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Standardised protocol for monitoring microplastics in seawater

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BASEMAN
MICROPLASTICS ANALYSES IN EUROPEAN WATERS

WP4 Sampling methodologies for microplastics in the marine environment: standardisation, suitability and intercomparison

Deliverable 4.1
Standardised protocol for monitoring microplastics in seawater
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Project coordinator
Gunnar Gerdts
Alfred Wegener Institute | Helmholtz Centre for Polar and Marine Research
Telephone: +49(4725)819-3245
E-mail: Gunnar.Gerdts@awi.de

Secretariat contact
John Hanus
E-mail: hanus@deutsche-meeresforschung.de
Tel. +32 (0) 2 626 16 77

Website: http://www.jpi-oceans.eu/baseman/main-page

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Contributors to the report: Ana Filgueiras*, Jesus Gago*, Maria Luiza Pedrotti*, Giuseppe Suaria, Valentina Tirelli, José Andrade, João Frias*, Róisín Nash, Ian O'Connor, Clara Lopes, Miguel Caetano*, Joana Raimundo, Olga Carretero, Lucía Viñas, Joana Antunes, Filipa Bessa, Paula Sobral, Alenka Goruppi, Stefano Aliani, Luca Palazzo, Giuseppe Andrea de Lucia, Andrea Camedda, Soledad Muniategui, Gloria Grueiro, Veronica Fernandez, Gunnar Gerdts.

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Corresponding authors(*): Jesus Gago (jesus.gago@ieo.es), Ana Filgueiras (ana.filgueiras@ieo.es), Maria Luiza Pedrotti (Maria-Luiza.Pedrotti@obs-vlfr.fr), Miguel Caetano (mcaetano@ipma.pt), João Frias (joao.frias@gmit.ie)

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Executive Summary

Microplastic litter is an omnipresent pollutant in marine systems across the globe; spread out from the water surface to benthic sediments. Furthermore, the current trend of microplastic accumulation in the marine environment will enable these particles to remain there for centuries to come, due to their persistence. Nevertheless, the impact of plastic particles on aquatic ecosystems is far from being understood. The consequences are estimated to be severe as microplastics can accumulate persistent organic pollutants from the environment and release toxic additives into the environment, which might pose a threat to marine organisms upon ingestion. Although microplastics are recognised as a contaminant of emerging concern in the environment, currently neither sampling, extraction, purification nor identification approaches are standardised, making microplastic studies difficult to compare, if at all, possible. Harmonization of protocols for determination of plastic particles is urgently needed in order to overcome this gap.

The JPI-Oceans BASEMAN project is an interdisciplinary and international collaborative research project that aims to overcome this problem and to undertake a profound and detailed comparison and evaluation of all approaches from sampling to identification of microplastics. The two overall goals of the project are the "*The validation and harmonisation of analytical methods*" which is indispensable for the "*Identification and quantification of MP*".

The BASEMAN project will try to answer questions like the abundance and distribution of microplastics in the environment. For this purpose, tools and operational measures will be proposed so that they allow evaluation Member States' compliance with existing and future monitoring requirements.

This document regards microplastic sampling, processing and analysis for surface and water column seawater samples.

Jesus Gago
28th January 2019

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Introduction

In the past hundred years, production of man-made debris has increased exponentially and consequently evidences of debris started to become relatively common throughout the environment. Marine litter is defined as 'any persistent, manufactured or processed solid material discarded, disposed or abandoned directly or indirectly, intentionally or unintentionally, in the marine and coastal environment', as described in the Marine Strategy Framework Directive (MSFD). Litter has been found in all marine habitats, from surface water down to the deep sea, including ice cores. Distribution and accumulation of marine debris are influenced by hydrography, geomorphology, winds, anthropogenic activities, and chemical characteristics of the materials themselves.

Marine litter is classified according to their origin as either land- or oceanic/maritime-based, depending on how the debris enters the water. Debris are discharged into the water or are transported by currents, creeks, rivers, storm drains, and sewers into the ocean from land sources. Maritime-based debris is largely the result of dumping or accidental discarding of material from ships or offshore platforms and the wear and tear of equipment deployed.

Marine debris is comprised of a wide range of materials, including timber, glass, metal, rubber, plastic and paper from many different sources; however, plastics are by far the most abundant material recorded (Arthur *et al.,* 2009). It is believed that plastics began to enter the ocean in significant quantities after the 1950s and currently is the most abundant material recorded (Arthur *et al.,* 2009).

