NET4mPLASTIC PROJECT

Activity 3.1

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Bibliographic Report on monitoring approach:
Marine litter and Microplastics

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1 Introduction

Numerous methods for marine litter and plastics (including microplastics) have been published in the litterature but none have been indentified as being the best method, sometimes creating a barrier in comparing studies to create a baseline of data. For example, some studies record the numbers of items, other the mass or both. Litter is genearlly classified according to the type of material, function or both. The size of marine litter sampling is also different and the limits of beach area may also be different from survey to survey.

In this present document, we report a review of works dealing with the monitoring marine litter and plastics in the different environments.

2 STUDIES

2.1 Rees G. and Pond k., 1995: Review: Marine Litter Monitoring Programmes – A review of methods with spatial reference to national surveys, Marine Pollution Bulletin, 30(2), 103-109

The authors suggest that for monitoring issues, marine debris can be classified according to size and according to composition, e.g. paper, netting, metal, etc. The authors identify the following

- estimate the types and quantities of solid waste generated by ships and pleasure craft, results being extrapolated to obtain the total amount of litter in the marine environ- ment
- assess the density and type of floating marine debris in different way by i) using seacraft to collect information on the distribution and amounts of floating debris, ii) collecting or counting objects within a specified distance from the side of the ship along strip transects (50 to 100 m wide), iii) line transects are preferable
- beach surveys used to determine the amount and type of debris on a beach in a specified area at a certain time that can also be done through beach cleans.

However, the authors didn't provide detailled explanation on the procedure used. They also described large scale surveys done in UK with volunteers. They concluded that the use of volunteers is the most appropriate way of conducting large scale surveys. (Rees & Pond, 1995)

2.2 Christine A. Ribic, 1998: Use of Indicator Items to Monitor Marine Debris on a New Jersey Beach from 1991 to 1996, Marine Pollution Bulletin, 36(11), 887-891

The US National Marine Debris Monitoring Program used indicator items from beach surveys to identify whether amounts of marine debris are changing over time. Indicator items were selected through expert opinion and assumed to reflect the trend of all debris. The author used monthly data from a 1991-1996 study of debris on a New Jersey beach to determine if indicator and non-indicator items showed similar trends.

In this study, the author used data obtained by volunteers trained by the Center for Marine Conservation (CMC) to pick up and identify all debris items larger than 2.5.cm in the 500 m study area. The data card used was the standard field form developed by CMC and an additional list was generated for miscellaneous items. Usually five observers collected data during any given survey. Quality assurance protocols included checking debris classifications of items collected during surveys and periodic checking of the study area by the survey coordinator after cleaning to ensure all debris items were collected. Debris found during monthly surveys was divided into two groups: indicator and non-indicator items. (Ribic, 1998)

Table 1: Indicator items used for marine debris found on the beaches (Ribic, 1998)

Probable source	Item
Ocean based	All gloves Plastic sheets ≥ 1m Ligh bulbes/tubes Oil/gas containers ≥ 1 quart Pipe-thread protectors Nets, traps/pots, fish baskets Fishing line

	Floats/buoys Rope ≥ 1m Salt bags Cruiseline logo items
Land-based	Syringes Condoms Meta bevverage cans 1 quart motor oil containers Mylar or rubber ballons Six-pack rings Straws Tampon applicatotd Cottom swabs
General	Plastic bags with seams Straps Plastic bottles

2.3 Ryan P.G., Moore C. J., van Franeker J. A. and Moloney C. L., 2009: Review - Monitoring the abundance of plastic debris in the marine environment, Phil. Trans. R. Soc. B (2009) 364, 1999–2012

The paper mainly deals on plastic (macro) survey. The authors indicate that the main questions regarding plastic litter in the environment are:

- What is the abundance, distribution and composition of plastic litter, and are these attributes changing over time?
- What are the main sources of plastic litter, and are they changing over time?
- What are the impacts of plastic litter (environmental and economic) and are they changing over time?

And therefore specific measures and monitoring program should be applied. The authors in this paper summarize monitoring protocols used to measure changes in plastic debris with a focus on the marine environment because accumulation and impacts of plastic litter appear to be most serious in marine systems providing a set of best-practice guidelines for monitoring the abundance and impacts of plastics. Since plastics in the marine environment derive from two main sources, it will be important to understand the dynamic linkages between litter sources to monitor plastic litter, considering also the spatio-temporal variability of the marine litter distribution. The authors reviewed the approaches used in the litterature considering three kinds of marine litter monitoring: 1) beach surveys, 2) at-sea surveys and 3) estimates of the amounts entering the sea. (Ryan, Moore, Van Franeker, & Moloney, 2009)

Beach surveys

Different methods have been deveoped and used for monitoring the litter accumulated on the beaches but only few studied have sampled buried litter. They identify:

- Standing-stock surveys that reflect the long-term balance between inputs (both local, land-based strandings) and removal (through export, burial, degradation and cleanups);
- Accumulation and loading rates requires an initial cleanup to remove all existing debris, followed by regular surveys that record and remove all newly arrived debris. Such surveys form the basis of major monitoring programmes established in the USA and western Europe.
- Sampling meso and microplastics (no targeted by cleanup efforts). Quantitative estimates of meso-debris have been obtained by sieving beach samples, typically to a depth of approximately 50 mm, and sorting samples including the use of floatation in sea water. The authors further report that point sampling is likely to miss old, buried lines and that sieving a strip transect from the most recent strandline to the back of the beach is a more reliable way to characterize meso-debris loads.

From their analysis, the authors indicate that the best approach is to record all litter (number and mass) from the sea edge to the highest strandline along at least 50/500 m. Items should be identified as accurately as possible, allowing them to be categorized according to both composition and function.

They suggest that meso-debris should be sampled by a combination of sieving, dry picking and floatation to locate the greatest proportion of plastic litter and surveys should sample the entire beach profile from the most recent strandline to the back of the shore to a depth of 50 mm.

Surveys at sea

Floating and suspended debris

The methodologies for defining the abundance of floating plastics include direct observation of large debris items used for instance by Thiel et al. (2003) or by net trawls for smaller items used by Moore, C. J. et al. (2001). The measurements provide an index of abundance (number of items per unit distance) or an estimate of abundance based on fixed-width or line transects.

Aerial surey can also be used to estimante the abundance of marine litter in the sea but only for large items. Net-based survey (aperture 1-2 m) is generally used for sampling at sea and the plastic debris sampled is determined by net mesh size. Floating debris typically is sampled with a neus- ton or manta trawl net lined with 0.33mm mesh

Litter on the sea bed

Surveys of macro-debris may be conducted with divers, submersibles and remote- operated vehicles and trawl surveys.

Monitoring changes in benthic plastic litter can be done by divers using beach sampling protocols in shallow water. Trawl nets also become clogged, reducing their efficiency and thus underestimating actual plastic abundance. Remote cameras may provide a more objective sampling strategy for benthic litter.

The authors concluded indicating that pilot studies should be used to estimate variability in sample data, and then power analysis should assess the numbers of samples necessary to detect a predetermined change.

Table 2: Comparison of survey protocols for monitoring the accumulation of beached litter in the USA and EUROPE (Sheavly, 2007 and OSPAR Commission, 2007)

	USMDMP	**OSPAR**
Type of beach	Sand/gravel	Sand/gravel
Beach slope	15-45° (not steep)	-
Beach length (m)	>500	>1000
Length of beach surveyed (m9	500	100 (all items) 1000 (items >0.50 m across)
Sample frequency (days)	28 +/- 3	90 (approx.)
Type of litter recorded	31 indicator items	All debris (111 categories)
Other criteria	No regular cleaning No impact on threatened species	Distant from source (rivers) Visuatlly/frequently littered

2.4 Ryan P. G., 2014: Litter survey detects the South Atlantic 'garbage patch' Marine Pollution Bulletin 79 (2014) 220–224

This study focuses on floating marine debris that was counted during a research cruise from 5 September to 9 October 2013. The method used by the author is reported below.

Observations were conducted throughout daylight hours while the ship was underway. Only debris on one side of the bow was counted. Most observations were made from the bridge wing or from the deck above the bridge, 12–15 m above sea level and 50 m from the ship's bow, but some observations were made from the ship's bow (elevation 6 m) during calm conditions. Litter was mostly detected with the naked eye, but regular scans of waters away from the ship were made with 8 32 binoculars to detect more distant debris. Binoculars or images taken with a digital SLR camera with a 500 mm telephoto lens were used to identify litter items. Observations were recorded continuously for up to 11.5 h per day, with location and environmental parameters (wind speed, direction, sea surface temperature, salinity) recorded from the ship's data logger at the start and end of each hour. Track length was calculated from the ship's positional record to measure the distance covered during observations. To compensate for the patchy nature of floating debris at sea, data were pooled into transects of roughly 50 km (2–3 h of transects), which sample 2.5 km^2 of sea surface given an effective transect width of 50 m.

The size of items and their distance from the side of the ship were estimated following Ryan (2013). Distance from the ship was placed into one of seven categories: 0 = 0–10 m from the side of ship, 1=11–20m, 2=21–30m, 3=31–40m, 4=41–50m, 5 = 51–100 m, and 6 = >100 m. The size of each debris item was allocated to one of five size classes based on its longest dimension: a<5cm, b=5–15cm, c=15–30cm, d=30–60cm, and e>60cm. Minimum item size was approximately 1–2 cm. Litter items were placed into one of the following categories based on the type of material and likely use of the item. Plastic items were divided into packaging, fishery-related plastic articles, other plastic user items, and finally, other plastic pieces. Non-plastic items were divided into glass jars/bottles, light bulbs, tins/aerosols, cardboard/paper, and wood. The effect of item size on detection distance was determined from the frequency of encounters in relation to distance from the ship (Ryan, 2013).

2.5 Galgani et al., 2010: Marine Strategy Framework directive - Task Group 10 Report Marine litter

Galgani et al. (2010) realized a comparison of the approaches used for monitoring the marine litter in different context (beach, sea surface, water column, sea floor) and for microplastic.
On coastline, the marine survey provides information on activities as the source of litter, changes in amounts of litter present in the marine environment and potential threats to marine biota and ecosystems.
The floating litter can be estimated either by direct observation of large debris items, by net trawls for smaller items or by aerial surveys

Table 3: Summary of approaches for assessing GES with regards to marine Litter (Galgani et al.*, 2010 and 2011).*

Compartment	Approaches	Positive aspects	Poorly covered and negative aspects
Coastline	Counts of the amount of litter items on known stretches of coast.	Allows for assessment of composition, amounts, sources, trends, social harm (aesthetic, economic).	Very small items and microparticles in sediments are not quantified. Not all coasts are accessible or appropriate.
Sea surface	Ship observers.	Precise evaluation at local scale.	Depending on weather. Not at large scale, small debris not considered, strong temporal variation.
Sea surface and water column	Trawling and water filtration.	Precise evaluation at local scale, consider smaller debris.	Costs, strong temporal variation.
Sea surface	Aerial counts of the number of litter items floating on the sea surface along transects.	Assessment of densities of litter on water surface over large areas possible; correlation with shipping or fisheries activities.	Smaller items not covered. Only counts of items from TetraPak size upwards are possible.
Sea floor shallow	Visual survey with divers.	All substrate types, replicability, feasible to account for detectability.	Depth limitation (<40 m).
Sea floor, deep sea litter	Trawling.	Replicability, possible standardization.	Only where trawling is possible.
Sea floor, deep sea litter	Submersibles and remote operated vehicles.	All sites accessible.	Only small areas, costs.
Microplastic on shorelines	Extraction of fragments from sediment samples and subsequent	Positive identification of specific polymers.	Analysis is time-consuming and is unlikely to detect all of the microparticles. This is especially true for very small fragments (<100 µm).

	identification using FT_IR spectroscopy.		
Microplastic at sea surface	Manta trawl (330 µm) and subsequent identification using FT_IR spectroscopy.	Positive identification of specific polymers.	Analysis is time-consuming and is unable to detect all of the microparticles.
Socio-economic	Assessment of direct costs through survey-based methods.	Provides indication of economic burden on marine and coastal sectors.	Does not capture full impact of degradation of ecosystem goods and services due to marine litter.

