain the swept area of the net tow and/or the amount of filtered water volume, as calculated by the following equations:

  - Swept area = net width × tow distance
  - Filtered water volume = (net width × net immersion depth) × tow distance
  * Net width is the horizontal dimension of the net aperture

- Refer to §2.4.4 for estimation of tow distance. Reporting the tow distance estimation methods is recommended, including equations and numeric figures used in calculation of the tow sweep area and filtered water volume.
2.4.4 Tow distance

Flow meter (RIGO.No.5571)  Neuston net with flow meter

Keynotes

· As the abundance of microplastics at the ocean surface is reported as particle quantity or weight per unit water volume or unit surface area, the filtered water volume or the swept surface area of each net sampling should be estimated in accordance with the units used in reporting.

· In prior research, the filtered water volume or the swept surface area have generally been obtained by multiplying the tow distance by the net immersion area or the net aperture width respectively. There are three methods for obtaining tow distances, as follows:

  ① Calculate from ground speed obtained from position information measured by GPS, etc.
  ② Calculate from the relative speed of the vessel to seawater (log speed), measured with a current meter.
  ③ Calculate using the rotation count of a flow meter installed in the net mouth and its calibration value.

· In CMSM2018, tow distances were measured using all three methods simultaneously during the same runs and the results were compared. Tow distance calculated using method ① showed large differences depending on the dominant direction of water flow when compared to methods ② and ③. Results for methods ② and ③ were similar, but the values estimated by method ③ were lower than those by method ②, almost reaching 70% at times when net resistance was large due to much floating matter encountered at the sea surface.

· Thus, method ③ appeared to generate the most accurate value for estimating the water volume passing through the net both theoretically and experimentally.

· It is recommended that Method ③ be used with a flowmeter set at the net mouth to obtain
the tow distance, concentration of microplastics per swept area and also concentration of microplastics per filtered water volume. Calibration of the flow meter is important. Location/vessel position at the start and end of each tow should be recorded.

- If a flow meter is not available, it would be desirable to estimate sampled water volume using an appropriate tow distance found by other alternative methods, such as using speed relative to sea water.

**Explanatory Notes**

- Earlier studies have reported large differences between tow distances calculated from ground speed and tow distances calculated by a flow meter (Suaria et al., 2016).
- In CMSM2018, the tow distance obtained from the vessel speed relative to ground using GPS, the speed of the vessel relative to seawater measured by flowmeter, and the rotation number and calibration value using a flow meter, were compared.
- A difference as great as a two-fold increase was observed in some cases when comparing these methods (Fig.2-5 and Fig.2-6). It is assumed that tow distance calculated from ground speed may not reflect the actual amount of filtering. On the other hand, when comparing distances calculated from the relative speed of the vessel to the seawater (log speed) and distances calculated using a flow meter, similar values were obtained when the floating matter caught in the net was scant and the resistance of the net could be assumed to be small (Fig.2-7). In CMSM2018, when an electromagnetic current meter was installed on the side of the vessel to measure the speed of the vessel relative to the sea, it was found that the water in the vicinity of the vessel was pulled by friction in the direction of travel, making the speed of the vessel relative to the sea water slightly slower than the actual speed.
- However, when high volumes of floating matter are caught in the net and the resistance of the net is relatively high, the tow distance calculated using a flowmeter is 10 to 30% less than the distance calculated from the relative speed of the vessel to the seawater (log speed) (Fig.2-7). The main reason for this is considered to be decreased filtering efficiency due to the resistance caused by the entrained floating matter. Thus, it is assumed that calculating the filtered volume using the tow distance would provide results closer to the actual filtered water volume.
- For this reason, it is desirable to estimate the amount of filtered water by attaching a flow meter to the net mouth.
- In cases with high waves that may cause the flow meter to pop above the water surface during towing, it would be desirable to maneuver the vessel to ensure the flow meter is submerged and prevent the propeller from getting idled and the tow distance to be overestimated.
Fig. 2-5. Relationship between tow distance calculated from relative speed of the vessel to the seawater (log speed) and distance calculated from ground speed using coordinates obtained by GPS in the CMSM2018 survey.

Fig. 2-6. Relationship between tow distance obtained using a flow meter and distance calculated from ground speed using coordinates obtained by GPS in the CMSM2018 survey.

For calculating these data, the tow distance obtained while towing a Neuston net with mesh openings of 0.35 mm was used.
Fig. 2-7. Relationship between the tow distance obtained from log speed and that obtained using a flow meter in the CMSM2018 survey.

Data from surveys with less floating matter in the net throughout the tow, with no change in net immersion depth observed and relatively small resistance on the net, are plotted in blue; while those with a lot of floating matter and gradual increase in net immersion depth and hence relatively large resistance on the net are plotted in red. For calculating these data, the tow distance obtained while towing a Neuston net (0.35 mm) was used.
2. 4. 5 Tow position

**Keynotes**

- In general, a sampling net is towed at one side of the vessel. However, in some cases it may be towed at stern by angling a rope to divert the net from the center line of the vessel and avoid its wake.

- In CMSM2018, the influence of the wake, screw propellers, etc. was investigated by conducting several tows at the side of the vessel and at the stern 20 m away from the vessel simultaneously. As a result, data obtained for the stern net had a tendency to be as low 50 to 80% less with respect to the data obtained from the side (Fig.2-8). It is thought that vertical mixing caused by the vessel influenced the results.

- It is desirable to conduct sampling at the side of the vessel with less influence from its turbulence.

- In case there is no option other than towing at the stern, sampling should be conducted by steering the net well enough or obliquely behind the stern to a location with minimal influence from both the wake and the screws.

**Explanatory Notes**

- Nets are generally positioned on either side of the vessel (port/starboard) or at the stern. In CMSM2018, densities of particles collected were compared by towing Neuston nets at the port, starboard and stern simultaneously.

- With the net set at the stern (vessel size 16 m, rope length 20 m, towed directly behind the hull), the density of microplastics >1 mm was less than that obtained by collecting at the vessel side, suggesting influence of vertical mixing of microplastics caused by the wake, etc.

- Since the results of CMSM2018 were obtained under conditions with relatively strong wind and waves and a turbulent sea surface, sampling under calm conditions may result in a bigger difference between the quantities of collected particles at the side of the vessel and at the stern.
These are divided into two figures according to density of particles due to wide disparities, reflected in the larger scale of the X-axis in (b): (a) 0-0.8 items/m$^3$, and (b) 0-8 items/m$^3$.

**Fig. 2-8. Particle density comparisons depending on tow position.**
2. 4. 6 Net immersion depth

**Keynotes**

- Net immersion depths have been recorded between 10 cm and 100 cm. Manta net immersion depth is measured as the height of the net's mouth, whereas a Neuston net is often set at about 1/2 to 3/4 of the height of the net's mouth.
- Recording immersion depth of the net during sampling is important as the section area of the net mouth under the sea surface is multiplied by the tow distance to estimate the filtered water volume.
- In CMSM2018, the Manta net tended to jump and skip above the sea surface when the waves were rough, whereas, there were cases when the Neuston net sank over time as a large amount of floating matter was collected and maintaining a constant immersion depth was considered difficult when the waves were rough or there was an abundance of drifting seaweed, etc.
- Therefore, it is most important to tow the net in a way that keeps the immersion depth constant, and measures such as attaching a moderate weight, adjusting the length of the tow rope and avoiding high wave conditions that may cause the Manta net to jump and skip on the water surface are recommended.
- Periodically recording the net immersion depth during each sampling run is considered effective for accurately calculating the filtered water volume, particularly when towing a Neuston net and in conditions when the immersion depth cannot be controlled effectively due to large amounts of floating matter.