Most plastics are extremely durable and persistent materials in the marine environment, and some estimates refer to a potential lifetime of hundreds of years in the environment (Arthur *et al.,* 2009; Galgani *et al.,* 2010; JRC, 2013; Gago *et al.,* 2016; Rocha-Santos *et al.,* 2017). The characteristics that make plastic materials so useful, such as high-resistance to corrosion, low electric and thermal conduction, high persistence, are the same that are prejudicial to the environment (Frias *et al.,* 2010; Shashoua, 2008). While in the ocean, plastic litter slowly deteriorates and fragments as a consequence of UV-radiation exposure (photo-degradation); of interaction with sand and rocks (abrasion) or interactions with marine organisms (bite marks) (GESAMP, 2016; UNEP; 2016) which leads to physical and chemical deterioration (ter Halle *et al.,* 2016). Fragmentation of larger items results in numerous plastic pieces of ever smaller dimensions, known as 'microplastics'. In

the early 1970s, Carpenter and Smith reported the first evidences of small pieces of floating plastics in the surface ocean (Carpenter and Smith, 1972), but the term 'microplastics' was only coined for the first time in 2004 to describe the accumulation of microscopic plastic particles retrieved from environmental samples (Thompson *et al.*, 2004). Since then, an upper size limit of 5 mm (Arthur et al., 2009) was proposed and this became the most common definition of microplastics. The definition was further refined, and MPs where distinguished as either *i)* primary: particles of microscopic dimensions directly released into the environment (e.g. industrial virgin resin pellets, microbeads, etc) or *ii)* secondary: particles which result from the fragmentation of larger plastic items (Cole *et al.,* 2011).

Nonetheless, the need for an all-inclusive definition that accurately encompasses all criteria and that could potentially describe what a microplastic is, created the following definition: "Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water" (Frias and Nash, 2019).

Microplastics, can act as a double transport vector for chemicals and species in the ocean, been a potential threat to ecosystems and human health. For example, ingestion of microplastics which may contain persistent organic pollutants adsorbed to them (Mizukawa *et al.,* 2013) may also provide a pathway for transport of harmful chemicals into the food web (Shim *et al.,* 2017).

To be able to assess the impact of microplastics, a holistic view of the problem must be taken into consideration. This vision should begin with the establishment of 'best practices' through devising a working protocol for microplastics which includes sampling collection, processing, and characterization of the isolated microplastics, as well as data management.

BASEMAN Project

Although MPs are recognised as an emerging contaminant in the environment, currently neither sampling, extraction, purification nor identification approaches are standardised, making the increasing numbers of MP studies hardly -if at all- comparable. The overall goal of this interdisciplinary and international collaborative research project is to overcome this problem through a profound and detailed comparison and evaluation of all approaches from sampling to identification of MP. Our collaborative research project combines experienced MP scientists (from different disciplines and countries) in a cutting-edge project addressing the JPI-O pilot call "Ecological aspects of MP in the marine environment". Our proposal tackles the two major themes of the call: 1) "The validation and harmonisation of analytical methods" which is indispensable for 2), the "Identification and quantification of MPs". The results of the project will provide EU authorities with tools and operational measures that may be applied to describe the abundance and distribution of MP in the environment. Such tools will allow JPI-Oceans Member States' compliance with existing and future monitoring requirements.

The comparison and intercalibration of field and laboratory methods for measuring MPs is required for consistent analysis, and robust assessment and reporting of MP pollution levels in the marine environment. However, this is currently precluded due to the lack of standardized MPs sampling methodologies. The goal of BASEMAN's WP4 is to develop robust methods for sampling seawater (both the water column and sea surface), sediments, and biota. This will produce Standard Operating Procedures (SOPs) for sampling sediments and seawater samples (from surface to the bottom) and biota (based on evaluations of field results in specific regions). In addition, the viability of alternative methods using platforms of opportunity will be evaluated in "pilot" regions.

#1 Collection

Material

1. Nets
2. Niskin bottles
3. Metal buckets
4. Metal sieves: 5mm and 200 µm
5. Vacuum pump with glass or metal flask
6. Water pressure sprayer
7. Glass beaker
8. Glass jars
9. Filter membranes of different size ranges
10. Glass Petri dishes
11. Metal forceps
12. Metal tweezers
13. Pencils, Datasheet, Labels
14. Camera
15. Flowmeter
16. GPS
17. Aluminium foil / Tin foil
18. Permanent Marker

Sampling and storage

Manta trawl and Bongo nets

Polymers have different buoyancies and some microplastics are positively buoyant (Table A3), which allows them to float and travel large distances from their origin. Due to the characteristics described in the introduction section (Shashoua, 2008; Mizukawa *et al.,* 2013; Rocha-Santos *et al.,* 2017; Shim *et al.,* 2017), microplastics have the tendency persist in the environment. In this section, focus will be given to microplastics sampling at sea either in the sea surface, sub-surface or water column.