2.6 M. Cole, P. Lindeque, C. Halsband, T.S. Galloway, 2011: Microplastics as contaminants in the marine environment: A review, Mar. Pollutt. Bull. 62, 2588-2597.

The paper presents a bibliographic reviewin order to: (1) summarise the properties, nomenclature and sources of microplastics; (2) discuss the routes by which microplastics enter the marine environment; (3) evaluate the methods by which microplastics are detected in the marine environment; (4) assess spatial and temporal trends of microplastic abundance; and (5) discuss the environmental impact of microplastics.
The authors report that a suite of sampling techniques has been developed that allow the presence of small plastic debris to be determined. These include: (1) beach combing; (2) sediment sampling; (3) marine trawls; (4) marine observational surveys; and (5) biological sampling. For 1
Beach combing: This technique is particularly useful for determining the presence of macroplastics and plastic resin pellets, termed 'Mermaid's Tears' by beach combers, but microplastics, especially those too small to be observed by.
Sediment sampling 2) allows benthic material from beaches, estuaries and the seafloor to be assessed for the presence of microplastics. To separate any plastics from the benthic material, saline water or mineral salts can be added to the sediment samples to increase water density; Visible,
denser plastic fragments can be removed by hand under a microscope. A lipophilic dye (e.g. Nile Red) can then be used to stain the plastics to assist identification using a range of microscopy techniques. Using Fourier-Transform Infrared Spectroscopy (FT-IR), items of interest can then be confirmed as plastic by comparing spectra of the samples with that of known polymers. (Cole, Lindeque, Halsband, & Galloway, 2011)
Marine trawls 3) Microplastics within the water column can be collected by conducting a trawl along a transect (i.e. manta trawls for sampling surface water, bongo nets for collecting mid-water levels and benthic trawls to assess the seabed) using fine meshes.
Marine observational surveys 4) allow divers or observers on boats and in submersibles to record the size, type and location of visible plastic debris. While this technique is effective at detecting macroplastics over relatively large areas, microplastics will often go undetected;
Biological sampling 5) involves examining plastic fragments consumed by marine biota. A number of marine organisms can mistake plastic debris for prey. By dissecting beached marine animals, or by instigating regurgitation in some seabirds, their gut contents can be analysed for the presence of plastics, which can then be identified and quantified.

2.7 Ryan, P.G., 2013. A simple technique for counting marine debris at sea reveals steep litter gradients between the Straits of Malacca and the Bay of Bengal. Mar. Pollut. Bull. 69, 128–136.

The method used by Ryan (2013) regarded floating marine debris counted during a research cruise. Observations were conducted throughout daylight hours while the ship was underway, and were made from the bridge wing or from the deck above the bridge, 10–13 m above sea level and 57 m from the ship's bow. Only debris on one side of the bow was counted. In addition, scans of the waters in a 330 arc around the vessel were made from the ship's helideck every 30 min while on station. Litter was mostly detected with the naked eye, but regular scans of waters away from the ship were made with 10 32 binoculars to detect more distant debris. Binoculars or images taken with a digital SLR camera with a 500 mm telephoto lens were used to identify litter items, but some submerged items could not be identified. Observations were recorded continuously in 10-min bins for up to 12 h, with location and environmental parameters (wind speed, direction, sea surface temperature, salinity) recorded from the ship's data logger at the start and end of each hour. The ship's position also was recorded
The distance, estimated using a simple range-finder, from the ship using the following seven category scoring system: 0 = 0–10 m from side of ship, 1 = 11–20 m, 2 = 21–30 m, 3 = 31–40 m, 4 = 41–50 m, 5 = 51–100 m, and 6 = > 100 m. The size of each debris item was allocated to one of five size classes based on its longest dimension: a < 5 cm, b = 5–15 cm, c = 15–30 cm, d = 30–60 cm, and e > 60 cm. Minimum item size was approximately 1–2 cm. Litter items were placed into one of the following categories based on the type of material and likely use of the item. Plastic items, which included foamed polystyrene, were divided into packaging, fishery-related plastic articles, other plastic user items, and finally, other plastic pieces. Non-plastic items were divided into glass jars/bottles, light bulbs (mostly fluorescent tubes), tins/aerosols, cardboard/paper, and wood (worked timber). Correction factors were also defined and applied. One advantage of this size-based counting technique is that it is possible to estimate the densities of different litter size classes at sea.
Ryan further indicated that standardised data collection protocols are needed for counts of floating debris, particularly as regards the size classes used, to facilitate comparisons among studies.

2.8 Lisbeth Van Cauwenberghe, Ann Vanreusel, Jan Mees, Colin R. Janssen, 2013: Microplastic pollution in deep-sea sediments, Environmental Pollution, 495-499

Microplastic extractions were performed on 11 sediments samples originating from several locations in the Atlantic Ocean and Mediterranean Sea ranging in depth from 1176 to 4844 m. The authors, in an attempt to investigate the presence of microplastics in deep sea, collected sediments in a surface area of 25 m2. After recovery, cores were cut into horizontal slices by extruding them and slicing the sediment with a metal plate. The cores were cut into 1-cm-thick slices and the top centimeter of the sediment cores was wet sieved, first on a 1-mm mesh sieve and sub-sequently on a 35-µm mesh sieve. The fraction remaining on the35-µm mesh sieve was used for further separation based on density flotation by adding a solution of NaI. The solids were then transferred to a centrifuge tube followed by shaking and centrifugation. A fter centrifugation the top layer containing the microplastics was vacuum filtered.

2.9 J.P. Desforges, M. Galbraith, N. Dangerfield, P.S. Ross, 2014: Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean, Mar. Poll. Bull, 79, 94-99

The authors document the abundance, composition and distribution of microplastics in sub-surface seawaters of the northeastern Pacific Ocean and coastal British Columbia. Sampling was conducted aboard two oceanographic research cruises in 2012. The sampling regime was developed to create a low cost, long-term monitoring program that is integrated into existing oceanographic programs.
Seawater was collected at 4.5 m below the surface using the saltwater intake system of the vessel. A flow-meter measured the volume of water pumped from the saltwater intake at each site, and the readings were converted to cubic meters of water filtered.
Water was typically pumped for 10–20 min at each station, but this varied as a function of other oceanographic sampling taking place aboard the vessel. Water was first passed through a coarse 5 mm filter to remove large debris and organisms before entering the intake system, then run through a series of copper sieves of diminishing pore size: 250 µm, 125 µm, and 62.5 µm. The material on each sieve was rinsed with seawater into labelled 20 ml glass vials and stored refrigerated with 5–10% HCl at 4 °C at the Institute of Ocean Sciences (Sidney, BC).

2.10 Peter G. Ryan, Annerie Lamprech, Debbie Swanepoel, Coleen L. Moloney, 2014: The effect of fine-scale sampling frequency on estimates of beach litteraccumulation, Marine Pollution Bulletin, 88, 249-254

First, a study area (250 m or 500 m) was demarcated, divided into ten equal subsections along the beach. Prior to each experiment, a team of volunteers removed all accumulated macro litter (articles >10 mm diameter) between the water line and the dune vegetation (including any litter visible in the vegetation) from each study area, and from adjacent buffer zones approximately 25 m wide at each end of the study sections. The following day (24 h, or two tidal cycles later), sampling of 'new' litter commenced at both sites. Teams of three to six observers worked systematically along each beach, collecting all litter items in each of the ten subsections. Each subsection was searched until no items had been found for several minutes, with searches of each section taking roughly 5–15 min, depending on the amount of litter present.
All litter samples were sorted and counted. Each debris item was identified and categorised by type of material (plastic, metal, glass, ciga- rette butts, etc.). Wood items were included if they were 'worked' rather than natural wood. Plastic items were further subdivided by function: packaging and other single-use items, user items including fishing gear, and plastic fragments of uncertain provenance. Foamed polystyrene items (including cups and fast-food trays as well as packing chips and moulded packaging) were placed in a separate category because of their lower density than most other litter items. Articles were dried and cleaned of sand before weighing.

2.11 T.R. Santos, A.C. Duarte: 2015: A critical overview of the analytical approaches to the occurrence, thefate and the behavior of microplastics in the environment, Trends in Analytical Chemistry, 47-53

The review addresses the analytical approaches to characterization and quantification of microplastics in the environment and discusses studies on their occurrence, fate, and behavior.

The authors indicate that sampling microplastics in the water column can be achieved by:
(1) using a trawl along a transect, such as manta net and Neustonnet for surface waters
(2) using bongo nets for mid-water levels;
(3) benthic trawls for seabed; and,
(4) vessels for surface and mid-water levels.
Typically, 333–335-µm mesh apertures are the most used for the net and, when a different size aperture is used, it can producelarge variations in the quantity of microplastics collected.
Sampling sediments can allow the benthic material from beaches, estuaries and seafloor to be assessed for the presence of microplastics. Using stainless-steel spoons or spatula and box-corer usually collects superficial sediments or sand from the beaches and estuaries, while seabed sediments are collected using coreand bottom trawl. There is a general lack of specific sampling protocols for collection of microplastics in sediments andwater.
Biological sampling involves examining microplastics in marine organisms through dissection of marine animals, and investigation of regurgitation in seabirds and analysis for the presence ofmicroplastics in their gut contents.
Separation of microplastics from samples has been done bydensity flotation, filtration and sieving. The specific density of plastics particles varies considerably, since it depends on the type of polymer and the manufacturing process, in the range 0.8–1.4 gcm-3. These values do not take into account the effect of adding several additives that might be incorporated into the production process, so they refer onlyto the virgin resin, biofouling and weathering. Since sand or sediments have densities around 2.65 gcm-3, the difference in density can be used to separate the lighter microplastics from the heavier sand or sediments. A salt-saturated solution (usuallyNaCl or NaI) is added to sand or sediments and mixed by shaking or using a vortex. After mixing, the sediment will settle to the bottom, while the microplastics will remain in suspension or float to thesurface of the solution. The supernatant is then extracted for further processing of the microplastics particles.
The Marine Strategy Framework Directive (MSFD) Technical Sub-group on Marine Litter recommended the use of NaCl for thes eparation of microplastics by density flotation, since it is an inexpensive, eco-friendly salt. However, the use of saturated so-lution of NaCl (1.2 g cm-3) or tap water may lead to underestimation of the microplastics content in sediments because the solution density is too low to enable the flotation of all polymers, princi-pally those containing additives. Instead, an NaI-saturatedsolution density (1.6 g cm-3) is enough to separate the polymers con-taining additives, so it is preferable to use NaI. (Rocha-Santos & Duarte, 2015)

2.12 Q. Qiu, Z. Tan, J. Wang, J. Peng, M. Li, Z. Zhan, 2016: Extraction, enumeration and identification methods for monitoring microplastics in the environment