**Explanatory Notes**

- Data on net immersion depth at the time of towing are required for accurate calculation of net sampling area and filtered water volume. It is also important to clarify the depth from the surface at which the water is collected, and it is necessary to keep the immersion depth as constant as possible.
- In CMSM2018, Manta nets were observed to jump off the sea surface when wind and waves were present, making them difficult to tow. Although it is possible to make some adjustments by attaching a heavier weight to the net mouth or by changing the direction of the net relative to the wind and current, accurate sampling is assumed to be difficult if the wind is strong and waves are high.
- When a Neuston net was used, there was a greater change in net immersion depth when large pieces of floating matter (seaweed, jellyfish, etc.) were caught in the net, especially when a net with finer mesh openings was used or large amounts of plankton were caught. In this case, the
change in net immersion depth could not be reversed even if the length of the rope was adjusted.

- Therefore, in surveys using a Neuston net, attention is required when comparing results, as significant changes in immersion depth can be expected when many large pieces of floating matter are also present. In addition, filtering efficiency may also decrease.

- When towing in a sea area with a lot of floating matter, it is helpful to set a marker at the net mouth to indicate the immersion depth, and record the immersion depth at the net mouth by video camera or by taking photographs of the net mouth periodically (Fig.2-9).
Fig. 2-9. Chronological changes in net immersion depth obtained through image analysis.

**Immediately after tow starts (net immersion depth normal)**

**13 min. after tow starts (net immersion depth increased).**

**Sample inside net with increased immersion depth (a large amount of sea buckthorn (*Zostera marina*) has been caught).**

(May 18, Tow No. 5, starboard side)
2.5 Recording metadata

**Keynotes**

- In general, wind speed and wave height have a large effect on the microplastic density of the ocean surface layer (e.g., Reisser et al., 2015, Suaria et al., 2016).
- In a chronological series of data obtained in CMSM2018 microplastic particle density at the ocean surface decreased when wind speed and wave height increased.
- Additionally as salinity was observed to decrease, density of microplastics at the ocean surface showed a tendency to increase (Fig. 2-10). This was thought to have been caused by sampling in water bodies that were affected by river water.
- Therefore, to ensure comparability, axillary metadata for each sampling event should be recorded where possible through in situ observations or onboard instruments. Data required include time of day and date (to account for seasonality), as well as environmental variables (e.g., weather conditions, wind speed, wind direction, wave height, Beaufort scale index, chlorophyll, fluorescence, salinity etc.) and sampling parameters (net type and dimensions, measured sampling water volume, vessels movements – heave, pitch, roll, vessel speed, etc.). For more details, refer to Chapter 4 (p. 62).

**Explanatory Notes**

- First, when towing, the survey position coordinates and survey method items introduced above through Section 2.5, such as tow time, tow speed, rotation number of the flow meter, net position and net immersion depth, must be recorded.
- In CMSM2018, a nearly ten-fold difference in microplastic density at the ocean surface was confirmed over a relatively short time (about 20 to 30 min.) and small spatial scale (distance of about 100 to 500 m). Some correlations were observed when chronological changes in density were compared with physical environment data (wind and waves) and water quality data (water temperature and salinity). Specifically, as wind speed and wave height increased, the density of microplastics tended to decrease (see § 2.2, Fig. 2-2), and when salinity decreased the microplastic density tended to increase (Fig. 2-10).
- It would be desirable to check and record the wind direction and wave height before and after each survey, as it is also possible that stormy weather the day before the survey may affect microplastic densities at the ocean surface.
- In CMSM2018, there were cases in which the density of microplastics may have increased due to an influx of river water. When rain is observed shortly before or on the day of the survey, data on precipitation would be beneficial because river flows are strongly influenced by rainfall which may influence both salinity and fresh water input.
• The influence of tidal current direction and flow rate on microplastic collection results is not clear, but it is beneficial to record these as they are useful in considering the influence of loads from land areas such as via rivers. If the vessel does not have a current meter, record survey conditions using publicly available oceanographic data for the surveyed area.

• As water temperature and salinity are generally characteristic for each water mass, water temperature and salinity are considered useful information in confirming whether the properties of the water mass have changed between tows, especially in coastal zones that are easily affected by rivers and tides.

• In addition, there are indications that it is possible to minimize variance in collection results by towing in a way that keeps the direction of the net relative to the wind direction constantly perpendicular. It would be desirable to record the direction of the net relative to the wind direction and ocean current.

• The presence of floating matter captured in the net can also be recorded.

• Variables may differ from sampling cruise to sampling cruise, or even sample to sample. If it is possible to average all variables during a sampling event, e.g., 20-minute tow, this is preferable over only recording the information at the beginning and the end of each tow.

Fig. 2-10. Tide level and salinity (a) and chronological changes in microplastic particle density (b).
2.6 Implementing blank tests

Keynotes

- Generally, in net sampling of microplastics, the net is cleaned thoroughly from its outside before the start of a sampling run to ensure no plastic particles are left inside the net. The influence of plastic particles remaining in the net on the survey results can be significant, especially in sea areas where the quantity of sampled microplastics is relatively small. Therefore, cleaning just before each sampling run is particularly important to prevent plastic particles from clothing, equipment, the vessel's paint, etc. from entering the net and affecting the results.

- In CMSM 2018, blank tests were carried out eight times in a similar manner to those reported (GESAMP, 2019) by washing the net before towing, comparable to washing after towing, by hanging the net with a crane and pouring pumped sea water from the outside the net, then counting the microplastics in the cod end. Two microplastic particles were observed on average.

- A similar blank test was carried out for a net that had been kept in a natural fiber bag for a long time after being thorough cleaning at the end of a survey. This specific net was observed to contain many particles (n=24 particles), indicating that contamination may occur during storage.

- Nets should be washed thoroughly just before each sampling run due to the risk of contamination during storage.

- A blank test is recommended to be conducted for at least one of several nets to be used for a survey, as it can confirm whether sampling procedures such as washing have been carried out properly without contamination. When towing multiple times, it would be desirable to periodically conduct blank tests to ensure particle contamination has been sufficiently controlled.

Explanatory Notes

- Generally, when net towing is completed, the rotation number of the flow meter is first recorded, then the net is hung using a crane or pulley and cleaned thoroughly from the outside. For washing the net, it is common to use sea water pumped up using a pump installed aboard the vessel. When doing so, care needs to be taken to avoid having the sea water enter the net via the mouth.

- In CMSM2018, blank sampling was conducted by washing unused nets in the same manner as for sampling, and on average two plastic particles (ranging from 0 to 5) were confirmed.

- Confirmed particles were all sufficiently shorter in shortest length than the mesh openings (0.35 mm), and the composition of the material also differed from those obtained in the
surveyed surface layer. Therefore, it is assumed that plastic particles smaller than the mesh openings do not cause contamination when washing from the outside of the net.

- Also, when a net that had been stored for a long time was used in a blank test without washing immediately before use, more particles were confirmed than when the net was washed in advance. This net had been thoroughly cleaned after the most recent past survey and stored in a natural fiber bag.

- Before using a net that has been stored for a long time, it would be desirable to wash it again, even if it was thoroughly washed after the previous survey, taking into consideration the possibility of contamination with plastic particles during storage.

- The quantity of particles collected each day in the survey area was around 100 to 2,000, so the several plastic particles collected in the blank test were considered not to have a significant influence on the survey results (above the limit of detection, LOD). However, a higher level of plastic particle contamination (24 pieces) was confirmed in a net stored for a long time, so the influence cannot be ignored when sampling in sea areas where the quantities collected are small. Therefore, it is necessary to pay attention and avoid contamination as the survey is conducted.

- When particles confirmed as plastic were found in the blank sample, a high proportion of particles were vinyl chloride, polystyrene and polyurethane. These materials are rarely found in the survey samples. Therefore, it is assumed that the net had been contaminated not only from its washing, but also from tape used to fix equipment and the vessel’s buoy and paint. For surveys, it is advisable to pay attention to plastic products on the vessel and take measures to prevent contamination, such as keeping them as far away as possible from places where samples are processed.