For surveying the sea surface, different nets can be used. The most common devices for microplastic sampling are the Manta trawl and the Neuston net, which have a maximum tow speed limit of 3 knots and allows sea surface to be mandatory sampled during relatively calm sea conditions. The principal difference between these nets consists in the width of the sampled water layer: Manta generally samples the first 15-25 cm while the Neuston net samples a larger water layer (generally slightly less than 50 cm). Either of these two trawling devices are recommended. The AVANI trawl, another sampling method, was designed to be used during long transects while sailing at normal cruise speed up to 8 knots in moderate seas, immersing only half of the rectangular mouth net. According to Eriksen *et al.,* (2018), AVANI collects similar amounts and types of microplastics as the Manta trawl and the DiSalvo Neuston net, allowing data among studies to be compared.

The main advantage of net sampling is that large volumes of water can be sampled relatively quickly. Their limiting factor is the mesh size that can strongly affect the size spectrum of collected particles. The net mesh sizes vary widely, ranging from 53 to 3000 µm, being the most usual mesh size from 300 to 390 µm (Hidalgo-Ruz *et al*., 2012). This is in accordance with the MSFD guidelines, which recommends a mesh size of 333 µm and a month aperture of usually 60 cm (Gago *et al.,* 2016).

Ideally, the device should be deployed from the side of the vessel (thus avoiding disturbance from the ship's wake, as well as contamination from the ship itself) during a period of 20 minutes; but the duration of the sample collection could vary between 10 and 60 minutes due to different *in-situ* factors (e.g. local productivity, intense boat traffic or weather conditions), as well as mesh size of the net. The GPS start and stop positions

should be recorded, and the sampled volume can be computed using a flowmeter placed at the centre of the net mouth (see section 3, page 21).

After each sampling event, the whole net must be rinsed thoroughly from the outside using a deck hose in order to concentrate all the natural and man-made materials to the cod-end. The cod-end sampler is removed and rinsed with filtered (200 µm or lower) seawater or tap water, in the wet laboratory on board.

First, larger pieces of biological material, including e.g. leaves, bugs, larger algae or wood are picked out of the samples with metal tweezers and carefully rinsed with filtered seawater (200 µm or lower), which is collected back into the container to avoid loss of microplastics. Larger plastic debris are picked out and rinsed in the same way, but instead of discarding them, they are counted and stored for further analysis. The material retained in the cod end is carefully transferred into glass or plastic bottles, previously rinsed 3 times with ultrapure Mili-Q or filtered seawater and frozen at -20ºC until subsequent analysis[1].

Surface and sub-surface bulk water sampling

For water column, Niskin bottles attached to a CTD-Rosette sampler is a common method used to collect water from different depths.

The procedure is as follows: water from the Niskin Bottles is transferred into jerrycans, previously rinsed 3 times with ultrapure Milli-Q or filtered seawater, to remove/minimise any potential contamination. The water from the jerrycans can be pre-filtered to reduce sample volume, using a metal with a variable size mesh depending on the targeted plastic size. This sample is then filtered directly onto stainless steel mesh, Anodiscs[2] or glass microfiber membranes using a vacuum pump. Filters are then placed into labelled Petri dishes until further processing. In the laboratory filters will be observed under a stereomicroscope or under another device (e.g. Raman or micro-FTIR spectroscopy).

Barrows *et al.*, 2017 have described alternative methods that can be used, such as bulk water sampling. This method is suitable for sampling microfibers from the water surface, nonetheless it is important to take into consideration that it collects relatively low volumes of water. In this case, samples are taken on the downwind side of the boat in the top 45 cm of the water, using a stainless-steel bucket or Niskin bottles horizontally operating - like the Van Dorn bottle used in the BASEXPEMIPS cruise (Carretero *et al.,* 2017) - and a natural fibre rope to prevent shredding from synthetic ropes. Before sampling, the device used must be rinsed 3 times with filtered-seawater at the time of sampling to remove any contamination.

The collected water is then filtered over 20 µm stainless-steel mesh filters, Anodisc or glass microfibres membranes using a vacuum pump. Filters are then placed into labelled Petri dishes and stored for subsequent analysis.

Procedural blanks are performed filtering the same amount of ultrapure Milli-Q or filtered water using the same filtering equipment used for sampling, both for Niskin and bulk water sampling. The disadvantage of this method is the low volume of water collected. Collecting enough water to compare to other methods would require more deployments in order to increase the volume of water.

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[1] Alternatively use seawater formalin solution at 4% or 70-80% ethanol to store an aliquot of the sample for plankton identification. We recommend deploying 2 mantas, one for plankton identification and another for microplastics characterization.

[2] Stainless steel mesh or Anodisc filter membranes are ideal for FTIR analysis.

Another method is the one described by Lusher *et al.,* (2015), where sub-surface water can be directly collected from the vessel's on-board seawater pump. This would allow higher volumes of water collected and therefore comparison of results among studies, which use a similar method.