In their review, the authors report that sediment samples are generally taken from beaches, estuaries and the seabed. Sampling on the beaches varies widely from the sampling location, stationing route to sampling depth. First of all, the sampling location could be far away from the center of human activities and lies between coastline and tide line, especially in parallels to the high tide line
They further indicate that there is a wide range of sediment sampling depths, such as 1 cm, 3 cm, 5 cm and 10 cm. It is expected that different pollution characteristics would be obtained by collecting samples from different depth sediments but there is little research available. Bottom sediment samples could be

collected by a box corer, while samples of the surface could be scooped out using iron spoons or non-plastic sampling spades. Then the sediment samples are put into a glass container or aluminum foil.
For the water samples, the authors indicate that samples could be collected from the surface, middle and bottom layer water through a trawl with a rectangular opening and a net con- nected with a collecting bag. The value of mesh size, ranging from 0.18 mm to 0.5 mm, is close to 0.333 mm. Larger mesh size would reduce fouling but give access to less abundant microplastics. A flow meter is used to calculate the volume filtered per tow. The sampling area is calculated by multiplying the length of sea surface trawled by the width of the trawl and the units of floating samples are particle/m3 and particle/m2 (p/m3 and p/m2).
During the sampling process, a strong net is essentially installed on the side of the boat to avoid the disturbance of the debris by the bow wave. The ship should be at constant speed so as to record the motion distance by a stopwatch. After sampling, it would be better to use seawater to wash samples to decrease the impurities on the surface. All samples could be also stored in the 2.5% formalin solution. The plastic particles can then be separated from organic tissue in graduated cylinders by gravity.
The volume of water could be obtained from the reading of flow meter converted to m3 filtered. Other auhtors directly collected the surface samples by using bottles. HCl or H_2O_2 solution was then used to remove natural organic matter so as to remove natural organic particles. Water samples were filtered in a laboratory and the filter papers dried at a certain temperature and stored in dishes. Plastic particles are identified, hand-separated, dried and kept in darkness at room temperature.
The zooplankton would be attached to plastic particles and therefore samples could be transferred into tubes so as to separate by gravity plastic particles from zooplankton. However, a major problem is that most studies in plankton take only in consideration the living portion and left behind the non-living portion, including microplastics
For marine organisms, the authors report different studies that used direct or indirect methods
Direct method
Marine organisms are firstly collected. Fishes, for instance, are wrapped in aluminum foil, frozen and thawed out at room temperature prior to examination. The relative data of fishes, including length, weight, girth, sex, maximum distance between dorsal and ventral sides, should be recorded. Then fishes are dissected to remove the gastrointestinal tracts to analyze.
Some other studies used flushed stomach of shearwaters to evaluate its ingested microplastics. Body mass, wing chord, head and bill length are determined by using a spring balance, a stopped ruler, and a vernier caliper, respectively. Seawater is pumped into the proventriculus to rush out any food and plastics particles, which are then cleaned with tap water, dried and stored.
Mussels and lug- worms were also used. Organisms are acquired and keed in glass jars containing filtered seawater for some days to clean the gut. Water must be renewed to ensure that previously egested material, including microplastics, would not be re-ingested. After depuration, organisms are removed from their shell. The soft tissues are left in the acid solution followed by boiling, diluting and filtering. Faeces were also collected by a sieve, then transferred to a tube and subjected to NaI-extraction.
Stranded large animals have been also investigated. Samples are firstly measured, sexed and examined for external lesions or other anomalies. Digestive tracts are removed and stored at 20 C prior to obtain the full digestive tract in a veterinary laboratory. Biopsies are taken and skin samples are detected for species confirmation and genetic relationships. Blubber and muscle samples are also collected for extracting the DEHP (di-(2-ethylhexyl) phthalate) and MEHP (mono-(2-ethylhexyl) phthalate).
Indirect method

Similarly, organisms are firstly collectedand then randomly divided into groups and starved to acclimate to the experimental conditions, including oxygen, temperature, photoperiod, electrical conductivity, water hardness and ammonia concentratio. All glass materials are washed with acid or H_2O_2, rinsed with acetone and distilled water. After the acclimation, organisms are fed with food mixed with visible microplastics, especially fluorescent microplastics, and then are dissected. Tracts are collected and frozen at 20 C. The pretreatment of tract is similar to samples in sediment, such as flotation, filtration and drying.

2.13 K.S. Edyvane, A. Dalgetty, P.W. Hone, J.S. Higham, N.M. Wace, 2004: Long-term marine litter monitoring in the remote Great Australian Bight, South Australia, Marine Pollution Bulletin 48, 1060–1075

In this study, annual survey involves the systematic clearance of all litter from the 26 km beach, foredunes and the frontal edge of the main dunes—and importantly, the sourcing of all litter into litter types and also, the origin of the litter. Litter was collected and removed for each 1 km section of beach, fore dunes and main dunes. Individual items of 'megalitter' (i.e. greater than 2 cm across) were cleared of sand, classified, counted and weighed.

The collected litter was classified as either hard (i.e. moulded) plastic, soft plastic (i.e. flexible and foamed), glass, metal or rubber. However, no metal was recorded. Driftwood was not included. If possible, the origin of the litter was also determined. Fishing related litter was identified and sourced by local volunteer fishermen. Individual items of fishing-related debris were not always weighed or counted. Items of biogeographic interest and also, natural marine flotsam was also recorded. This included many derelict wedge-tailed shearwaters, tagged wildlife and also stranded sealife. The annual ocean litter survey was generally conducted over 3 days (followed by 1–2 days of sorting), generally during spring.

2.14 Iván A. HinojosaMartin Thiel, 2009: Floating marine debris in fjords, gulfs and channels of southern Chile Marine Pollution Bulletin 58 (2009) 341–350

The authors used ship sur-veys to examine the abundance, composition and distribution of Floating Marine Debris during the years 2002–2005 inthe fjords, gulfs and channels of southern Chile. They used visual observations obtained during daytime navigation by a skilled observer that surveyed the sea surface from the bridge of the research vessel (~4 m above sea level, ship velocity ~10 knots.). Using a handheld GPS and binoculars, the observer recorded all FMD on one side of the ship, taking notes of their position and distance to the vessel. The distance of FMD to the vessel was estimated using known distances (e.g. width or length of vessel). In order to estimate the abundance of FMD, we used the strip transect method, which is based on the number of items seen and the width and transect length. A preliminary examination of the data indicated that the number of observed items decreased substantially at distances >20 m from the vessel, therefore only a transect width W of 20 m from the vessel was consiedered in order to not underestimate the abundance of floating items due to overlooked items.

Seven categories of FMD were distinguished: styrofoam (expanded polystyrene, particles of 2–60 cm diameter); plastic fragments (fragments of various non-identified hard and soft plastic items >2 cm); plastic bags (typical plastic grocery bags), lines (principally polypropylene ropes), food sacks (food sacks commonly used in salmon aquaculture), wood tables (wood boards used for construction or transport crates), and others (glass and plastic bottles, tetra pack, plastic cup, cigarette boxes, etc.).

2.15 Amandine Collignon, Jean-Henri Hecq, François Glagani, Pierre Voisin, France Collard, Anne Goffart, 2012: Neustonic microplastic and zooplankton in the North Western Mediterranean Sea, Marine Pollution Bulletin 64 (2012) 861–864

Neustonic microplastic and zooplankton abundance was determined in the North Western Mediterranean Sea during a summer cruise between July 9th and August 6th 2010. During this survey, the samples were collected with a manta trawl net lined with0.333 mm mesh. The size of the rectangularnet opening was 0.60.2 m2. The trawl sampled the top 10 cmof the sea surface at an average speed of 2.5 knots for 20 minfor each sample. The trawl was towed from a boom installedon the side of the boat to prevent the disturbance of the debrisby the bow wave.

2.16 Bjørn E. Grøsvik, Tatiana Prokhorova, Elena Eriksen, Pavel Krivosheya, Per A. Horneland1 and Dmitry Prozorkevich, 2018: Marine Science, 5:72.

The study of Grosvik et al. (2018), presents a large-scale monitoring of marine litter performed in the joint Norwegian–Russian ecosystem monitoring surveys in the period from 2010 to 2016 and contribute to documentation of the extent of marine litter in the Barents Sea. The distribution and abundance of marine litter in upper 60 m are based on pelagic trawling with a small meshed pelagic trawl "Harstad trawl" with a mouth opening of 20 × 20 m, with seven panels and a cod end. The panels have mesh sizes varying from 100 mm in the first part to 30 mm in the end. Pelagic trawling was carried out at three depths, each over a distance of 0.5 nautical mile, with the headline of the trawl located at 0, 20, and 40 m, respectively, and with trawling speed of three knots.
Visual estimated volume of the floating marine debris has also been recorded for mapping and correlations analyses only. Indeed, during transit between stations (35nm miles), observations of floating marine debris at surface were recorded by whale observers, and material types and volumes were noted. Visual observations were taken only during day time and when weather and visibility was suitable. Observers recorded approximate volume of the same categories of the floating marine debris. In total, 784 visual observations of floating marine debris were recorded during the period. Marine litter has been categorized according to volume or weight of the material types plastic, wood, metal, rubber, glass, paper, and textile. (Grøsvik et al., 2018)

2.17 Giuseppe Suaria; Stefano Aliani, 2014: Floating debris in the Mediterranean Sea, Marine Pollution Bulletin, 86, 494-504

Wihtin this study, visual surveys of floating debris were carried out between May and October 2013 (Fig. 1). Observations were all made by the same observer during regular navigation of the ship at a speed of 10 knots. The observer scanned the sea surface from the bearing deck of the research vessel (~5 m above sea level and ~30 the bow) and recorded UTC time, GPS coordinates, size and type of all macro-debris items (>2 cm) sighted off the starboard side of the track-line. Sightings were all performed during daylight hours, by naked eye and only under good weather conditions. 7 × 50 binoculars were used to clearly identify more distant objects. Data collected in poor visibility conditions were removed and not considered. Meteo-marine data such as wind speed and direction, sea surface temperature and salinity, were automatically recorded by the ship's data logger. The survey effort was split into 30-min transects (mean length: 9.21 ± 1.05 km) in order to standardize fatigue for the observer, enhance the number of

replicates and better account for the patchy distribution of debris at sea. The exact distance covered during each transect was calculated from GPS start and stop positions using the haversine formula.
After being recorded, every item was allocated to one out of four size classes (<10, 10–50, 50–100, >100 cm) and to one of two major type categories: Anthropogenic Marine Debris (AMD) and Natural Marine Debris (NMD). AMD was further subdivided into styrofoam (expanded polystyrene), plastic (mainly fragments, plastic bags, bottles and containers) and others (e.g. manufactured wood, aluminum cans, rubber strips, glass bottles, paper and cardboard), while NMD was classified as wood, algae or others. The survey area was subdivided into 14 sectors following the METEOMEDTM subdivisions of the Mediterranean Sea for marine weather forecasts. The number of surveyed transects per sector varied from 3 to 38, mainly due to different weather conditions, navigational schedules and operational needs during the cruises. (Suaria & Aliani, 2014)

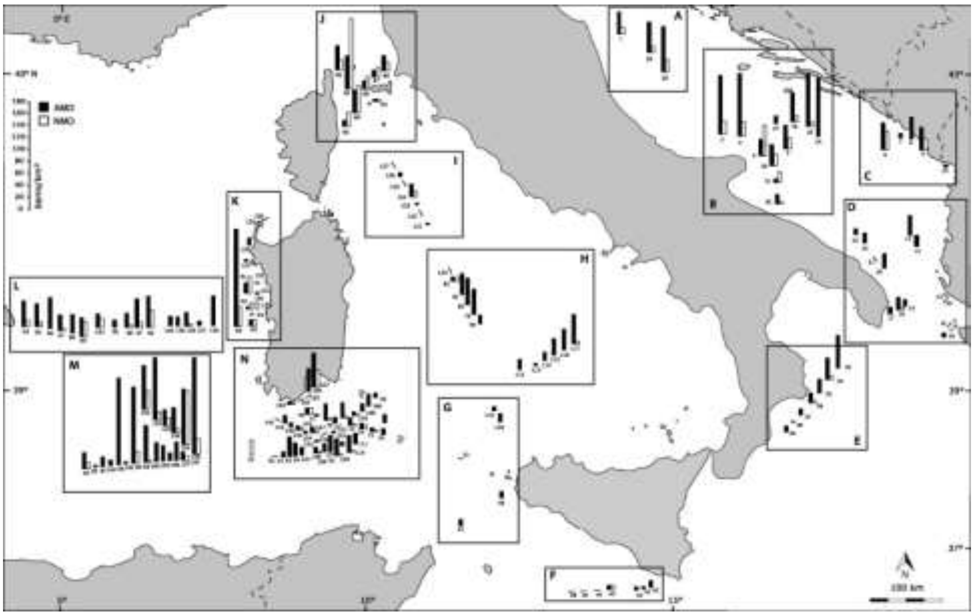


Fig. 1: Map of the central-western Mediterranean Sea showing the study area, the location of all transects and sectors and the distribution of AMD (black bars) and NMD (white bars) densities (expressed as number of items/km2) in all surveyed transects (after Suaria and Aliani, 2014).