- To understand how accurately procedures such as washing are carried out, implementing a blank test for at least one out of several nets is recommended.
3. Laboratory analysis of microplastics

3.1 Outline

- In general, analysis of samples that include microplastics obtained by trawling a net through the ocean surface layer is carried out in the following order: pretreatment (separation of non-plastic material other than microplastics), picking out microplastics, counting and measurement, and material identification. Depending on the purpose of the study, their weight may also be measured.

- The order of the pretreatment process, i.e. density separation, biological digestion and sample splitting, may differ depending on the purpose of the survey and the state of the sample.

- Prior to all analytical processes, fractionation of the samples, including non-plastic material, by sifting through sieves of various sizes is sometimes performed before pretreatment.

![Diagram](image)

Fig. 3-1. General flow of microplastic analysis.

※Prior to all analytical processes, fractionation of the samples, including the non-plastic material, by sifting through sieves of various sizes is sometimes performed as a pretreatment.

※Counting and measurement of sizes and weights conducted based on purpose of the study.
• When comparing the densities of microplastics obtained through laboratory procedures, care should be taken to note which method was used, as oversight or loss of microplastics may occur depending on the pretreatment or separation methods. The proficiency level of the analysts may also be a source of errors.

• Prior to preparation of these Guidelines, an international collaborative analysis with the participation of 12 laboratories from 10 countries (ILC2017) was conducted using standard samples to ascertain the extent of variation in results depending on the various analytical methods used. Each of the standard samples contained plastic particles of the same quantity, size and weight and some non-plastic material (plankton, seashells, wood pieces, crustacean shells, etc.). Two samples were sent to each laboratory, one with a large amount of microplastics and non-plastic material simulating a sample from an inner bay, and the other with few microplastics and non-plastic materials, simulating a sample from the outer ocean. These samples were analyzed according to the analytical methods of each laboratory, and the results and analytical procedures used were reported. The differences between the results reported from each laboratory and the design value of the standard samples were compared and discussed in terms of whether the differences were of a systematic nature.

• Recommendations and points to be noted in each analytical process are introduced in this chapter based on the results of ILC2017 and with regard to harmonization.

• These Guidelines focus on the microplastics present in sea water and do not cover analysis of microplastics taken in by lower organisms such as plankton.

• The results of this joint analysis (ILC2017) have been submitted to an academic journal for publication.
3.2 Preprocessing for analysis

- Samples obtained by net towing contain various natural particles as well as plastic particles. Removing the non-plastic particles as much as possible through pretreatment, improves the accuracy of subsequent processing for plastic particles such as picking, material identification, counting and weighing.

- For that reason, pretreatment may be performed when sampled particles include non-plastic material.

- Fractionation of the samples, including the non-plastic material, by sifting through sieves of various sizes is sometimes performed before pretreatment.

- Pretreatment methods include density separation, mainly to remove inorganic particles, and digestion of organism-derived organic substances by oxidation, hydrolysis or enzymatic reactions.

- When there are many plastic particles or non-plastic items per sample, the sample may be subsampled to reduce the amount of counting, measuring and other work at the time of analysis.

- In ILC2017, nine out of the 12 laboratories conducted pretreatment: three laboratories conducted only density separation, two conducted only digestion of organic matter, and four carried out both pretreatments. Sample splitting was not conducted at any of the laboratories.
3.2.1 Biological digestion and chemical treatment

This figure illustrates implementation of WPO (wet peroxide oxidation). To digest the organic matter, hydrogen peroxide (H$_2$O$_2$) is added to the sample. In this process, Fe (II) is also added as a catalyst. The photographs show addition of these solutions to the sample to obtain the reaction time.

**Keynotes**

- When there are many non-plastic materials such as plankton (in the sample), pretreatment to digest organic substances with chemicals or enzymes is performed in many cases to remove the non-plastic material as well as biofilms that have formed on the surface of the sampled plastic particles. The intent is to minimize the possibility of misidentifying plastic particles, improving the accuracy of the picking out process and overall work efficiency. If improperly conducted, however, it may lead to deterioration (deformation and/or weight reduction) of plastic particles from chemicals added or from heating.

- The purpose of digesting organic substances is not limited to removal of non-plastic material to simplify subsequent processing but may also include analyzing microplastics ingested by organisms in the lower trophic levels such as plankton, although the latter purpose is not covered by these Guidelines.

- Digestion of organic substances is effective when biofilms are formed on the surfaces of plastics and non-plastic material in the sample to the extent that they may interfere with weight measurement and material identification using spectral optical instruments. It should be noted that the size of microplastics incorporated into organisms in the lower trophic levels, such as plankton, is often in the order of 10 μm (Botterell et al., 2018).

- In ILC2017, there was no systematic difference in the measurement results of plastic particles (larger than 1 mm and less than 5 mm) between the laboratories that performed organic matter digestion on the standard samples and the laboratories which did not. For particles < 1 mm,
the results for the quantity and weight of particles were underestimated by all of the laboratories but the values obtained by laboratories performing digestion treatments tended to be closer to the original value.

- On the other hand, there was one laboratory conducting digestion that was unable to measure the quantity of particles correctly because the particles aggregated due to biological residue caused by insufficient digestion.
- From the viewpoint of harmonizing monitoring methods for particle quantity density of particles larger than 1 mm and less than 5 mm in size, it is not always thought to be necessary to digest organic matter as a pretreatment.
- On the other hand, when analyzing particles of less than 1 mm in size, it would be preferable to digest the organic substances to obtain more accurate analytical results.

**Explanatory Notes**

- Digesting organic matter contained in the sample through oxidation, hydrolysis or enzymatic reactions makes separation of plastic particles easier.
- Removing biofilms formed on the surface of plastic particles or non-plastic material, if present, is expected to make material identification by spectral optical instruments more accurate (see §3.5).
- On the other hand, there are reports of plastics deteriorating when strong acids are used to digest organic substances (e.g., Miller et al., 2017; Hurley et al., 2018; GESAMP 2019).
- In ILC2017, six out of the 12 laboratories conducted organic matter digestion as a pretreatment: among these, three laboratories conducted digestion using hydrogen peroxide and divalent iron solvent (H₂O₂, Fe²⁺), one laboratory used only hydrogen peroxide (H₂O₂), one laboratory conducted alkaline digestion using potassium hydroxide (KOH), and one laboratory conducted biochemical digestion using corolase enzyme. Advantages and disadvantages of various biological digestions and chemical treatments are shown in Table 3-1.
- When comparing errors in the measurement results between laboratories that conducted organic substance digestion and laboratories that did not, there was no systematically significant difference in particle quantity measurement results. Consequently, from the viewpoint of harmonization, microplastic measurement results can be compared regardless of whether digestion was performed or not.
- It should be noted that, however, that in ILC2017, there was a case in which aggregation of particles due to the gluing effect of biological residue was observed because of insufficient digestion.
- Among the particle quantity and weight measurements in ILC2017, the results for particles of less than 1 mm among laboratories that did not conduct digestion tended to be underestimated compared to the design value.
Laboratories using digestion of organic substances reported more accurate values for particles <1 mm. It is assumed that digestion makes it easier to pick out plastics.

When conducting digestion, depending on the purpose and equipment of the study and the state of non-plastic material in the sample, care should be taken to select conditions that do not cause deterioration of the plastics and avoid influence from digested biological residue (appropriate reagents, temperatures, digestion times, etc.).

Table 3-1. Advantages and disadvantages of various biological digestions and chemical treatments (reproduced from GESAMP, 2019).