Cross-contamination controls

During sampling and sample handling, it is also important to identify potential sources of plastic contamination and cross-contamination of the environmental samples collected. The highest risk is associated with airborne contamination, such as synthetic fibres stemming from clothing, gear, and atmospheric fallout; thus, it is suggested that background contamination should be always taken into consideration during the whole sample collection process. Nonetheless, ensuring reduction of cross-contamination while sampling at sea can be extremely difficult, and precautionary measures should be taken into consideration. For mitigating these cross-contaminating risks, the sources of contamination should be eliminated and/or substantially reduced by cleaning all equipment prior to sampling; covering samples and equipment in use; ideally wearing polymer-free clothing or cotton coveralls and gloves; taking notes of the type and colour of clothes each person is wearing; working inside a fume hood (if one is available); employ consumables directly from packaging; and ideally use non- plastic materials.

During long cruises, sometimes is not easy caring glass items due to weight limitations, and plastic containers are the most cost-efficient alternative. In such cases, it is recommended to fill some bottles with filtered seawater to serve as blank samples. These will follow the same laboratory procedures as all other samples, and they can serve as a contamination evaluation while on-board.

Any additional contamination sources can be quantified by using protective environmental filters or running procedural blanks in parallel during all phases of the analytical procedure (Graça *et al.,* 2017). As described in the previous section, during Niskin bottle or bulk water sampling, procedural blanks can be performed by filtering the same volume of ultrapure Milli-Q or filtered swater, using the same sampling equipment.

Another important aspect to reduce cross-contamination is linked to the collection of paint samples from the sampling vessel. These paint scrapes will be a valuable control measure while processing environmental samples.

In order to quantify and account for airborne contamination, control filters are placed around the sampling working area.

#2 Processing

Material

1. Glass Petri dishes
2. Glass beakers
3. Metal forceps
4. Filtration Kit including vacuum pump
5. Filters: Glass microfibre or Nitrate cellulose
6. Temperature controlled oven

Reagents

Hydrogen peroxide 30 % solution (CAS no. 7722-84-1)
Potassium hydroxide 10 % (w/V) solution (CAS no. 1310-58-3)
Sodium iodide (1.8 $g \cdot cm^{-3}$) (CAS no. 7681-82-5)
Sodium chloride (CAS no. 7647-14-5)

Glass decontamination

Nitric acid 1 % solution (CAS no. 7697-37-2)[3]
Glass needs to be rinsed with water before use. Ideally it should dry up-side-down for airborne microplastics not to accumulate in it.

Washing and rinsing

Ultrapure water[4]

Note: All solutions and rinsing liquids need to be filtered (1 µm) prior to use to reduce potential contamination. Please take extra care while preparing all the solutions and follow the health and safety guidelines according to your institute or organisation.

Microplastic separation

Low organic matter content in seawater samples

(clear water samples)

If samples are frozen, defrost them at room temperature. If the quantity of organic content is low, samples are filtered through a glass fibre filter (e.g. GF/C 1.2 µm/pore and Ø=47 mm) or through an Anodisc or a nitrate cellulose membrane. After this, analysis can be directly made at the stereomicroscope or under another device (e.g. micro-FTIR spectroscopy), once the samples are filtered. Filters are stored in a labelled Petri dish until examined under a stereomicroscope or under another device (e.g. Raman or micro-FTIR spectroscopy).

Medium and high organic matter content in seawater samples

(water with eggs and larvae, plus zoo- and phytoplankton)

If samples are frozen, defrost them at room temperature. If the quantity of organic content is too high to allow direct examination, samples are pre-filtered using a 100 µm sieve and carefully transferred to a 200 mL conical flask. A sample pre-treatment consisting of digestion of the soft tissues in potassium hydroxide should be performed. Potassium hydroxide solution is added at 1:3 volume sample:solution ratio to digest the biological material (10 % KOH is prepared and filtered through a 0.2 µm filter). The mixture is placed in a temperature-controlled oven at 40ºC. It is very important to not exceed this

[3] Alternatively use 1 µm filtered denatured alcohol (CAS no. 64-17-5).

[4] Alternatively use either 0.45 µm or 1 µm filtered tap or distilled water.

recommended temperature. The treatment of the samples continues until all visible organic material is digested or up to a maximum of 72 h.

Following this step, if all organic matter was not digested, an additional step using hydrogen peroxide could be necessary. Hydrogen peroxide (15% solution) at 1:1 volume sample:solution ratio is added to oxidize and digest the biological material. The mixture is placed in a temperature-controlled oven at 40ºC.

After this, and in order to minimise filtration time, 100 ml of density separation solution (table A1) is poured into the sample. The mixture is poured onto a filter (e.g. glass microfibre, stainless steel or Anodisc membranes) and the filters are stored in a labelled Petri dish until examined under a stereomicroscope or under another device (e.g. micro-FTIR spectroscopy).

If the digestion of organic matter has not been completed, a further step of density separation might be necessary.

Note: According to Karami *et al.* (2017) high temperature could degrade and reduce the recovery rate of some polymers. Therefore, incubation in this protocol uses low temperatures (40 ºC), as recommended in that paper.