2.18 Bum GunKwon, Keiji Amamiya, Hideto Sato, Seon-Yong Chung, Yoichi Kodera, Seung-Kyu Kim, Eung Jae Lee, Katsuhiko Saido, 2017: Monitoring of styrene oligomers as indicators of polystyrene plastic pollution in the North-West Pacific Ocean

The paper deals on the Styrene oligomers as global contaminants as little is known about the SOs chemicals generated from PS plastic in the coastal ocean surface and deep seawaters. In this study the authors analyzed water samples from the coastal ocean surface and deep seawaters inthe North-West Pacific Ocean in order to map the distribution of the SOs derived from PS plastic polymer and in particular on styren trimer, styrene dimer and styrene monomer. This study aims to provide a new direction for the assessement of the fate and behavior of PS plastic discarded into the ocean. The method used allowed to collect water samples. At the sampling points, 12 L seawater samples were collected using 12 bottles of a

Niskin seawater sampler which was composed of polyvinyl chloride material. Furthermore, material containing PS plastic was excluded from all sampling and extraction procedures to eliminate any errors. In the field, the seawater samples collected were subjected to cotton plug filtration. The seawater sample spiked with surrogate biphenyl prior to extraction was immediately extracted four times with 100 mL dichloromethane using a portable shaker. In the laboratory, about 100 mL of the DCM extract was mixed with approximately 10 g anhydrous sodium sulfate (ACS grade, Sigma-Aldrich, USA) and left overnight. The extract was evaporated by a rotary evaporator at 30°C until it was dry. After adding 0.5 mg L^{-1}phenanthrene as an internal standard, the eluate was completely dissolved in 1 mL of benzene. The result shows evidence that SOs chemicals leached from PS plastic are spreading into the marine environment. From the monitored results, SOs are found at concentrations that are higher than those expected based on the durability of PS. Finally, these SOs quantitatively provide an environmental indicator for assessing the scope of marine pollution arising from the PS disposal.

3 Macro marine litter on the beach

Several national and regional bodies have developed protocols for conducting beach surveys, which have been designed to reduce variability and bias in the observations. Generally, the protocols are based on simple counts of the number or the weight or volume, of litter items found on a given length of beach or water line. Such surveys have their limitations but because the abundance of beach litter is very much influenced by water currents, prevailing winds and the exposure of the beach, the use of exactly defined stretches of coast is vital when using this type of survey if trends in the amount of litter over time are to be measured.

3.1 NOWPAP, 2007. Guidelines for Monitoring Marine Litter on the Beaches and Shorelines of the Northwest Pacific Region, 12pp.

The guidelines are designed for the NOWPAP member states and help to identify the types, amounts and sources of marine litter but also for favoring the sharing of the results of the different monitoring surveys. First the guideline specifies the criteria to select the survey site. In general, the floowing areas are NOT suitable for surveys:

- Areas within 1 km of river mouths, harbors, ports and swimming beaches
- Rocky beaches and breakwaters

For the sampling of the marine litter, the area should be defined (10 x 10 m or 4 x 100 m) and the positions should be recorded. In this area, all the marine litter should be collected and recorded onto an appropriated form (example is reported below in Fig. 2). All collect items should be put into litter bags or place into suitable containers.

Annex: Examples of Data Collection Forms

a) International Coastal Cleanup Data Card, The Ocean Conservancy

INTERNATIONAL COASTAL CLEANUP™ DATA CARD

Data collected during The Ocean Conservancy's International Coastal Cleanup™ is used to educate people and create solutions to the problems of solid waste and litter. Through partnerships with business, government, environmental groups, and citizens, we are helping to change the behaviors and practices that create debris. Thank you for being part of this very important process.

20th ANNIVERSARY International Coastal Cleanup The Ocean Conservancy

CLEANUP LOCATION

Type of Cleanup: ☐ Shoreline/Beach ☐ Underwater Location of Cleanup: State______ Country______

Zone or County Cleaned:______ Beach Site Name:______

Today's Date: Month____ Day____ Year____ Name of Coordinator:______

Number of People Working on This Card:______ Distance Cleaned:______ miles or______ km

Number of Trash Bags Filled:______ Total Estimated Weight Collected:______ lbs. or______ kgs.

NAMES OF PARTICIPANTS IN YOUR GROUP

If you are interested in becoming a member of The Ocean Conservancy and/or joining our Ocean Action Network (OAN) to make your voice heard on important ocean conservation issues, please check the box(es) below your name and address. ***Thank you for helping to protect our oceans!***

1. Name:______ Age:____
Address:______
City:______ State:______
Zip Code:______ Country:______
Phone: (____) ______
Email:______
I would like information on: ☐ The Ocean Conservancy ☐ The OAN

2. Name:______ Age:____
Address:______
City:______ State:______
Zip Code:______ Country:______
Phone: (____) ______
Email:______
I would like information on: ☐ The Ocean Conservancy ☐ The OAN

3. Name:______ Age:____
Address:______
City:______ State:______
Zip Code:______ Country:______
Phone: (____) ______
Email:______
I would like information on: ☐ The Ocean Conservancy ☐ The OAN

4. Name:______ Age:____
Address:______
City:______ State:______
Zip Code:______ Country:______
Phone: (____) ______
Email:______
I would like information on: ☐ The Ocean Conservancy ☐ The OAN

ENTANGLED ANIMALS: (☐ Dead or ☐ Alive). List all entangled animals found during the Cleanup. Tell us what they were entangled in (fishing line, rope, net, etc.) ______

WHAT WAS THE MOST PECULIAR ITEM YOU COLLECTED?______

The following national and international organizations endorse and/or support the International Coastal Cleanup:

- U.S. Environmental Protection Agency
- IUCN – The World Conservation Union
- Intergovernmental Oceanographic Commission (IOC) of the United Nations' Educational, Scientific, and Cultural Organization (UNESCO)

Please return this card to your area coordinator or mail it to:

The Ocean Conservancy Office of Pollution Prevention and Monitoring
1432 N. Great Neck Road, Suite 103
Virginia Beach, VA 23454 USA
Phone (757) 496-0920 Fax (757) 496-3207
www.oceanconservancy.org

100% post-consumer recycled paper. Chlorine free.

Fig. 2: Example of data Collection form, (NOWPAP, 2007)

1. Rubber Items	Weight (kg) :	Number (ea) :	Volume (ℓ) :	
	Land-origin	Ocean-based	Land/Ocean origin	Sub-total(ea)
rubber glove, rubber band, balloon, rubber belt				
tire, rubber boot				
others				
Sub-total(ea)				

2. Metal Items	Weight (kg) :	Number (ea) :	Volume (ℓ) :	
	Land-origin	Ocean-based	Land/Ocean origin	Sub-total(ea)
wire, nail, tool, steel plate, aluminum plate (re-used item)				
can, aluminum foil (re-used item)				
fishhook, sinker made of lead and copper, lead for fishing net				
paint barrel and others (non-re-used items)				
Sub-total(ea)				
1) can: beverage can, gas can, spray can, good can, bottle-cap, can ring, etc.				

3. Wood Items	Weight (kg) :	Number (ea) :	Volume (ℓ) :	
	Land-based	Ocean-based	Land/Ocean-based	Subtotal(ea)
Wooden box, Wooden chopstick, Basket, Bamboo mat				
Wooden material, Palette (Wooden carrier)				
Wood for fishery use				
Others				
Subtotal(ea)				
1) Unprocessed wood, leaves and stems are excluded.				

Fig. 3: Example of data Collection form, (NOWPAP, 2007)

		Code of the beach	
Name of Beach		Code of the Desinated Zone	
Date	Time ~	A note-taker	

(1)Plastic		Domestic	Foreign C	K	R	O
①Bags						
	for food, or package					
	of shopping					
	for snack					
	others					
②Bottles						
	for drinking					
	for detergent or bleach					
	for seasonings or sources					
	others					
③Containers						
	for food, dish					
	food tray					
	for seasonings					
	caps, rids					
	others					
④Line						
	string					
	rope					
	tape					
⑤Miscellaneous goods						
	drinking straw					
	cigarette filter					
	lighter					
	toy					
	stationery					
	others					
⑥Fishing gear						
	fishing line					
	lure, float					
	bouy					
	others					
⑦Broken pieces						
	sheet or bag					
	plastic					
⑧Resin pellets (plastic grain)						
⑨Others (be specific as possible)						
Subtotal	Quantity					
	Weight					

(2)Rubber		Domestic	Foreign C	K	R	O
①Balls						
②Balloon						
③Gloves						
④Rubbber bands						
⑤Broken pieces						
⑥Others (be specific as possible)						
Subtotal	Quantity					
	Weight					

(3)Styrene form		Domestic	Foreign C	K	R	O
①Containers/packages						
	food trays					
	cups					
	for lunch box or noodle					
	packing materials					
②Buoys						
③Broken pieces						
④Others (be specific as possible)						
Subtotal	Quantity					
	Weight					

(4)Paper		Domestic	Foreign C	K	R	O
①Containers						
	cups					
	drinking packages					
	plates					
②Packages						
	bags					
	cigarettes					
	for snack					
	cardboard box					
	heavy paper box					
③Cardboard tube for fireworks						
④Pieces of paper						
	newspaper/magazine/leaflet					
	tissue					
	broken pieses					
⑤Others						
Subtotal	Quantity					
	Weight					

Fig. 4: Example of data Collection form, (NOWPAP, 2007)

3.2 UNEP, 2009. Marine Litter: A Global Challenge.

When undertaking beach litter assessments as part of a regional programme there is a need to:

1. Identify and select suitable beaches to allow the establishment of appropriate sampling units; and
2. To develop a survey schedule to ensure that data are collected as required over the lifetime of the study.

The basic beach selection criteria should include:

- A minimum length of 100 m although beaches with small amounts of litter may need to be longer (e.g. 1 km);
- Low to moderate slope (15 – 45º), which precludes very shallow tidal mudflat areas;
- Clear access to the sea;
- Accessible to survey teams year round;
- Ideally the site should not be subject to any other litter collection activities;
- Survey activities should be conducted so as not to impact on any endangered or protected species.
- it is recommended that the location of sampling sites should be stratified such that samples are obtained from beaches subject to different litter exposures, including:
    - Urban coasts (i.e. mostly terrestrial inputs);
    - Rural coasts (i.e. mostly oceanic inputs);
    - Within close distance to major riverine inputs.