<table>
<thead>
<tr>
<th>Purification method</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative digestion</td>
<td>• Inexpensive</td>
<td>• Temperature needs to be controlled</td>
<td>Masura et al. (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Several applications may be needed</td>
<td></td>
</tr>
<tr>
<td>Acid digestion</td>
<td>• Rapid (24 h)</td>
<td>• Can attack some polymers</td>
<td>Claessens et al. (2013)</td>
</tr>
<tr>
<td>Alkaline digestion</td>
<td>• Effective</td>
<td>• Damages cellulose acetate</td>
<td>Dehaut et al. (2016)</td>
</tr>
<tr>
<td></td>
<td>• Minimal damage to most polymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzymatic digestion</td>
<td>• Effective</td>
<td>• Time-consuming (several days)</td>
<td>Löder et al. (2017)</td>
</tr>
<tr>
<td></td>
<td>• Minimal damage to most polymers</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.2.2 Density separation

Density separators
Density separation is often performed by pouring the sample and a dense solvent into a funnel or a separating funnel.

Floating plastic particles in a density separator
Plastics with lower specific gravity than the solvent float on the surface.

Keynotes

- As a part of pretreatment, density separation may be performed to remove non-plastic material in the sample. Unlike in the case of analyzing microplastics contained in sediment, which requires removing sand, mud, etc., it is not commonly performed in analyzing microplastics at the ocean surface.

- In ILC2017, there was no systematic difference in analytical results among laboratories that performed density separation on the standard samples and laboratories that did not perform density separation.

- In cases where there is a lot of non-plastic material, density separation would be effective as it enables efficient separation of plastic particles, but from the viewpoint of harmonizing the methods of monitoring microplastics at the ocean surface, it is not necessarily required.

Explanatory Note

- Density separation is an effective method of fractionating low-density plastic particles and high-density natural particles of inorganic matter.

- In general, density separation is conducted by mixing the sample into a solution with a higher specific gravity than that estimated for the collected plastic particles, letting high-density inorganic substances settle out and recovering and fractionating the floating low-density plastic particles. Commonly employed solutions for density separation of microplastics are shown in Table 3-2.
Density separation is a particularly effective process for measuring microplastics in bottom and coastal sediment samples that include heavy materials such as sand, seashells, etc. It is not necessarily a common practice in analyzing floating microplastics samples collected with nets at the ocean surface, but it pays to be aware that there may be lots of plankton.

In ILC2017, density separation was carried out at seven out of the 12 laboratories, using aqueous solutions of sodium chloride (NaCl) or sodium metatungstate hydrate (Na₂WO₄) for the separation.

However, there were no systematically significant differences in the measurement results between laboratories that did or did not perform density separation. Consequently, from the viewpoint of harmonization, the results of surface layer microplastic density per filtered water volume can be compared for microplastics that are 1 mm or larger and less than 5 mm regardless of whether density separation was performed or not.

Table 3-2. Solutions commonly used for the density separation of microplastics (reproduced from GESAMP, 2019)

<table>
<thead>
<tr>
<th>Salt</th>
<th>Density (g cm⁻³)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Chloride (NaCl)</td>
<td>1.2</td>
<td>Hidalgo-Ruz et al. 2012</td>
</tr>
<tr>
<td>Sodium Polytungstate (PST)</td>
<td>1.4</td>
<td>Hidalgo-Ruz et al. 2012</td>
</tr>
<tr>
<td>Sodium Iodide (NaI)</td>
<td>1.6</td>
<td>Claessens et al. 2013</td>
</tr>
<tr>
<td>Zinc Chloride (ZnCl₂)</td>
<td>1.7, 1.6</td>
<td>Imhof et al. 2012, Zobkov &amp; Esiukova, 2017.</td>
</tr>
</tbody>
</table>
### 3.2.3 Sample splitting

<table>
<thead>
<tr>
<th>Folsom splitter</th>
<th>Use of Folsom splitter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Splitter is moved back and forth to mix thoroughly, then the sample is divided.</td>
</tr>
</tbody>
</table>

**Keynotes**

- Sample splitting before counting is often performed in analyses for zooplankton, especially where the quantities sampled are large, but it is not common in the analyses of microplastics.

- In ILC2017, the standard samples and samples obtained in actual sea areas were divided with a splitter (Folsom splitter) and measured. The estimated values of total quantity of particles from the divided samples had about ± 10% error with respect to the total measured quantity of particles. When the splitting was repeated, a tendency for the error to increase was observed.

- Using a splitter may be effective when the quantity of particles in one sample is large (e.g., when it exceeds 1,000 or so) or when there are time or personnel constraints, keeping in mind that a certain level of error is expected.

**Explanatory Notes**

- In a small proportion of analyses for zooplankton and microplastics, the samples are divided and only a part of each sample is analyzed to improve the efficiency of the analysis. In one case (Fossi et al., 2016, Di Mauro et al., 2017) the samples were divided using a Folsom splitter, known for its high splitting accuracy (Guelpen et al., 1982).

- After confirming no loss of particles when using a splitter on the standard samples in ILC2017, a splitter (Folsom splitter) was used in a trial with samples obtained in an actual sea area.

- The samples obtained in the actual sea area were divided using the splitter and the quantity of particles was counted. Compared to a sample for which all particles were counted, the error was about ± 10% for a sample divided into two using the splitter once, and ± 20% for a sample divided into four using the splitter twice. It is assumed that almost the same level of accuracy was achieved in the splitting process for plankton (Guelpen et al., 1982).
As described above, dividing microplastic samples using a splitter is an effective means of improving efficiency when large amounts of samples need to be divided and time is limited, or when analyses need to be conducted with limited human resources or time, keeping in mind that some error is expected. When dividing the sample with a splitter, it is necessary to stir it thoroughly and sufficiently wash and collect the sample sticking to the wall of the splitter. It would also be desirable to verify the degree of error for the sum of measurements obtained from each portion of a sample divided by the splitter compared to the measurement of the original sample.

### Table 3-3. Error due to use of splitter.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Average (Coefficient of Variation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>$N_{\text{total}}$</td>
<td>873</td>
</tr>
<tr>
<td>$N_{\text{sub}} \times 2 / N_{\text{total}}$</td>
<td></td>
</tr>
<tr>
<td>sub-R</td>
<td>1.03</td>
</tr>
<tr>
<td>$N_{\text{sub}} \times 4 / N_{\text{total}}$</td>
<td></td>
</tr>
<tr>
<td>sub-LR</td>
<td>1.20</td>
</tr>
<tr>
<td>sub-LL</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Note: 
- ' $N_{\text{total}}$ ' indicates the total quantity of microplastics in all sub-samples.
- ' $N_{\text{sub}}$ ' indicates the quantity of microplastics in each of the sub-samples.
- 'sub-R' indicates one of the sub-samples split into two.
- 'sub-LR' and 'sub-LL' indicate one of the sub-samples split into four.
- 'R' indicates the right side of the splitter, 'L' indicates the left side of the splitter.
- 'Coefficient of variation': (standard deviation) / (average)

### Fig. 3-2. Ratios of quantity of particles estimated from the result of counting divided samples to the quantity of particles when the total quantity was counted.
3.3 Picking out microplastic particles

Picking out process
To separate microplastics, it is common to pick them out manually under a stereomicroscope.

Microplastics on a petri dish
Microplastic particles collected in CMSM 2018.

Keynotes

- Picking out particles is an important process that greatly affects the accuracy of microplastic analysis.
- There are several methods of separating plastic particles from a sample, such as picking plastic particles out after fractionating the sample by size using sieves of various sieve mesh opening sizes such as 5 mm, 1 mm, and 0.3 mm, and picking the plastic particles from the filter paper after directly filtering the sample. Stereomicroscopes are commonly used to facilitate picking out microplastics.

- In ILC2017, despite the use of stereomicroscopes by all the laboratories for hand-picking, the quantities of separated plastic particles <1 mm from the standard samples were underestimated by 40 to 80%, and the variance in reported results among the laboratories was also large. This was thought to have resulted from the loss of particles in the pretreatment process and incomplete picking due to overlooking small particles masked by contaminants.