Microplastic density separation

This procedure will be carried out whenever the digestion of organic matter has not been completed.

From the wide range of polymers and density separation solutions described in literature, Tables A1 and A2 in the appendix, compile important information about density separation solutions and polymer densities.

These tables combined will provide relevant information needed to conduct a safe decision-making process for both monitoring and scientific research on microplastics.

For density separation, it is recommended to use a saturated sodium chloride solution (NaCl – 1.2 g cm^{-3}), as it is both an economical and reliable method that allows polymers to float, thus facilitating their separation. However, this method will not allow the separation of denser polymers such as polyvinyl chloride (PVC) or polyethylene terephthalate (PET) (Table A2). If you are interested in recovering denser polymers, such as polyvinyl chloride (PVC) or other polymers with a density > 1.5 g cm^{-3} an alternative solution is will be the use of NaI-solution (4.4 M), with a density of approximately 1.6 g cm^{-3}.

The material collected onto the filter is transferred in glass beakers where the density separation solution is added in a ratio of about three times the volume of the original sample and stirred vigorously. After mixing, the sample is allowed to settle for 1 hour. The supernatant is then filtered with a vacuum pump through a fibre glass filter (GF/C), Anodisc or similar membrane. Wash and rinse the walls of the filtration system with ultrapure water to ensure that all particles are recovered on the filter. Filters are then placed in covered and labelled Petri dishes, dried in a temperature-controlled environment (stable room temperature) to reduce degradation during storage until examination under a stereomicroscope or under another device (e.g. micro-FTIR spectroscopy).

The work scheme of the separation process for isolating microplastics from seawater samples as it follows:

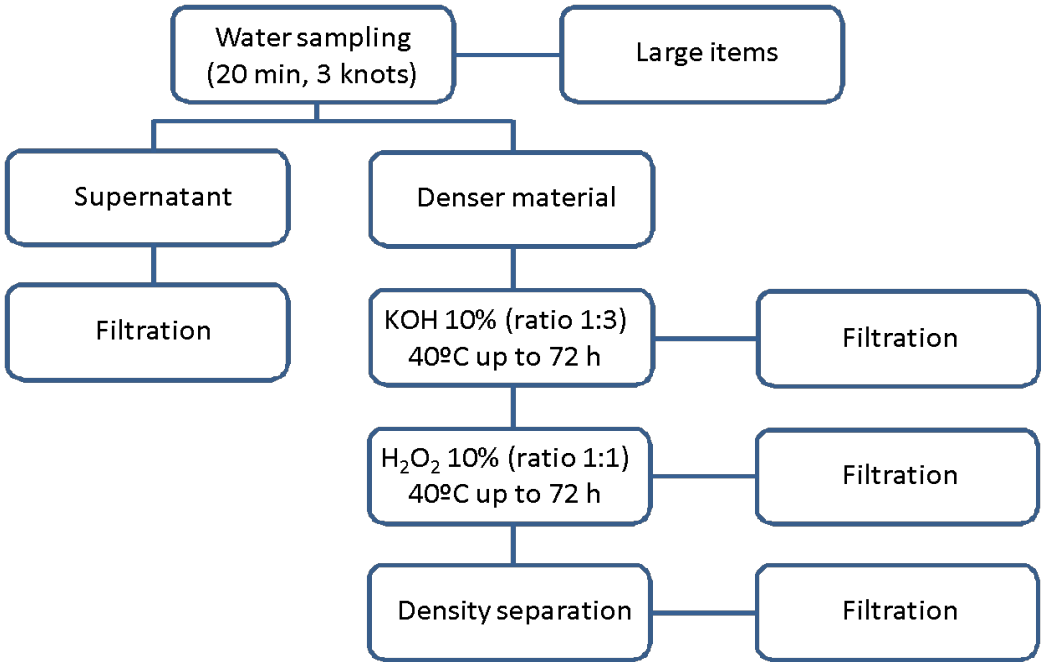

Figure 1 – Laboratory processing diagram for water samples

#3 Identification

Relevant criteria to take into consideration during identification include physical (size, type, colour) and chemical properties. These criteria were established during the BASEMAN WP4 workshop held in Lisbon, Portugal, in September 2017.

Physical properties

Size classification

Table 1 describes the most common size categories for marine anthropogenic litter, including microplastics.

Table 1 – Size ranges of marine anthropogenic litter.
Adapted from Van Cauwenberghe *et al.,*2015 and Gigault *et al.,* 2018

Terminology	Size range
i. Macroplastics	>2.5 cm
ii. Mesoplastics	0.5 – ≤ 2.5 cm
iii. Large microplastics	1 – ≤5 mm
iv. Small microplastics	1 µm – ≤ 1000 µm
v. Nano plastics	1nm – ≤ 1 µm

Currently, it is globally accepted that the lower size value for small microplastics (iv) is 1 µm (van Cauwenberghe *et al.,* 2015; Gigault *et al.,* 2018; Frias and Nash, 2019). The current processing technologies allows the researcher to identify particles as small as 10 µm (µ-FTIR with an FPA detector) or down to 1-2 µm (µ-RAMAN).