At each location data need to be collected relating to the depositional environment and proximity to litter sources including:

- Aspect.
- Prevailing wind (from meteorological data).
- Beach curvature.
- Total beach length.
- Nearest river – name, distance, direction and whether or not it inputs directly to the beach.
- Nearest town – name, distance and direction.
    - Estimated number of person visits per year.
    - Main beach usage.
    - Access (vehicular, pedestrian and/or boat only).

Beach slope should be measured at the start and end point of each transect. The shape of the beach profile should be described at transect start and end points.

The guidelines further report that sampling units (Fig. 5) of 100-1000 m will achieve the most pragmatic balance between areal coverage and the amount of effort required to complete the survey within an acceptable time allocation (i.e. preferably less than three hours, OSPAR 100m, Australia 1000 m).

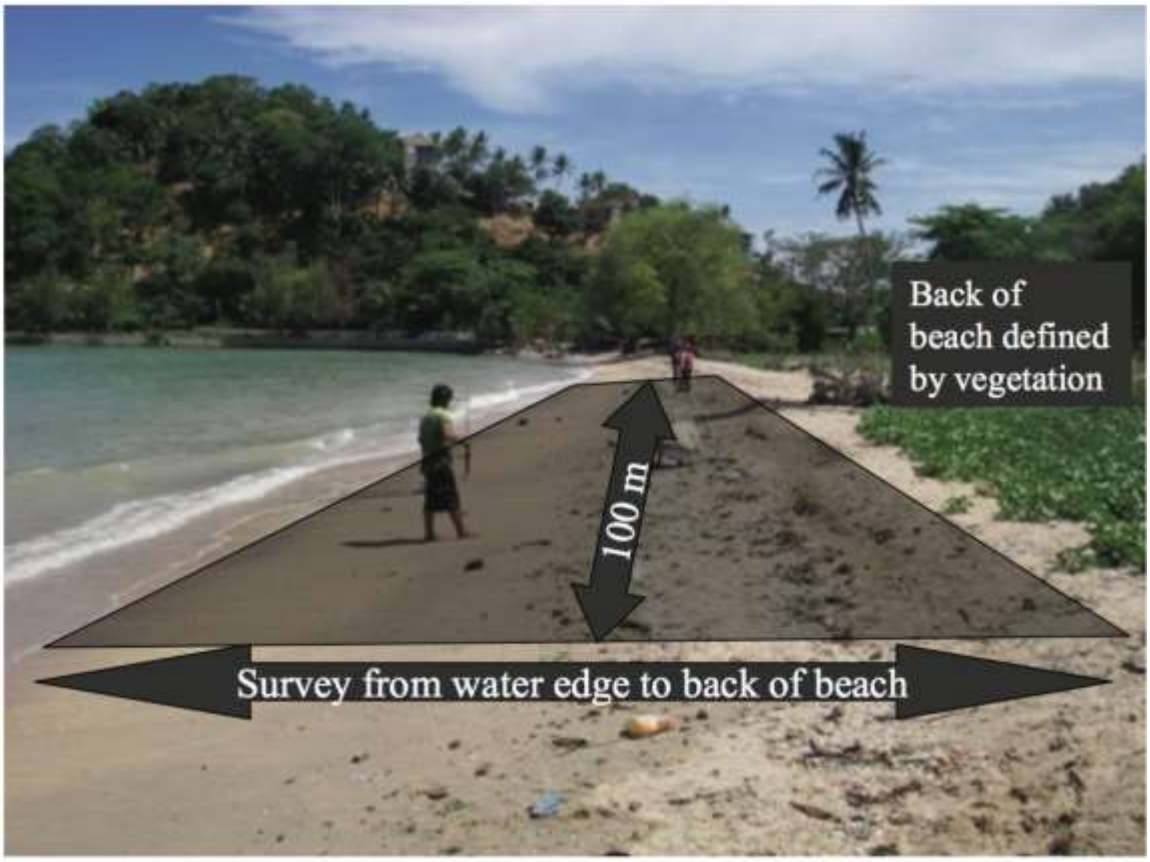


Fig. 5: UNEP (2009): Relationship between a typical sampling unit and the beach on which it is positioned. All litter from the water's edge to the back of the beach is collected along the length of the sample unit (e.g. 100m). If more than one sampling unit occurs on a beach the minimum separation distance shall be at least 50 m

The minimum sampling frequency for any site should be annually. Ideally it is recommended that locations be surveyed every three months. In order to obtain data on litter flux rates there is a need to undertake an initial beach clearance in order to remove all accumulated litter.

As reported in Fig. 6, the survey process can be undertaken in either of two parallel to the coast or perpendicolar. All litter, within the sampling unit, that is larger than 2.5 cm in the longest linear dimension should be collected into carry bags. Information of the beach and on the litter collected should be recorded in a specific form (Fig. 7 and Fig. 8).

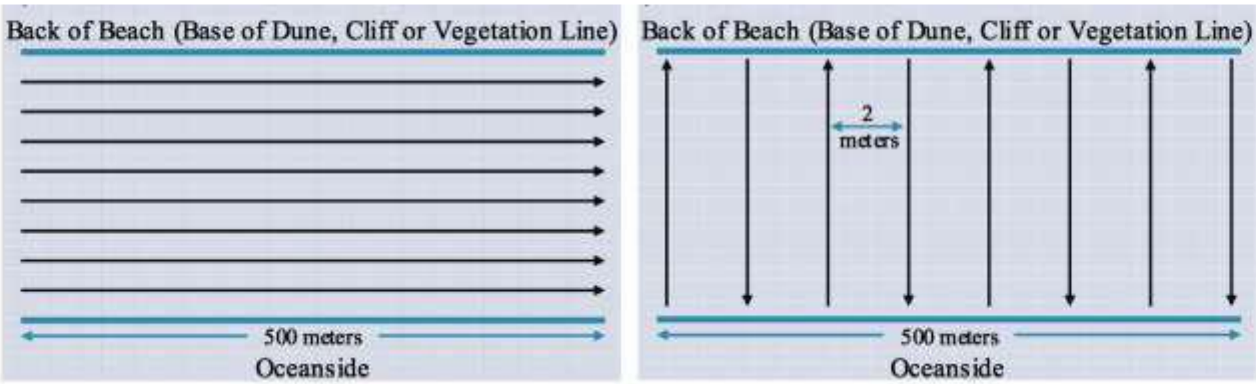


Fig. 6: Beach litter surveys can be undertaken in either of two ways; a) parallel to the coast or b) at right angles to the coast.

The additional data include:

- Survey date.
- Survey start and end times.
- Date on which the transect was last cleaned either as a survey or as part of broader beach maintenance programme.
- Distance along beach covered by the survey.
- Width of the beach at the time of the survey t).
- Number of persons on the survey team). Etc…

Large immoveable objects (abandoned cars, very large fishing nets, baulks of timber, etc) should be recorded on an additional datasheet, with information collected on the nature and location (preferably GPS fixed) for each large item.

BEACH LITTER **Beach Data Sheet** **BC01**	Organization		Name of the organization responsible for collecting the data
	Surveyor Name		Name of the surveyor (person responsible for filling in this sheet
	Phone number		Phone contact for surveyor
Completed **ONCE** for each site	Date		Date of this update to the data
SAMPLING AREA			
BeachID			Unique identity code for the beach (office use only)
Beach name			Name by which the beach is commonly known (include country)
Region name			Name for the region (office use only)
LME			Name for the LME in which the Beach is located (office use only)
Co-ordinate system			Datum and coordinate system used to record latitude and longitude
BEACH CHARACERISTICS – considered from the start point of the transect			
Slope			Slope of the beach – distance for 1 m of fall from mid point of beach
Aspect			Compass direction perpendicular to the beach facing the sea (nnn degrees)
Prevailing wind			Direction of prevailing wind for the beach system (nnn degrees)
Beach curvature			Concave, convex, sinusoidal, straight
Horizontal profile			Horizontal shape of the beach (Linear, Concave, Convex, Mixed)
Total beach length			Length measured along the mid point of the beach (kilometres)
Substratum type			Defines whether predominantly a sandy or gravel beach (pebble, rock etc)
Substrate Uniformity			An indication of the coverage by the predominant substrate type (Percent)
Offshore reefs			Presence of offshore reefs (yes/no)
Offshore seagrass			Presence of offshore seagrass beds (yes/no)
Tidal range			Max – min vertical tidal range (metres)
Tidal distance			Horizontal distance (metres) from the lowest tide to back of the beach
Back of beach			Describe the landward limit (Rock wall, Cliff, Dune, Anthropogenic)
Terrestrial vegetation (describe if any)			

Fig. 7: Beach data sheet (UNEP, 2009)

BEACH LITTER Sample and Beach litter data BC02 Completed **ONCE** for each survey			
	Organization		Organization responsible for the survey
	Surveyor Name		Name of the surveyor (person responsible for filling in this sheet)
	Contact		Phone contact for surveyor
	Region		Name for the region
	BeachID		Unique identity code for the beach (office use only)

Sample unit information

Beach Name			Unique Name by which the beach is known
Latitude/longitude start			Recorded as nnn.nnnnn degrees at the start of the sample – indicate NSEW
Latitude/longitude end			Recorded as nnn.nnnnn degrees at the end of the sample – indicate NSEW
Coordinate system			Datum and coordinate system for latitude and longitude
Survey date			Date survey was started for the sample (generally today's date)
Time start/end			Time taken to complete the survey (h)
Season			Spring, Summer, Autumn, Winter, NE Monsoon etc
Date of last survey or cleaning			Date on which the beach was last cleaned either by survey or maintenance clean up
Storm activity			Has there been any significant storm activity since the last survey
Number of persons			Number of persons collecting litter
Sample unit length			Length of sample unit along the beach (m)
Width of beach			Width of beach at the time of survey (m)
Sub-units (if used)			Number and distance along beach
Quality assurance			Is the sample for quality assurance purposes (either YES or leave blank)
Large items	Collate data on large items using the ML01 datasheet		

LITTER DATA (continue over page if more space required)

Item code (standard list)	Description	Count (# items)	Weight (kg)	Item code (standard list)	Description	Count (# items)	Weight (kg)

Notes (e.g. entangled fauna, important events – storms, shipwrecks etc, conditions that may affect survey)

Fig. 8: Sample and beach litter data (UNEP, 2009)

3.3 OSPAR, 2010. Guideline for Monitoring Marine Litter on the Beaches in the OSPAR Maritime Area

OSPAR (2010) has developed a guideline for monitoring marine litter on beaches as a tool to collect data on litter in the marine environment in order to provide information on amounts, trends and sources of marine litter. Such information is useful to assess the efficiency of the mitigating measures and the effectiveness of existing legislation and regulations. The guideline defines the marine litter as "*Marine litter (marine debris) is any persistent, manufactured or processed solid material discarded, disposed of, abandoned or lost in the marine and coastal environment*" (UNEP, 2005). This also includes such items entering the marine environment via rivers, sewage outlets, storm water outlets or winds.
The proposed method requires first to select the beach's sites according to specific criteria:
• be composed of sand or gravel and exposed to the open sea;
• be accessible to surveyors all year round;
• be accessible for ease of marine litter removal;
• be a minimum length of 100 metres and if possible over 1 km in length;
• be free of 'buildings' all year round;
• Ideally not be subject to any other litter collection activities.