- To obtain fairly accurate results, conducting the picking process carefully is recommended even when a stereomicroscope is used, and exerting caution to avoid losing particles in pretreatment. Be especially careful not to overlook particles smaller than 1 mm.
- Therefore, as in case with sampling errors arising due to mesh sizes, to maintain comparability of results, reporting results for both particles smaller than 1 mm and for particles larger than 1 mm separately is recommended.
Explanatory Notes

- The accuracy of picking out particles greatly affects microplastic analysis results, as plastic particles picked out from the sample, whether pretreated or not, are used for subsequent measurement and analysis.
- Particle fractionation using sieves with various mesh openings, such as 5 mm, 1 mm, 0.3 mm may be carried out before pretreatment when the samples include non-plastic materials.
- In ILC2017, many laboratories filtered the samples through sieves with mesh openings of 5 mm or 0.3 mm for fractionation and then suction filtered using filter paper of about 0.8 μm. Many laboratories used glass fiber filters and polycarbonate filters. These laboratories used stereomicroscopes to pick out particles from the sieves or filter paper. However, the analysis results for quantities of particles smaller than 1 mm were less than the design value (about 40-80% of the design value). This was seen across all laboratories, and the variation in the reported values between these laboratories was also great.
- This may reflect the difficulty in visually finding small particles that are mixed in with non-plastic material. Also, as glass petri dishes were used in many cases when picking out the particles, they may have caused difficulty in picking out transparent particles.
- For improvement, laboratories participating in ILC2017 have suggested that it would be advisable to work with a microscope as much as possible, using not only the backlight but also the incident light when confirming the existence of particles.
- It is necessary to pay special attention when picking out fibrous particles as they can be mistaken for other materials (natural fibers, etc.).
- At the same time, it is also important to build capacity among analysts to improve the accuracy of the picking process. Therefore, recovery tests and duel identification procedures are recommended.
- Although it is convenient to use sieves before picking out the particles, special care should be taken to avoid losing particles that have longest lengths greater than the sieve openings, which may nonetheless pass through the sieve (see §3.4, Fig. 3-3).
- When separating with a sieve, it is desirable to re-collect the sample passed through the finest sieve on filter paper.
- In ILC2017, the research institute that conducted re-collection reported a value closer to the design value for fine particles smaller than 1 mm.
3.4 Counting and measuring sizes of particles

Measurement of microplastics
Measurement using photos of plastic particles and image processing software. The longest length is measured from the captured image and quantities of particles are aggregated by size.

Multi-staged sieve with various mesh openings
The photo shows a multi-staged sieve with 4 mm, 1 mm, 0.3 mm and 0.1 mm openings. Plastic particles are poured from the top, and the quantity of particles remaining in each sieve is aggregated as the quantity of particles by size.

Keynotes

- Microplastic abundance at the ocean surface is most commonly reported in quantities of particles by size.
- There are two common methods for counting the quantity of particles by size: (1) directly measuring the longest diameter (maximum Feret’s diameter) of separated particles individually, and, (2) counting the quantity of particles remaining in the sample after fractionating by size using sieves of various mesh opening sizes.
- In ILC2017, many laboratories measured the longest length using image processing software or calipers by method (1) and summed up the quantity of particles by size. There were some laboratories that fractionated with sieves of different mesh openings as in method (2), counted the quantity of particles remaining in each sieve and reported them as quantities of particles by size.
- For particles sampled during CMSM2018, the quantities of plastic particles that were measured for their longest length and particles that were sieve fractionated were compared, with the hypothesis that plastic particles of up to 7 mm, which is around the diagonal length of a 5 mm square mesh opening, would pass through a 5 mm sieve. It was found that the quantity of particles smaller than 5 mm obtained by the latter method (using sieves) was about 1.25 times larger than by the former. The above findings indicate that in sieve fractionation, the quantity of particles smaller than 5 mm counted using sieves would be overestimated.
because particles with a shortest length smaller than the diagonal length of the mesh may pass through the sieve, even if their longest length exceeds 5 mm.

- In terms of harmonization, measuring the longest length of each particle using image processing software is recommended.
- When estimating the quantity of particles and/or weight of particles by size using only sieves, it is necessary to keep in mind that it would be difficult to compare the results with those of particles measured directly for longest length.
- We also recommend providing classification of plastic particles by morphological traits such as beads, fragments foams, pellets and fibers, noticing the difficulty of distinguishing between natural and synthetic fibers.

**Explanatory Notes**

- Size fractionation by sieving is effective from the viewpoint of efficiency. However, in the case of using a 5 mm lattice sieve mesh, particles up to 7.0 mm, which is the diagonal length of the openings, or fibrous particles having a shortest length may pass through the sieve (Fig. 3-3).
- For one sample collected during CMSM2018, the quantity of particles by size was compared. The results showed that the quantity of particles smaller than 7 mm that could pass through the diagonal line of a sieve with a 5 mm lattice was 1,974. When maximum diameters were measured for the same sample, the quantity of particles obtained smaller than 5 mm was 1,574. When using a 5 mm mesh sieve, the result is overestimated by about 20%, as particles with a longest length of more than 5 mm could also pass through the sieve.
- These findings suggest the possibility that sieve fractionation may lead to overestimation of particles < 5 mm in size.
- When only sieving is used, care should be taken to note the fact that the obtained results cannot be compared to fractionation results obtained by measuring longest lengths. Consequently, it would be desirable to measure longest lengths and aggregate the quantity of particles by size.
- Recently, image capturing devices and software/applications capable of image processing can be obtained inexpensively, and particle size measurement by image processing is relatively easy. Therefore, it would be desirable to measure particle sizes using these devices and software.
- When measuring longest lengths with image processing software, it would be preferable to record the shortest lengths and projected area simultaneously.
- Depending on the purpose of the study, the shape and color of the plastic particles may need to be recorded, and there are also guidelines that recommend recording these (EC, 2013, etc.).
- The shape and color of plastic particles are valuable information for identifying sources. Also, as the color of plastics is considered to be related to uptake by organisms (e.g., Des forges et al., 2015, Steer et al., 2017.), it is important to acquire these data for future study.
- In many studies performing classification by shape, commonly seen shape categories include fragments, beads, foam, pellets and fibers.
If the projected area of individual particles is measured after classifying the particles by shape, it may be possible to convert the projected area to weight with a conversion formula using volume, weight and plastic density.

**Fig. 3-3. Relationship of the mesh openings of a sieve to the particle sizes that may pass through.**
3.5 Identifying microplastics

Keynotes

- Spectral optical instruments such as IR/Raman spectroscopy are used most commonly to separate microplastics from non-plastic materials and identify polymers.

- In ILC2017, many laboratories used Fourier Transform Infrared Spectroscopy (FTIR, including ATR-FTIR) for material identification, while others used Raman spectroscopy.

- The accuracy of hand-picking relying solely on visual observation with a stereoscopic microscope was particularly low, although there were only a few laboratories that did not use spectral optical instruments to pick out microplastics. Meanwhile, the laboratories that applied other methods such as pushing the particles with a needle, etc., in addition to visual observation, reported values close to the design value.

- From the viewpoint of harmonization and accuracy, it is essential to confirm the material of plastic particles using the spectral optical instruments to ensure accuracy of separation by hand-picking.

- Even when using such spectroscopy, knowledge of chemistry is recommended and appropriate training is required when conducting separation because it may be difficult to determine whether particles are made of plastic or not.

- In addition, when confirming materials by visual inspection without using spectral optical instruments such as FTIR, having an analyst who is skilled at separating plastic particles is recommended.

Explanatory Notes

- Visually identifying microplastics from non-plastic material is generally difficult at the hand-picking stage, especially with particles <1 mm. Confirming that the particles picked out are plastics and correcting the counting or measurement results is desirable.
Even if it is difficult to analyze characteristics of all microplastic particles by spectroscopy, confirming the characteristics of some of the particles by spectroscopy is recommended.