Nonetheless, for monitoring purposes the recommended lower size limit for microplastics by BASEMAN WP4 is **100 µm** (Frias *et al.,* 2018). Microplastic data should be recorded in three size classes, which reflect current sampling and processing practices namely: 1 ≤ 100 µm; 100 ≤ 350 µm and from 350 µm to ≤5 mm, as this would allow for studies to be more easily compared. (Frias and Nash, 2019).

Type

This criterion is targeted to the most common microplastic types described in peer-reviewed publications and the categories suggested are the following:

1. Pellet
2. Fragment
3. Fibre
4. Film
5. Rope and filaments
6. Microbeads (perfect spheres)
7. Sponge/foam
8. Rubber

Figure 1 illustrates six of the eight categories of microplastics commonly identified in visual identification.

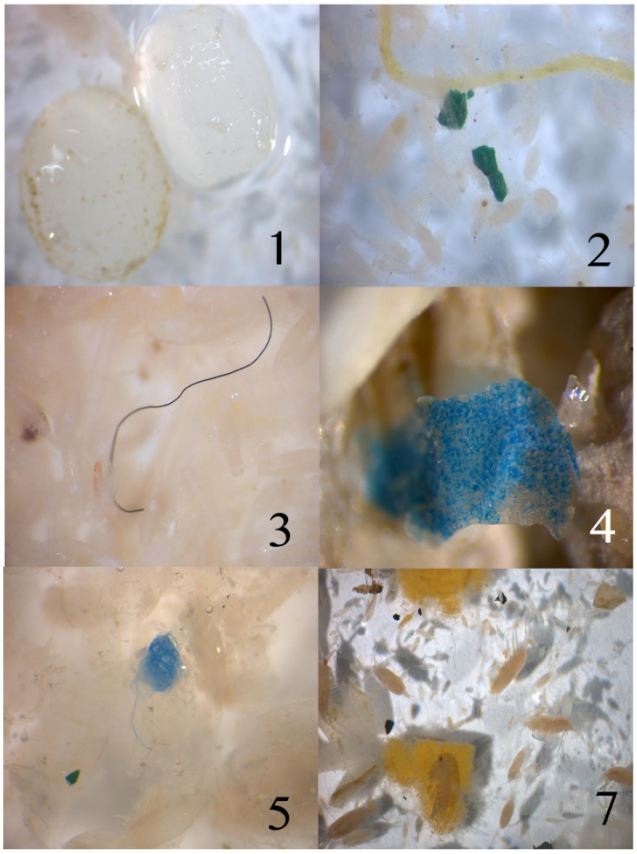

Figure 1 – Examples of microplastic types. Numbers correspond to the previous mentioned categories, i.e. 1. Pellets; 2. Fragments; 3. Fibre; 4. Film; 5. Rope and filaments; 7. Sponge/foam. Microbeads (6) are no commonly found in environmental samples, therefore they are not represented in the photo (credits: João Frias)

Colour

The classifications for this criterion were collected from the most common microplastic colours reported in peer-reviewed publications (Pham *et al.,* 2017) and might be considered relevant according to the goals of different projects (e.g. geographical influence; impact on marine species, etc.). The most common colours identified are showed below:

1. Black ■
2. Blue ■
3. White
4. Transparent
5. Red ■
6. Green ■
7. Multicolour ■
8. Others ■ ■ ■ ■ ■

In this criterion, we have attributed a separate class to multicolour. This represents microplastics that have one colour in one side and another colour on the other side (e.g. neoprene or paint particles), or rope or filaments that might contain more than one colour. The difference between white and transparent is the opacity, white is opaque and transparent is translucent. Colours such as purple, pink, grey, yellow or brown should be included in the category "Others", unless they have relevance for a specific project.

Chemical properties

Identification of polymer type can be done through the following techniques: 1) micro-Fourier Transform Infrared spectroscopy (in brief micro- or μ-FTIR); 2) Attenuated Total Reflection Fourier Transform Infrared spectroscopy (ATR-FTIR); 3) micro-Raman spectroscopy (μ-RAMAN) and 4) Pyrolysis-gas chromatography-mass spectrometry (Py-GCMS), to name the most common methodologies.

From the above-mentioned techniques, it is recommended to use micro-FTIR and ATR- FTIR[5], to clearly identify the polymer type. They are cost-effective and more easily available than others.

It is important to note that Py-GCMS provides results in mass and not in number of particles, besides permanently destroying the sample. Therefore, it should be used as a complementary technique to microplastic processing. We recommend the use of micro-FTIR before using Py-GCMS.