After the selection of the beach, the survey beach stretch is identified and cover the whole area between the water edge to the back of the beach. OSPAR protocol uses two sampling units:

- 100-metres: all marine litter items (Fig. 11), must be part of the 1-km stretc;
- 1-km: objects generally larger than 50 cm (Fig. 11), this survey may be optional.

If more than one sampling unit occurs on a beach the minimum separation distance shall be at least 50 m.
The reference beaches are surveyed 4 times a year (winter, spring, summer and winter).
All items found on the sampling unit should be entered on the survey forms provided (Fig. 9).

OSPAR ID	Unep ID	Items	Total
Plastic • Polystyrene			
1		4/6-pack yokes	
2		Bags (e.g. shopping)	
3		Small plastic bags, e.g., freezer bags	
112		Plastic bag ends	
4		Drinks (bottles, containers and drums)	
5		Cleaner (bottles, containers and drums)	
6		Food containers incl. fast food containers	
7		Cosmetics (bottles & containers e.g. sun lotion, shampoo, shower gel, deodorant)	
8		Engine oil containers and drums <50 cm	
9		Engine oil containers and drums > 50 cm	
10		Jerry cans (square plastic containers with handle)	
11		Injection gun containers	
12		Other bottles, containers and drums	
13		Crates	
14		Car parts	
15		Caps/lids	
16		Cigarette lighters	
17		Pens	
18		Combs/hair brushes	
19		Crisp/sweet packets and lolly sticks	
20		Toys & party poppers	
21		Cups	
22		Cutlery/trays/straws	
23		Fertiliser/animal feed bags	

Fig. 9: Survey form (OSPER, 2010)

OSPAR ID	UNEP ID	Example items	Total
		Plastic • Polystyrene	
1		Buoys	
2		Fish boxes	
22		Gloves (industrial/professional gloves)	
3		Packaging, plastic sheeting	
4		Rope (diameter more than 1 cm)	
23		String and cord (diameter less than 1 cm)	
5		Jerry cans	
6		Nets and pieces of nets (including fishing nets and fishing line)	
7		Oil drums	
8		Strapping bands	
9		Other large plastic/polystyrene items *(please specify in other item box*)*	
		Metal	
10		Oil drums	
11		Other large metal items *(please specify in other item box*)*	
		Wood (machined)	
12		Crab/lobster pots	
13		Crates	
14		Pallets	
24		Fish boxes	
15		Other large wooden items *(please specify in other item box*)*	
		Rubber	
17		Tyres & belts	
18		Other large rubber items *(please specify in other item box*)*	
		Cloth	
20		Clothing and shoes	
21		Other large cloth/textile items *(please specify in other item box*)*	

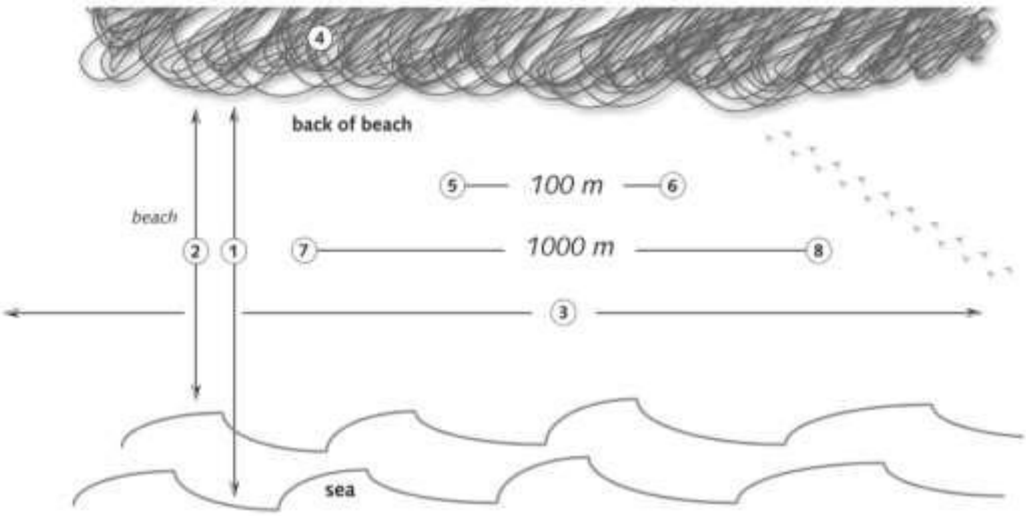
Fig. 10: Classification of the items observed in 1 km area (OSPAR, 2010)

OSPAR Marine Litter Beach Questionnaire

Name of beach: ....

OSPAR beach ID: ....

Country: .... to be filled in by national coordinators



① Beach width at mean low spring tide: .... (m) ② Beach width at mean high spring tide: .... (m)

③ Total length of beach: .... (m) ④ Back of beach (example dunes): ....

⑤ GPS coordinates start 100 m: ....

⑥ GPS coordinates end 100 m: ....

⑦ GPS coordinates start 1 km: ....

⑧ GPS coordinates end 1 km: ....

Coordinate system used: .... Date position measured: ..... / ..... / ..... (d/m/y)

Prevailing currents off the beach*: ☐ N ☐ E ☐ S ☐ W Prevailing winds*: ☐ N ☐ E ☐ S ☐ W

When you look from the beach to the sea, what direction is the beach facing*: ☐ N ☐ E ☐ S ☐ W

Type of beach material (% coverage): .... *(e.g. sand 60%, pebbles 40%)*

Beach topography: .... *(e.g. slope 20%)*

Are there any objects in the sea (e.g. a pier) that influence the currents: ....

Major beach usage (local people, swimming and sunbathing, fishing, surfing, sailing etc):

1. .... seasonal or whole year round: ....
2. .... seasonal or whole year round: ....
3. .... seasonal or whole year round: ....

Access to the beach: ☐ Vehicle ☐ Pedestrian ☐ Boats *you may tick one or two boxes

OSPAR Beach Questionnaire 2010.010

1/3 pages

Fig. 11: Extract of the beach questionnaire

3.4 NOAA, 2012. Marine Debris Shoreline Survey Field Guide, only for marine litter (macro).

The Marine Debris Shoreline Survey Field Guide illustrates the method used in USA to monitor marine litter acculumated on the beach. This shoreline protocol was developed and tested by the NOAA Marine Debris Program. The authors highligthed the differences between the two types of survey (standing stock and accumulation, Table 4)

Table 4: Salient characteristics of standing-stock and accumulation surveys.

Characterisitic	**Standing-Stock**	**Accumulation**
Debris removed during surveys?	No	Yes
Time required per survey	Less	More
Length of shoreline site	100 m	100 m or longer
Is a set survey interval required (e.g., once per week or per month)?	Yes	Yes
Types of data that can be collected	*Debris density (# of items / unit area) * Debris material types	* Debris deposition rate (# of items / unit area / unit time) * Debris material types * Debris weight

According to the authors the accumulation studies provide information on the rate of deposition of debris onto the shoreline while the standing-stock studies provide information on the amount and types of debris on the shoreline. Once the type of survey is defined, the next step consists in selecting the pilot site according to the objectives of the study.
The shoreline sites should have the following characteristics:

- Sandy beach or pebble shoreline;
- Clear, direct, year-round access;
- No breakwaters or jetties;
- At least 100 m in length parallel to the water (note that standing-stock surveys require a 100-m shoreline site);
- No regular cleanup activities.

The authors suggest that the site should be characterized before the marine litter collection by compiling a specific sheet.

Table 5: Site charaterization for marine litter collection

SHORELINE DEBRIS Shoreline Characterization Sheet	Organization		Name of organization responsible for collecting the data
	Surveyor name		Name of person responsible for filling in this sheet
	Phone number		Phone contact for surveyor
Complete this form ONCE for each site location	Date		Date of this survey
SAMPLING AREA			
Shore ID			Unique code for the shoreline
Shoreline name			Name by which the section of shoreline is known (e.g., beach name, park)
State/County			State and county where your site is located
Coordinates at start of shoreline section	Latitude	Longitude	Recorded as XXX.XXXX (decimal degrees) at start of shoreline section (in both corners if width > 6 meters)
Coordinates at end of shoreline section	Latitude	Longitude	Recorded as XXX.XXXX (decimal degrees) at end of shoreline section (in both corners if width > 6 meters)
Photo number/ID			The digital identification number(s) of photos taken of shoreline section
SHORELINE CHARACTERISTICS – from beginning of shoreline site			
Length of sample area (should be 100 m if standing-stock survey)			Length measured along the midpoint of the shoreline (in meters)
Substratum type			For example, a sandy or gravel beach
Substrate uniformity			Percent coverage of the main substrate type (%)
Tidal range			Maximum & minimum vertical tidal range. Use tide chart (usually in feet).
Tidal distance			Horizontal distance (in meters) from low- to high-tide line. Measure on beach at low and high tides or estimate based on wrack lines.

Back of shoreline			Describe landward limit (e.g., vegetation, rock wall, cliff, dunes, parking lot)
Aspect			Direction you are facing when you look out at the water (e.g., northeast)
LAND-USE CHARACTERISTICS – within shoreline location			
Location & major usage	Urban		Select one and indicate major usage (e.g., recreation, boat access, remote
	Suburban		
	Rural		
Access			Vehicular (you can drive to your site), pedestrian (must walk), isolated (need a boat or plane)
Nearest town			Name of nearest town
Nearest town distance			Distance to nearest town (miles)
Nearest town direction			Direction to nearest town (cardinal direction)
Nearest river name			If applicable, name of nearest river or stream. If blank, assumed to mean no inputs nearby
Nearest river distance			Distance to nearest river/stream (km)
Nearest river direction			Direction to nearest river/stream (cardinal direction from site)
River/creek input to beach	YES	NO	Whether nearest river/stream has an outlet within this shoreline section
Pipe or drain input	YES	NO	If there is a storm drain or channelized outlet within shoreline section
Notes (including description, landmarks, fishing activity, etc.):			

The survey can be done parallel or perpendicular to the shoreline and all the debris over 2.5 cm in the longest dimension must be counted or collected and fill a specific sheet.
The protocol for a standing-stock survey is the following:

1- Divide the 100 m shoreline survey site into 5-m segments, each section is then numbered from 1 to 20 (left to right). Each 5-m segment should run from the water's edge to the back of the shoreline
2- Then select four numbers from the Random Number Table (Table 6). These numbers correspond to the 5-m segments that will be sampled (i.e., 20% coverage of the area, Fig. 12).
3- A specific sheet is used to classify the marine litter observed during the survey.