The EC guideline (EC, 2013) recommends spectroscopic analysis for a subsample of 10% of the identified particles to verify visual identification, and this method has been applied in several reports (e.g., Lusher et al., 2018). The European Union’s Marine Strategy Framework Directive (MSFD) also recommends that a proportion (5·10%) of all samples be routinely checked to confirm the accuracy of visual examination (Gago et al., 2016).

At the International Experts Meeting held to examine these guidelines, it was pointed out that a best-case scenario would be testing all particles for chemical composition with FTIR or similar devices. In cases where time and resources do not allow this, a representative subsample would be a part of the total sample that reflects the composition of particles, both in shape and color. For example, if there are both fragments and fibers in a ratio of 1:5, at least one fragment to every 5 fibers should be assessed to a value which exceeds 20% of the overall total.

In ILC2017, laboratories that identified the material only by visual confirmation reported larger quantities than the design value of the standard samples due to errors in mistaking natural particles for plastic ones.

Particularly for small particles with a longest length of less than 2 mm, it would be easy to misidentify plastics and non-plastic material when conducting visual inspection only (Isobe et al., submitted), so even when studying plastic particles larger than 1 mm and less than 5 mm, if there are many small particles of 1 to 2 mm, use of a spectral optical instrument for composition analysis is recommended.

Understanding the composition of plastics using spectral optical instruments such as FTIR, ATR-FTIR, Raman spectroscopy, or infrared cameras, is useful not only for separating the particles from other substances/materials but also for obtaining useful information in regard to the sources of the plastics.

It is necessary to note that biofilms adhering to particle surfaces may make it difficult to identify materials or analyze composition using spectral optical instruments and that a certain amount of experience is required to be able to determine if the obtained spectra reflect the characteristics of plastics or not. If uncertain about particle analysis, it would be desirable to check the results with an experienced analyst.

Advantages and disadvantages of recent microplastic characterization methods, including identification of polymer types, are shown in Table 3·4.

In recent years, equipment such as ATR-FTIR that can perform counting, shape measurement and material identification simultaneously has started coming into use.
Table 3-4. Advantages and disadvantages of microplastic characterization methods, including identification of polymer types (reproduced from Shim et al. (2017)).

<table>
<thead>
<tr>
<th>Identification method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>Simple</td>
<td>No chemical information for confirming composition</td>
</tr>
<tr>
<td></td>
<td>Low cost</td>
<td>High possibility of false positives</td>
</tr>
<tr>
<td></td>
<td>Color and morphological information</td>
<td>High possibility of missing small and transparent particles</td>
</tr>
<tr>
<td>Microscopy + spectroscopy (sub-set)</td>
<td>Polymer composition of a sub-set of the sample</td>
<td>Possibility of false positives</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Possibility of missing small and transparent particles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sub-set may not be representative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Potential bias in sub-set selection</td>
</tr>
<tr>
<td>Microscopy + FTIR spectroscopy</td>
<td>No false positives – confirmation of all plastic-like particles</td>
<td>Manual selection of particles means some plastic may be missed</td>
</tr>
<tr>
<td></td>
<td>Reduction in false negatives</td>
<td>Expensive instrument</td>
</tr>
<tr>
<td></td>
<td>Non-destructive</td>
<td>Laborious and time-consuming for identification of all particles</td>
</tr>
<tr>
<td></td>
<td>20 μm particle detection limit</td>
<td>Requires expertise in spectral interpretation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contact analysis (ATR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Need to transfer particles from filter paper to metal plate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Removal of organic material a prerequisite</td>
</tr>
<tr>
<td>Microscopy + Raman spectroscopy</td>
<td>No false positives – confirmation of all plastic-like particles</td>
<td>Manual selection of particles means some plastic may be missed</td>
</tr>
<tr>
<td></td>
<td>Reduction in false negatives</td>
<td>Expensive instrument</td>
</tr>
<tr>
<td></td>
<td>1 μm particle detection limit</td>
<td>Laborious and time-consuming for identification of all particles</td>
</tr>
<tr>
<td></td>
<td>Non-destructive analysis</td>
<td>Requires expertise in spectral interpretation</td>
</tr>
<tr>
<td></td>
<td>Non-contact analysis</td>
<td>Interference by pigments</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Risk of laser damage to particles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Removal of organic material a prerequisite</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exact focusing required</td>
</tr>
</tbody>
</table>
Table 3-4 (2). Advantages and disadvantages of microplastic characterization methods, including identification of polymer types (reproduced from Shim et al. (2017)).

<table>
<thead>
<tr>
<th>Identification method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semi-automated spectroscopy (mapping based)</td>
<td>No manual particle selection error</td>
<td>No visual image data on single particles</td>
</tr>
<tr>
<td></td>
<td>High automation potential</td>
<td>Production of a large volume of data</td>
</tr>
<tr>
<td></td>
<td>In principle no false negatives</td>
<td>Long post-processing time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Still requires expertise in spectral interpretation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Efficient removal of interfering particles a prerequisite</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Still lacks validation for smaller particles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Expensive instrument</td>
</tr>
<tr>
<td>Semi-automated spectroscopy (image analysis directed point analysis)</td>
<td>High automation potential</td>
<td>Production of a large volume of data</td>
</tr>
<tr>
<td></td>
<td>Fewer false negatives</td>
<td>Long post-processing time</td>
</tr>
<tr>
<td></td>
<td>Potential for faster sample throughput</td>
<td>Still requires expertise in spectral interpretation</td>
</tr>
<tr>
<td></td>
<td>Size and morphology of single particles</td>
<td>Efficient removal of interfering particles a prerequisite</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Still lacks validation for smaller particles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Expensive instrument</td>
</tr>
<tr>
<td>Thermal analysis</td>
<td>Simultaneous analysis for polymer type and additive chemicals (Pyro-GC/MS)</td>
<td>Destructive analysis</td>
</tr>
<tr>
<td></td>
<td>Mass-based information</td>
<td>No quantity or size-based information</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Limited polymer type identification (DSC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Complex data (Pyro-GC/MS)</td>
</tr>
</tbody>
</table>
3.6 Weight measurement

Sample drying
Generally, drying prior to weighing is performed at room temperature.

Weight measurement
Weighing of microplastics in a glass vial.

Keynotes

- Weight measurement is carried out because it is important to understand the mass balance and also due to the difficulty of estimating the actual abundance of microplastics from the quantity of the particles only, because even if the same amount of microplastics exists at the ocean surface by weight, the quantity of particles may differ depending on fragmentation processes. Recommendations and guidelines on weight measurement have been issued by the EC and NOAA (EC, 2013., Masura et al., 2015.).

- All laboratories participating in ILC2017 provided weight measurements of the standard samples irrespective of whether or not they normally carried out weight measurements.

- In measuring the weight of the standard samples, for particles 1 mm or larger and less than 5 mm, there were no significant differences between the results reported from each laboratory and the design value. On the other hand, for smaller particles of less than 1 mm, results from the laboratories that had conducted digestion of organic matter before measuring the weight were closer to the design value than those from other laboratories.

- In response to a questionnaire distributed after the survey in ILC2017, many of the laboratories reporting relatively low accuracy in weight values suggested that in all probability insufficient drying affected the weight measurement.

- From the viewpoint of harmonization, it is important to wash each sample with distilled water and dry it thoroughly before measuring its weight. Attention should be paid to humidity and the laboratory atmosphere.

- Also, to obtain more accurate results, digesting organic matter in the pretreatment process is recommended (see §3.2).
• Reporting weight for both particles smaller than 1 mm and particles larger than 1 mm separately is recommended.