Micro-RAMAN can also be a destructive technique, unless the excitement energy is low. This quantitative technique is highly time consuming but Cabernard *et al.,* 2018 pointed out that micro-RAMAN quantified even two-times higher microplastic number compared to FTIR with particles below 500 μm.

[5] For large microplastics and above (see Table 1)

Reporting results

Microplastics reporting units

Reporting units are extremely important to allow comparison among studies. The proposed reporting units for microplastics retrieved from water samples are:

1. no. MPs per area (# particles km^{-2} | # particles m^{-2})
2. no. MPs per volume (# particles m^{-3})
3. mass of MP per area (g MP km^{-2} | g MP m^{-2})
4. mass of MP per volume (g MP L^{-1} | g MP m^{-3})

Note: It is suggested and highly encouraged that authors, always, report both the count and weight of microplastic particles when using different models of trawl or other sampling techniques.

Please note that visual identification by itself **is not enough** and it **does not replace** ATR-FTIR, micro-FTIR or micro-RAMAN. In fact, as stated before, it is recommended to use these techniques in order to correctly identify the polymer type.

Flowmeter vs GPS

During sampling with nets, attention should be given to the method used to measure tow length, filtered volume or area sampled. The filtered volume is calculated by multiplying the area of the mouth of the net by the distance covered during the tow or by applying the appropriate formula of the flowmeter. The sampled area is measured considering the distance covered during the sampling, which can be computed using GPS start and stop positions. GPS and flowmeter however, can give very different results, highly affecting the computation of the sampled area/volume and potentially halving (or doubling) MP abundances (Suaria *et al.,* unpublished data). Therefore, we suggest to always use both methods during net sampling, so that eventual differences between the two can be always taken into consideration.

#4 Environmental variables

This section illustrates other environmental variables that might help elucidate the influences on the recorded presence and concentration of microplastics in seawater;

Some of the most important ones are mentioned here but not described in detail:

- Wind speed and direction;
- Sea State; Wave height
- Depth in case of seawater from Rosette;
- Amount of macrodebris;
- Proximity to coast, river streams and/or estuaries;
- Proximity to wastewater treatment plants (submarine emissary);
- Surface temperature, salinity, oxygen, chlorophyll-a, turbidity, etc.

#5 Appendix

Tables

Table A1 – Common density separation solutions (Credits: Frias et al. 2018)

Chemical formula	Reagent name	CAS no.	Density solution (g cm^{-3})	Health Hazard (Toxicity)*	Average price (€ per 250g) †	Safety-Price Index
NaCl	Sodium chloride	7647-14-5	1.0 – 1.2	1 (low)	€ (3)	■
$Na_2WO_4 \cdot 2H_2O$	Sodium tungstate dihydrate	10213-10-2	1.40	2 (low)	€ (70)	■
NaBr	Sodium bromide	7647-15-6	1.37-1.40	2 (low)	€ (3-5)§ €€€€ (430)§	■ ■
$3Na_2WO_4 \cdot 9WO_3 \cdot H_2O$	Sodium polytungstate	12141-67-2	1.40	2 (low)	€€€€ (276)	■
$Li_6(H_2W_{12}O_{40})$	Lithium metatungstate	127463-01-8	1.6	1 (moderate)	€€€€ (360)‡	■
$ZnCl_2$	Zinc chloride	7646-85-7	1.6 – 1.8	3 (high)	€ (45)	■
$ZnBr_2$	Zinc bromide	7699-45-8	1.71	2 (high)	€€€ (200)	■
NaI	Sodium iodide	7681-82-5	1.80	2 (moderate)	€€€ (130)	■

*Health hazard retrieved from NFPA/HMIS forms and toxicity values retrieved from MSDS forms; † quotes for Ireland dated from March 2018, **please note that price values may vary in other countries;** § The cost of Sodium bromide (NaBr) is one example of the price fluctuation between countries – in Germany is very cheap (■) and in Ireland is extremely expensive (■) which would drastically affect the Safety-Price index ‡Lithium metatungstate quotes only available for a volume of 250 ml.

Table A2 - Densities of common polymers (adapted from Enders et al., 2015 and Crawford and Quinn, 2017)
**Density limit using: ■ Sodium chloride and ■ Sodium tungstate dihydrate and all above 1.40 g cm^{-3}*
(Credits: Frias et al., 2018)

Abbreviation	Polymer	CAS no.	Density (g cm^{-3})
PS	Polystyrene (expanded)	9003-53-6	0.01 – 1.06
PP	Polypropylene	9003-07-0	0.85 – 0.92
LDPE	Low-density polyethylene	9002-88-4	0.89 – 0.93
EVA	Ethylene vinyl acetate	24937-78-8	0.93 - 0.95
HPDE	High-density polyethylene	9002-88-4	0.94 – 0.98
PA	Polyamide	63428-84-2	1.12 – 1.15
PA 6,6	Nylon 6,6	32131-17-2	1.13 – 1.15
PMMA	Poly methyl methacrylate	9011-14-7	1.16 – 1.20
PC	Polycarbonate	25037-45-0	1.20 – 1.22
PU	Polyurethane	9009-54-5	1.20 – 1.26
PET	Polyethylene terephthalate	25038-59-9	1.38 – 1.41
PVC	Polyvinyl chloride	9002-86-2	1.38 – 1.41
PTFE	Polytetrafluoroethylene	9002-84-0	2.10 – 2.30

*Polymers before the marked lines are retained by the solutions. Please note that this is a theoretical model and some polymers with higher densities could potentially be found in sediments even using a solution with density **lower to 1.40 g cm^{-3}**.