Table 6: Random Number table

Random Number table					
	1	2	3	4	5
1	4	8	17	9	1
2	7	19	2	12	20
3	18	14	6	16	11
4	3	5	15	10	13

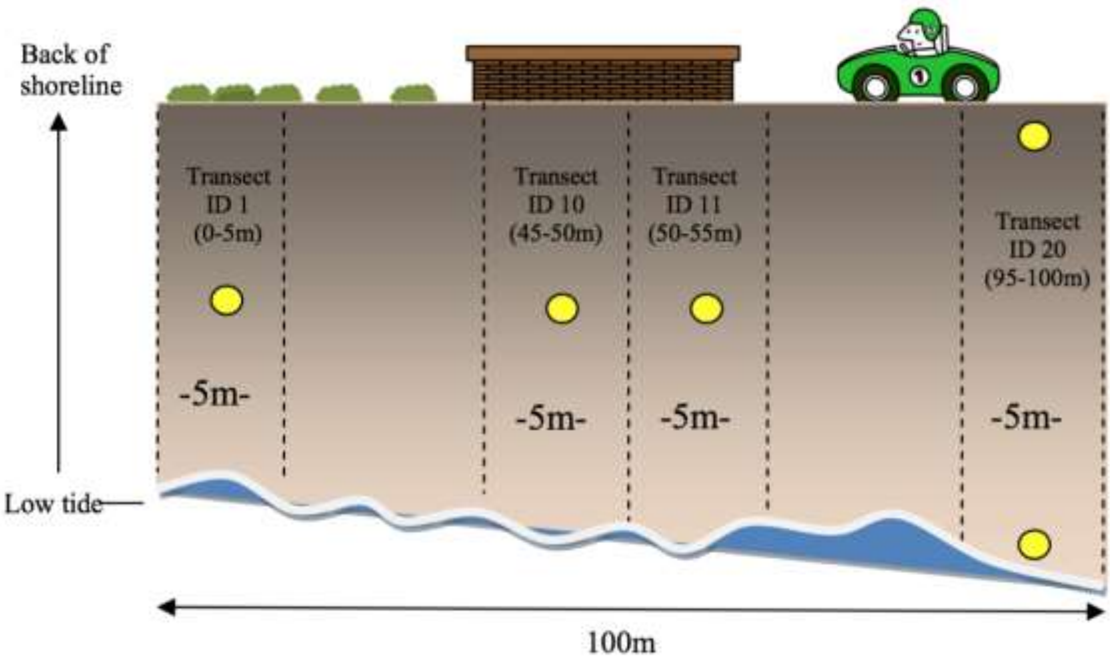


Fig. 12: Example of a shoreline section (100m) with yellow circles indicating marked GPS coordinates. Width determines location of GPS coordinates.

3.5 NOAA, 2013 – Marine Debris Program - Marine Debris Monitoring and Assessment: Recommendations for Monitoring Debris Trends in the Marine Environment.

Futher details are provided by NOAA (2013) and indicated that surveyors should walk each transect tallying debris items according to material type and subcategory. Debris items should only be recorded if they are at least 2.5 cm in size on the longest dimension (as adopted by UNEP (Cheshire et al., 2009) and MSFD (2011, 2013)) in order to ensure that the same size items are counted across surveys and to maintain consistency in survey results.

Information on large macro-debris items (> 30 cm or about 1 ft), reporting separatly, should include the debris type, the status of the large item, the coordinate, and the approximate debris size. Any item that is partially within a transect should be tallied. Multiple fragments of what may have originally been a whole item should be tallied separately.

The macro-debris item concentration (number of debris items/m^2) per transect is calculated as follows:

C= n/(w . l)

C = concentration of debris items (# of debris items/m^2)
n = # of macro-debris items observed
w = width (m) of shoreline section recorded during sampling (i.e, transect width)
l = length (m) of shoreline sampled = 5 m
The shoreline width is essential for calculating debris concentrations. For a given sampling event:

- Calculate debris concentrations for each individual transect surveyed (a minimum of four per survey).
- Take the mean of the concentrations at each transect to calculate an overall site concentration (± standard deviation) for that date.

3.6 MFSD Technical Subgroup on Marine Litter JCR, 2013. Guidance on Monitoring of Marine Litter in European Seas.

The criteria for selecting the beach are the following:

- A minimum length of 100m.
- Low to moderate slope (15 – 45°), which precludes very shallow tidal mudflat areas that may be many kilometres wide at low tide.
- Clear access to the sea.
- Accessible to survey teams year round.
- Ideally the site should not be subject to any other litter collection activities
- Survey activities should be conducted so as not to impact on any endangered or protected species.

Presently the guideline does not indicate a minimum number of site that may be representative for a certain length of coast. Four surveys per year are recommended (spring, summer, autumn and winter) but initially a higher frequency of surveys may be necessary in order to identify significant seasonal patterns. The guideline also recommends to use the Marine Litter Beach Documentation and Characterization Form of the OSPAR Marine Litter Beach Questionnaire to characterize the site survey.

At least 2 sections of 100m on the same beach are recommended for monitoring purposes on lightly to moderately littered beaches, and of 50 m for heavily littered beaches. Items present on the section should be collected or recorded and the unit in which litter is assessed can be number, weight or volume, or a combination of these units. Counts of items are recommended as the standard unit of litter to be assessed on the coastline, weight is more problematic as indicated by Jambeck & Farfour (2011). All items with a dimension superior to 2.5 cm in the longest dimension found on the sampling unit should be entered on the survey forms; the items are recorded according a unique identification number (Table 7). Unknown litter or items that are not on the survey form should be noted including a short description and eventually digital photos should be taken of unknown items.

Table 7: Master list of categories of litter items (JCR, 2013)

Master List of Categories of Litter Items											
TSG_ML General-Code	**OSPAR-Code**	**UNEP-Code**	**General Name**	**Level 1 - Materials**	**Core**	**Beach**	**Seafloor**	**Floating**	**Biota**	**Micro**
G1	1	PL05	4/6-pack yokes, six-pack rings	Artificial polymer materials	x	x				
G2		PL07	Bags	Artificial polymer materials	x		x	x		
G3	2	PL07	Shopping Bags incl. pieces	Artificial polymer materials		x				
G4	3	PL07	Small plastic bags, *e.g.* freezer bags incl. pieces	Artificial polymer materials		x				
G5	112		Plastic bag collective role; what remains from rip-off plastic bags	Artificial polymer materials		x				
G6	4	PL02	Bottles	Artificial polymer materials	x		x	x		
G7	4	PL02	Drink bottles <=0.5l	Artificial polymer materials		x				
G8	4	PL02	Drink bottles >0.5l	Artificial polymer materials		x				
G9	5	PL02	Cleaner bottles & containers	Artificial polymer materials	x	x				
G10	6	PL06	Food containers incl. fast food containers	Artificial polymer materials	x	x	x			
G11	7	PL02	Beach use related cosmetic bottles and containers, *e.g.* Sunblocks	Artificial polymer materials		x				
G12	7	PL02	Other cosmetics bottles & containers	Artificial polymer materials	x	x				
G13	12	PL02	Other bottles & containers (drums)	Artificial polymer materials	x	x				
G14	8		Engine oil bottles & containers <50 cm	Artificial polymer materials		x				
G15	9	PL03	Engine oil bottles & containers >50 cm	Artificial polymer materials		x				
G16	10	PL03	Jerry cans (square plastic containers with handle)	Artificial polymer materials		x				
G17	11		Injection gun containers	Artificial polymer materials		x				
G18	13	PL13	Crates and containers / baskets	Artificial polymer materials		x	x	x		
G19	14		Car parts	Artificial polymer materials		x				
G20		PL01	Plastic caps and lids	Artificial polymer materials			x			
G21	15	PL01	Plastic caps/lids drinks	Artificial polymer materials		x				
G22	15	PL01	Plastic caps/lids chemicals, detergents	Artificial polymer materials	x	x				

Example: Michael Prevenios, Christina Zeri, Catherine Tsangaris, Svitlana Liubartseva, Elias FakirisGeorge Papatheodorou, 2018: Beach litter dynamics on Mediterranean coasts: Distinguishing sources andpathways, Marine Pollution Bulletin, 129, 448-457

Over a period of 16 months, from July 2014 to October 2015 beach litter (≥2.5 cm) was sampled twice a month (every15 ± 5 days). All collections were done by the same person in order to achieve high degree of sampling objectivity. Three transects of 100 m long were monitored at different greek beaches. Transects were set parallelto the water line and extended to the back of the beach where vegetation or built constructions appear. Individual items were identified and categorized according to the methodology proposedby the EU MSFD TGML D.10 'Guidance on Monitoring of Marine Litterin European Seas'(Galgani et al., 2013). The protocol classifies litter in 8 marine litter types (artificial polymers, rubber, textile, paper, metal, wood, glass, unidentified) and in 213 detailed item categories each having a unique identification code (G1-G213). All litter items were counted, cleaned from any sand and weighed to the nearest 0.01 g.
Beach litter was expressed as number of items or mass per 100 m transect (N/100 m; kg/100 m).

3.7 DEFISHGEAR, 2014 http://mio-ecsde.org/wp-content/uploads/2014/12/Beach-litter_monitoring-methodology_updated_final-1.pdf

The IPA-Adriatic funded DeFishGear project aims to facilitate efforts for integrated planning to reduce the environmental impacts of litter-generating activities and ensure the sustainable management of the marine and coastal environment of the Adriatic-Ionian macroregion. The method used in the framework of this project is based on the EU MSFD TG10 "Guidance on Monitoring of Marine Litter in European Seas (2013)", the OSPAR "Guideline for Monitoring Marine Litter on the Beaches in the OSPAR Maritime Area (2010)" and the NOOA "Marine Debris Monitoring and Assessment: Recommendations for Monitoring Debris Trends in the Marine Environment (2013), taking into consideration the draft "UNEP/MAP MEDPOL Monitoring Guidance Document on Ecological Objective 10: Marine Litter (2014)".

The method includes the following steps:

- Selection of the sites to be monitored considering that should be situated in the vicinity of ports or harbors, of river mouths, of coastal urban areas; of tourism destinations; in relatively remote areas. In addition, as for the other guidelines, the selected beaches should:
  - Have a minimum length of 100m;
  - Be characterized by a low to moderate slope,
  - Have clear access to the sea;
  - Be accessible to survey teams throughout the year;
  - Ideally not be subject to cleaning activities. the timing of non-survey related beach cleaning must be known so that litter flux rates (the amount of litter accumulation per unit time) can be determined.

The sampling unit should be a 100-metre stretch of beach along the strandline and reaching to the back of the beach. Furhtermore, two sampling units (100-metre stretches) on the same beach should be monitored. They should be separated at least by a 50-metre stretch.
Sampling of items as small as 2.5 cm in the longest dimension should be bone four times a year and

shoreline characterization should be completed for each sampling unit by filling a specific sheet (Beach Identity Sheet). Items found in the sampling unit will be classified by type according to the 'Master List' and accordingly entered in the 'Beach Litter Monitoring Sheet' (Fig. 13). During the survey, all litter items should be sorted by 'category type', weighed and then removed from the beach.