Explanatory Notes
• Weight measurement (dry weight) of microplastic particles may be carried out based on the purpose of the survey, such as for detailed analysis of plastic particle distribution in sea areas.
• In the preliminary questionnaire, only four laboratories out of the 12 laboratories participating in ILC 2017 reported that they measured the weight (or measured the weight and quantity) in their routine measurements. In ILC2017, all laboratories were requested to measure weight.
• Out of the 12 laboratories, drying prior to weight measurement was done at room temperature at 11 laboratories, and at 60°C at one laboratory.
• Results of testing showed no significant differences among values reported from each laboratory regarding the weight of microplastic particles larger than 1 mm and less than 5 mm contained in the standard samples.
• Meanwhile, results of weight measurement of microplastics smaller than 1 mm in ILC2017 closer to the design value were obtained by laboratories that conducted digestion of organic substances compared to those that did not. This is thought to have been the result of improvement in the precision of picking out small particles and size fractionation through removal of non-plastic material by digestion of organic substances.
• Also, with respect to microplastics larger than 1 mm and smaller than 5 mm, digestion of organic substances is considered an effective process for achieving better accuracy in weight measurement because samples obtained from actual sea areas may contain particles with sessile animals or biofilms adhering to the surface.
• Furthermore, laboratories with large errors in weight measurement results in ILC2017 reported insufficient drying as the major factor influencing weight measurement.
• From the viewpoint of harmonizing monitoring methods, performing adequate digestion of organic substances and drying the particles thoroughly are thought desirable for achieving accurate weight measurement.
• However, measuring the weight of particles smaller than 1 mm is prone to error at the separation process, even with adequate digestion of organic substances and thorough drying (see §3.3). Hence, reporting is not considered essential as difficulty is expected in obtaining comparable results based on the analytical methods presented in these Guidelines.
3.7 Laboratory analytical process quality control

Keynotes

- In laboratory analysis, countermeasures, for preventing predictable airborne contamination such as with fibrous matter and contamination from washing water in the fractionation and filtration processes, are important, such as conducting blank tests in the laboratory or using filtered water to wash the equipment (EC, 2013, Masura et al, 2015). In recent articles, specific steps have been proposed to reduce and quantify this kind of contamination for accurate output (see Table 3-5 reproduced from Lusher, 2018).

- Hermsen et al. (2018) reviewed many cases of quality control for surveys and experiments on microplastics. This serves as a very useful reference and referring to it before surveys is recommended.

- Spiked recovery tests using relevant reference particles (similar properties as sample particles but still clearly distinguishable, e.g. by distinct colors) are also effective for assessing extraction efficiency or loss in digestion protocols or density separation (GESAMP, 2019).

- Also, the experience and ability of the analysts are thought to be very important in accuracy control.

- In preliminary questionnaires for participating laboratories in ILC2017, some laboratories responded that they used specific facilities such as clean benches and others excluded fibrous materials as potential contaminants. In addition, in ILC2017, there were several laboratories using specified water filtered through 0.7 to 1μm filters for washing in the fractionation process.

- It would be desirable to include information in the report on measures taken to prevent contamination that may affect the accuracy of the analysis. It would be also desirable to record the humidity and temperature of laboratory atmosphere.

Explanatory Notes

- In the discussions at the International Experts’ Meetings, reporting of quality assurance/quality control data was recognized as important.

- Examples of quality assurance/quality control data include blank tests in the analytical process, recovery rates, repeatability, etc.

- In the preliminary questionnaire given to the laboratories in ILC2017, eight laboratories reported using a clean bench, etc., and one laboratory was excluding fibrous particles as a measure.

- Examples of contamination risks and the measures against them are shown in Table 3-6.

- When ILC was implemented, there were at least two laboratories using water filtered using 0.7-1 μm filters for washing the mesh prior to fractionation.
What has been particularly prominently mentioned in past research has been airborne contamination in which cloth-derived synthetic fibers adhere to analytical instruments and samples via the air in the laboratory (Nuelle et al., 2014., Wesch et al., 2017.). Careful attention is necessary when analyzing fibrous microplastics.

Table 3-5. Examples of steps to prevent microplastic contamination. (modified from Lusher et al. (2018)).

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>All sample containers should be prewashed with filtered distilled water before use.</td>
</tr>
<tr>
<td>2</td>
<td>Samples should be kept covered as much as possible using aluminum foil or glass lids.</td>
</tr>
<tr>
<td>3</td>
<td>All equipment used in the processing and analysis stages should be rinsed and checked under a microscope for any microplastic particles adhering to them. The vacuum filtering apparatus should be rinsed with filtered water between each sample.</td>
</tr>
<tr>
<td>4</td>
<td>All reagents should be vacuum filtered through Whatman GF/D filter papers immediately prior to use.</td>
</tr>
<tr>
<td>5</td>
<td>Sample processing should be performed in a sterile cabinet.</td>
</tr>
<tr>
<td>6</td>
<td>Several procedural blanks should be performed as negative control samples through the sample processing and analytical stages in order to test for laboratory contamination.</td>
</tr>
</tbody>
</table>

Table 3-6. Examples of contamination risks and preventive measures.

<table>
<thead>
<tr>
<th>Contamination risks</th>
<th>Preventive measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contamination with plastic particles adhering to analytical instruments/apparatuses</td>
<td>Pour purified water into the apparatus used for analysis beforehand and conduct the same analytical process as for sample treatment to confirm the presence or absence of microplastic particles.</td>
</tr>
<tr>
<td>Contamination with fibrous microplastics during operations</td>
<td>Wear clothing that is not plastic-derived and remove any loose fibers from clothing with a lint roller before sampling and analysis. For example, wear clothing of a unique and visible color so that the fiber can be distinguished even if it contaminates the sample.</td>
</tr>
<tr>
<td>Contamination with plastics from air</td>
<td>Use of clean benches and clean rooms. Implementation of blank tests in the laboratory.</td>
</tr>
</tbody>
</table>
4. Reporting

- Observed abundances of ocean surface microplastics are commonly reported in terms of density, or quantity or weight of particles per unit area (/m², /km²) or unit volume of water (/m³).
- Densities of microplastics per unit area need to be reported together with the sampling depth to allow comparisons to be made between those per unit area and those per unit volume of water.
- Reports on the distribution of ocean surface microplastics should include not only their quantities or weight per unit area or per volume of water, but also their particle sizes and materials, and metadata at the time of their sampling.
- For example, collected quantities of microplastics plus the shapes of individual particles make it theoretically possible to convert them to weight. If data on wind speeds and wave heights are available for estimating the intensity of vertical mixing of water, abundances of underwater microplastics can also be estimated by sampling at the surface layer (Kukulka et al., 2012, Kooi et al., 2016).
- It is also necessary to record and report how each sample was stored and analyzed. Upon completion of these analyses, maintaining visual representations (pictures, etc.) obtained at the time of measurement would be desirable.
- In these Guidelines, data to be reported to ensure harmonization of ocean surface microplastic monitoring are summarized in Tables 4·1 to 4·3.
- As for the scale of samplings required to obtain the typical density of microplastics in a certain sea area, Dr. Cózar (personal communication) suggested 120 tows (one tow usually ranges between 500 and 2000 m²) for 174,000 m², while Goldstein et al. (2012) recommended 250 tows for 165,000 m². The total area surveyed may be more important than the number of samplings in studying the typical density of microplastics in certain sea areas, and further consideration is required.
<table>
<thead>
<tr>
<th>Items</th>
<th>Data necessary to ensure comparability</th>
<th>Essential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling date and location</td>
<td>Sampling date, time and location</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>GPS log (coordinates at the start and end of trawl, geodetic system)</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Season</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Tow time (daylight hours or night)</td>
<td>●</td>
</tr>
<tr>
<td>Sampling equipment</td>
<td>Net type (Manta or Neuston net), model number, manufacturer</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Shape and size of net aperture</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Length of net</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Mesh openings of net used for the survey</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>※It would be preferable to confirm whether the mesh opening size of the net used in the survey indicates side length or diagonal length.</td>
<td></td>
</tr>
<tr>
<td>Tow parameter</td>
<td>Tow duration (minutes)</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Vessel speed (speed relative to water, knots)</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Trawl sweep area, filtered water volume and calculation formulas</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>※Reporting the equations and numerical values used in calculating the swept area and filtered water volume is recommended.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tow distance (m) and calculation method (using flow meter or speed relative to water.)</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Tow position (starboard, port, stern)</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>※If towing is conducted at the stern, it is preferable to record measures for avoiding the influence of the vessel’s wake.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distance from vessel (m)</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Net immersion depth (cm, m)</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>※Recording the following items is recommended when using a Neuston net:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage of net immersion depth relative to the size of the net frame and scale position on the net frame (cm), whether a video was shot or an investigator monitored, whether there was any change in the net immersion depth</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Tow direction</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Whether or not blank tests were conducted and results</td>
<td>●</td>
</tr>
</tbody>
</table>

※Essential......