Table A3 – Buoyancy of common polymers (adapted from Crawford and Quinn, 2017; Credits: Frias et al., 2018)*

Abbreviation	Polymer	Density (g cm^{-3}) *	Buoyancy
PS	Polystyrene	0.01 – 1.06	Positive (↑)
PP	Polypropylene	0.85 – 0.92	Positive (↑)
LDPE	Low-density polyethylene	0.89 – 0.93	Positive (↑)
EVA	Ethylene vinyl acetate	0.93 - 0.95	Positive (↑)
HPDE	High-density polyethylene	0.94 – 0.98	Positive (↑)
Seawater		**1.025**	
PA	Polyamide	1.12 – 1.15	Negative (↓)
PA 6,6	Nylon 6,6	1.13 – 1.15	Negative (↓)
PMMA	Poly methyl methacrylate	1.16 – 1.20	Negative (↓)
PC	Polycarbonate	1.20 – 1.22	Negative (↓)
PU	Polyurethane	1.20 – 1.26	Negative (↓)
PET	Polyethylene terephthalate	1.38 – 1.41	Negative (↓)
PVC	Polyvinyl chloride	1.38 – 1.41	Negative (↓)
PTFE	Polytetrafluoroethylene	2.10 – 2.30	Negative (↓)

Polymer density might vary with additives added during production.

Forms

1.- Seawater sampling datasheet

Cruise _____

Station	Sample	Date	Initial time (GMT)	Final time (GMT)	INITIAL				FINAL				T (ºC)	Salt	Wind (kts)	Direct. Wind	Sea State	Depth (m)
					LATITUDE		LONGITUDE		LATITUDE		LONGITUDE							
					GRAD	MIN	GRAD	MIN	GRAD	MIN	GRAD	MIN						

Notes:

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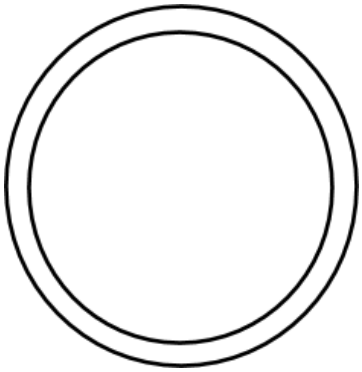
2.- Filter observation datasheet

Date: ____/____/20___

Sample code _____ Filter no. _____

Date of collection: ____/____/20___ Amplification _____ x _____



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Notes:

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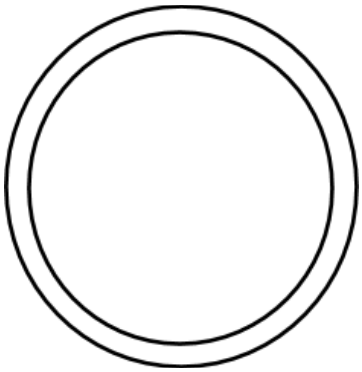
Sample code _____ Filter no. _____

Date of collection: ____/____/20___ Amplification _____ x _____



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Notes:

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3.- Identification datasheet

STATION	FILTER#	PHOTO	TYPE	COLOUR	AREA(µm)	LENGTH (µm)	WIDTH (µm)	PERIMETER (µm)	PERIMETER (mm)	SIZES (mm)					
										>5mm	2-5mm	1-2mm	0.5-1mm	0.3-0.5 mm	<0.3mm

Station: name and code of stations

Filter#: contamination control (CC) or sample (S) filter

Photo: ID photo from stereomicroscope

Type: fibre, filament, fragment, pellet, microbead, film, foam, paint sheet, rubber, macrofibres

Colour: blue, black, red, white, green, transparent, others (yellow, orange, pink, purple, tan), multicolour

Measurements[6]: area, length, width and perimeter in microns

Size: <0.3 mm; 0.3-0.5 mm; 0.5-1.0 mm; 1.0-2.0 mm; 2.0-5.0 mm; >5 mm

Note for measurements:
Fibre and filament: length and diameter
Fragment, film, paint sheet and rubber: perimeter, area, width and length
Pellet and microbead: perimeter and diameter

[6] Data could be processed with ImageJ software (open source) to determine the microplastic's dimensions.

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