Among data described in the above table, those with "●" in the "Essential" column are minimum requirements to make the survey results comparable. Data without "●" in the "Essential" column are those that may be obtained optionally depending on the specific purpose of individual surveys or instrument availability. When obtained, they should be reported.
## Table 4-1(2) List of data to be reported when sampling floating microplastics.

<table>
<thead>
<tr>
<th>Items</th>
<th>Data necessary to ensure comparability</th>
<th>Essential</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metadata</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Weather, sea conditions,)</td>
<td>Wind direction and speed</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Significant wave height (measure using an onboard wave meter.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>❧When a wave-height meter is not available on the sampling vessel, wave height data from nearby tide stations or websites can be recorded instead.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beaufort scale (visual observation)</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Sea surface temperature and salinity</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Current direction and speed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>State of floating debris on the sea surface</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>(large floating debris, drifting algae, etc.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vessels movements (heave, pitch, roll)</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Other of types of water quality data (chlorophyll, fluorescence, etc.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>❧If there are other collected data, record them</td>
<td></td>
</tr>
</tbody>
</table>

※Essential......

Among data described in the above table, those with "●" in the "Essential" column are minimum requirements to make the survey results comparable. Data without "●" in the "Essential" column are those that may be obtained optionally depending on the specific purpose of individual surveys or instrument availability. When obtained, they should be reported.
### Table 4-2. List of data to be reported for laboratory analysis of microplastics.

<table>
<thead>
<tr>
<th>Items</th>
<th>Data necessary to ensure comparability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density separation</td>
<td>Whether or not density separation conducted</td>
</tr>
<tr>
<td></td>
<td>Type and concentration of solution used for density separation (NaCl (%, g/kg), ZnCl₂ (%, g/kg), etc.)</td>
</tr>
<tr>
<td></td>
<td>Density separation processing time (minutes or hours)</td>
</tr>
<tr>
<td>Biological digestion and chemical</td>
<td>Whether or not biological digestion or chemical treatment conducted</td>
</tr>
<tr>
<td>treatment</td>
<td>Methods used for digesting organic matter (acid treatment, alkali treatment, enzyme treatment, etc.)</td>
</tr>
<tr>
<td></td>
<td>Temperature (°C) during processing and reaction time (hours)</td>
</tr>
<tr>
<td>Sample splitting</td>
<td>Whether or not sample splitting conducted</td>
</tr>
<tr>
<td></td>
<td>※In the case of splitting the sample, it would be desirable to confirm and report the error caused by use of the splitter.</td>
</tr>
<tr>
<td>Picking out microplastic particles</td>
<td>Pretreatment before picking out particles (fractionation by size including non-plastic material by sieve, etc.)</td>
</tr>
<tr>
<td></td>
<td>Method of picking (whether or not a stereomicroscope was used)</td>
</tr>
<tr>
<td>Counting and measuring sizes of</td>
<td>Method of size fractionation (whether maximum diameter was measured or sieves were used)</td>
</tr>
<tr>
<td>particles</td>
<td>Diameters of the measured particles (maximum and minimum Feret’s diameter, area)</td>
</tr>
<tr>
<td>Identification of microplastics</td>
<td>Whether or not composition analysis was conducted</td>
</tr>
<tr>
<td></td>
<td>Method of composition analysis (FTIR, Raman spectroscopy, etc.)</td>
</tr>
<tr>
<td></td>
<td>※When checking the material using methods other than spectroscopy (pricking with a heated needle, grinding with a forceps, etc.), describe them.</td>
</tr>
<tr>
<td>Weight measurement</td>
<td>Temperature and processing time of sample drying</td>
</tr>
<tr>
<td></td>
<td>Method of weight measurement (weighing the particles directly on a scale, weighing the mass of the vial and microplastics together and subtracting the mass of the tared vial to provide the mass of the microplastics)</td>
</tr>
<tr>
<td>QA/QC</td>
<td>Whether or not blank tests or spiked recovery tests were conducted and results</td>
</tr>
<tr>
<td></td>
<td>※It would be desirable to describe any processing carried out to prevent contamination during analysis.</td>
</tr>
<tr>
<td></td>
<td>Humidity and Temperature of laboratory atmosphere.</td>
</tr>
</tbody>
</table>

※Essential...

Among data described in the above table, those with “●” in the “Essential” column are minimum requirements to make the survey results comparable. Data without “●” in the “Essential” column are those that may be obtained optionally depending on the specific purpose of individual surveys or instrument availability. When obtained, they should be reported.
Table 4-3. List of data to be reported for results of microplastic survey.

<table>
<thead>
<tr>
<th>Items</th>
<th>Data necessary to ensure comparability.</th>
<th>Essential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight and quantity of plastic particles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plastic particles having a maximum Feret's diameter of less than 5 mm but 1 mm or more.</td>
<td>Weight and quantity of particles per unit area and unit water volume</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Weight and quantity of particles by size</td>
<td>●</td>
</tr>
<tr>
<td>Plastic particles having a maximum Feret's diameter of less than 1 mm.</td>
<td>Weight and quantity of particles by size</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weight and quantity of particles per unit area and unit water volume</td>
<td></td>
</tr>
<tr>
<td>Properties of the plastic particles</td>
<td>Shape (fragments, beads, etc.)</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Material (PP, PE, etc.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colors of microplastic particles</td>
<td></td>
</tr>
</tbody>
</table>

※Essential……

Among data described in the above table, those with “●” in the "Essential" column are minimum requirements to make the survey results comparable. Data without "●" in the "Essential" column are those that may be obtained optionally depending on the specific purpose of individual surveys or instrument availability. When obtained, they should be reported.
5. Conclusions

- The Guidelines summarize recommendations for harmonization of ocean surface microplastic survey methods to facilitate the generation of comparative results with the assumption that that various sampling and processing methods will be still used in future. Those recommendations are also useful for surveys conducted in freshwater systems.
- Many studies are expected to be carried out involving microplastic monitoring at the ocean surface for various purposes. Application of the harmonized methods proposed in the Guidelines will facilitate result generation in a comparable manner, enabling researchers to analyze, consolidate and integrate all the available data.
- Data gaps are expected to be filled in the future by surveys in various countries and areas where surveys have yet to be conducted, and at the same time, comparison of the results obtained from surveys conducted worldwide to date and accumulation of data measured using harmonized methods are expected to facilitate understanding of the global status of microplastic pollution.
- Current data on the abundances of microplastics in the ocean obtained to date suggest the existence of some unknown mechanism for their removal and identifying the distributions of ocean surface microplastic densities is expected to elucidate the process of their generation through to their disappearance via migration.
- It is important to tackle the following technological challenges for improving the efficiency and accuracy of identifying the status of oceanic microplastic pollution.

- Automation of microplastic analysis (size measurements and composition analyses) and ocean sampling for efficiency and speed, including faster speedier analysis. The turnaround time from sampling to data acquisition could be shortened, allowing prompt confirmation of the comparability and adequacy of the samplings, so that complementary samplings can be conducted as required for improving overall accuracy.
- Development of techniques to improve the accuracy of measuring tiny microplastics smaller than 1 mm
References


12. GESAMP (2019). Guidelines for the monitoring and assessment of plastic litter in the ocean (P.J.