ARTIFICIAL POLYMER MATERIALS			
Code	**Items name**	**Item counts**	**Total**
G1	4/6-pack yokes, six-pack rings		
G3	Shopping bags, incl. pieces		
G4	Small plastic bags, e.g. freezer bags, including pieces		
G5	Plastic bag collective roll; what remains from rip-off plastic bags		
G7	Drink bottles <=0.5l		
G8	Drink bottles >0.5l		
G9	Cleaner/cleanser bottles & containers		
G10	Food containers incl. fast food containers		
G11	Beach use related cosmetic bottles and containers, e.g. Sunblocks		
G12	Other cosmetics bottles & containers		
G13	Other bottles & containers (drums)		
G14	Engine oil bottles & containers <50 cm		
G15	Engine oil bottles & containers > 50 cm		
G16	Jerry cans (square plastic containers with handle)		
G17	Injection gun containers		
G18	Crates and containers / baskets		
G19	Car parts		
G21	Plastic caps/lids from drinks		
G22	Plastic caps/lids from chemicals, detergents (non-food)		
G23	Plastic caps/lids unidentified		
G24	Plastic rings from bottle caps/lids		
G25	Tobacco pouches / plastic cigarette box packaging		
G26	Cigarette lighters		
G27	Cigarette butts and filters		
G28	Pens and pen lids		
G29	Combs/hair brushes/sunglasses		
G30	Crisps packets/sweets wrappers		
G31	Lolly sticks		

G92	Bait containers/packaging		
G93	Cable ties		
G95	Cotton bud sticks		
G96	Sanitary towels/panty liners/backing strips		
G97	Toilet fresheners		
G98	Diapers/nappies		
G99	Syringes/needles		
G100	Medical/Pharmaceuticals containers/tubes		
G101	Dog faeces bags		
G102	Flip-flops		
G124	Other plastic/polystyrene items (identifiable)		
		Total weight (kg)	

CLOTH/TEXTILE			
Code	**Items name**	**Item counts**	**Total**
G137	Clothing / rags (clothes, hats, towels)		
G138	Shoes and sandals (e.g. leather, cloth)		
G139	Backpacks & bags		
G140	Sacking (hessian)		
G141	Carpet & furnishing		
G142	Rope, string and nets		
G143	Sails, canvas		
G144	Tampons and tampon applicators		
G145	Other textiles (incl. rags)		
		Total weight (kg)	

Fig. 13 Extract of the items master list. Note that pieces of litter that are recognizable e.g. as a shopping bag (G3) should be registered as such. Pieces of materials that are not recognizable as an item e.g. plastic and/or polystyrene pieces should be counted according to their size (G75 – G83) (DEFISGEAR 2014)

4 Microplastic in beach sediment

4.1 Method within the project Plastic Beach Project (5 Gyres Institutes, https://www.5gyres.org)

The method focuses on the presence of microplastic in beach sediment.
Within the Plastic Beach ProjectIdeally, two beaches should be selected, one in a frequented beach, the other one should be less frequented, a more “natural” or “remote” beach. Beaches can be sandy or rocky. Beaches should be clearly described and maps and/or GPS locations for each beach should be included as part of the background information for each beach.
Once the beach is selected, four transects from within a 100-meter section will be selected.
12 quadrants (1 m^2) should be identified along the 100 m beach (Fig. 14). Then, three to 12 beach quadrants should be analyzed to evaluate beach litter and plastic pollution. Transects should be placed on the wrack line, the line on the beach that represents where the last high tide reached, mid beach and back beach.
To better evaluate microplastic pollution in the area, you will first randomly select. You will then analyze four 1-square meter quadrats, three along each transect line. Therefore, three quadrants will be placed along the transect in the wrack line, mid beach and back beach.
At each Quadrant, big pieces of natural debris, like seaweed, leaves and wood are removed and then sediment from the surface evenly with a scoop or shovel is removed and put in gallon bucket that represents approximately 3cm of the surface. The sediment is then sieved through the 4.75mm and 1mm sieves. Successively the items are measured with a ruler and all fragments are recorded in a specific data sheet (number of fragments and weigth of the fragments). All the fragments are keep for further analysis.

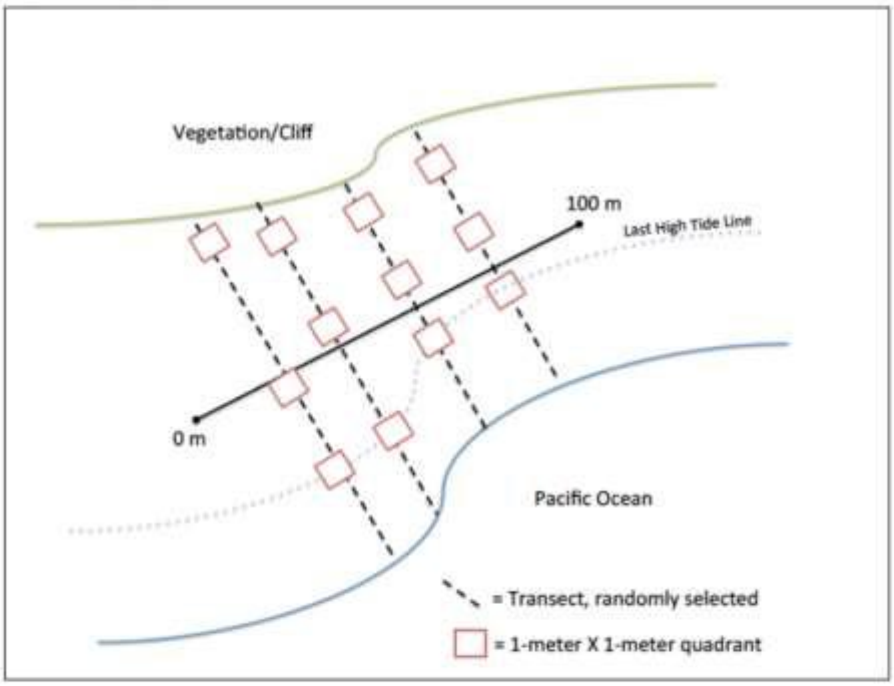


Fig. 14: Setup protocol for The 5 Gyres Institute’s Plastic Beach Project microplastic sampling methodology.

4.2 Modified methodology (Losh S., 2015)

The previous method has been modifed to develop meso- and microplastic debris survey methods that are practical for volunteers to perform on a regular basis and the volunteers only have to survey one transect, instead of four, with two quadrats, instead of three. The methods were split into two parts: debris collection and data analysis. Ideally, volunteers should "adopt" one beach to survey monthly, quarterly or biannually (winter and summer) at the very least.

Once the beach is selected, at least one random transect (but not more than four) will be surveyed, with two 1-meter by 1-meter quadrats. The quadrats will change each time the 100-meter transect is surveyed depending on where the high tide line is situated, the width of the beach, if a storm tide line is present, etc. Fig. 15 gives a general overview of what the 100-meter survey area may look like, keeping in mind only one transect must be established

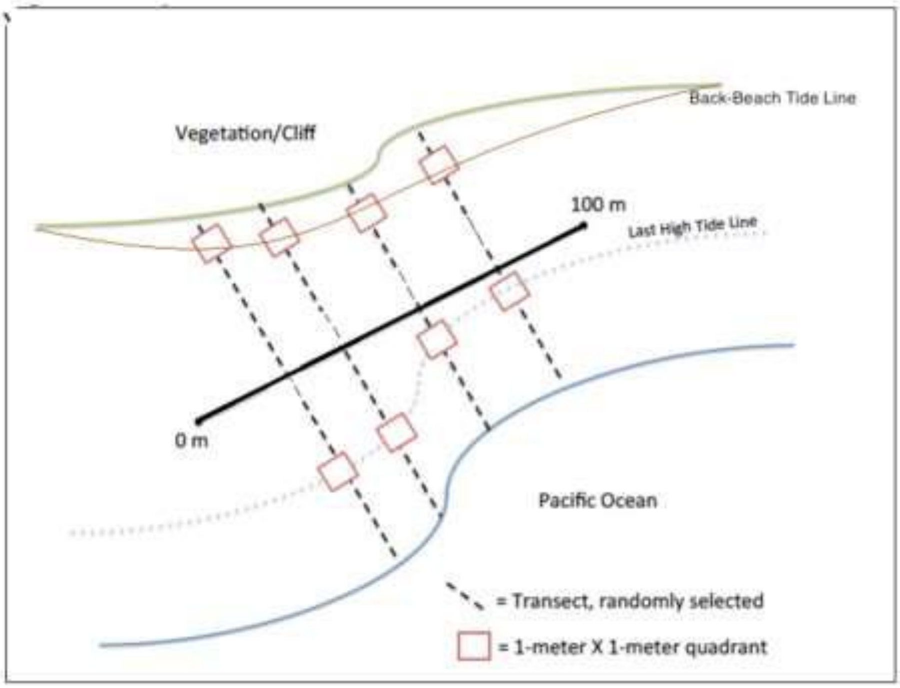


Fig. 15: Modified setup protocol for the microplastic sampling methodology

Similarly, the big pieces of debris are removed but the plastic debris should be collected to be disposed of properly. Sediment are sampled with a scoop or shovel and then sieve through the 1⁄2-inch mesh sieve over the 5- gallon bucket. The bucket should only be filled about half way. This will be approximately 3cm of the surface. Then any pieces of plastic debris are sieved through the 1⁄2-inch and 1/30 inch sieves and keep all items collected in the sieves. Successively, the empty 5-gallon bucket halfway is filled with seawater and the collected PMD from the 1/30-inch sieve are dumped into the seawater. In this way, the remaining sand sink, effectively cleaning the PMD. Other natural debris may sink as well, but any woody debris will float and will need to be separated out and the fragments are transfered into collection bags for further analysis. The material collected for each quadrant is also kept separately.

4.3 MSFD JRC, 2013. Guidance on Monitoring of Marine Litter in European Seas

Harmonised Protocol
The comparison of beach litter data between assessment programmes is the primary aim of a harmonised protocol. Comparison is difficult if different methods, different spatial and temporal scales, different size scales of litter items and different lists or categorisation of litter items recorded on beaches are used within the regional seas and the EU as a whole.
The type of survey selected depends on the objectives of the assessment and on the magnitude of the pollution on the coastline. A single survey method is recommended here – with different spatial parameters for light to moderately polluted coastline and for heavily polluted coastlines.
Amounts of litter on the shore can be relatively easily assessed during surveys carried out by non-scientists using unsophisticated equipment. Coastal surveys are thus a cost effective way of obtaining large amounts of information. The litter deposited on the coastline can vary greatly between sites and seasons, affected by hydrographical and geomorphological characteristics of the area (e.g. prevailing winds and currents, exposure of the beach to the sea) but also depending on the use of the coast (e.g. larger amounts can be deposited during the tourist season or during special events). Therefore, coastal surveys should focus on fixed sites, which fulfil the requirements of the protocol, and the timing of the survey (i.e. season) should take into account the potential sources of litter to the site (e.g. flooding in rainy seasons may increase the amounts). Sites can be placed far from known sources, in order to better reflect reference values for background litter pollution levels, or close to potential sources. By using temporal trends for assessments, both of the survey strategies give important information for managers.

4.4 Guidance on Monitoring of Marine Litter in European Seas 2013 MSFD Technical Subgroup on Marine Litter

Ideally the selected sites should represent litter abundance and composition for a given region. Not any given coastal site may be appropriate, as they may be limited in terms of accessibility, suitability to sampling (sand or rocks/boulders) and beach cleaning activities. If possible the criteria below should be used:

- A minimum length of 100m.
- Low to moderate slope (15 – 45º), which precludes very shallow tidal mudflat areas that may
- be many kilometres wide at low tide.
- Clear access to the sea (not blocked by breakwaters or jetties) such that marine litter is not screened by anthropogenic structures.
- Accessible to survey teams year round, although some consideration needs to be given to sites that are iced-in over winter and the difficulty in accessing very remote areas.
- Ideally the site should not be subject to any other litter collection activities, although it is recognized that in many parts of Europe large scale maintenance cleaning is carried out periodically; in such cases the timing of non-survey related beach cleaning must be known such that litter flux rates (the amount of litter accumulation per unit time) can be determined.
- Survey activities should be conducted so as not to impact on any endangered or protected species such as sea turtles, sea birds or shore birds, marine mammals or sensitive beach vegetation; in many cases this would exclude national parks but this may vary depending on local management arrangements.

- Within the above constraints, the location of sampling sites within each zone should be stratified such that samples are obtained from beaches subject to different litter exposures, including:
- Urban coasts, may better reflect the contribution of land-based inputs;
- Rural coasts may better reflect background values for litter pollution levels
- Coasts close to major rivers, if downstream from the prevailing drift, may better reflect the contribution of riverine input to coastal litter pollution.

Frequency and timing of surveys

At least four surveys per year in spring, summer, autumn and winter are recommended. However, because of the large seasonal variation in amounts of litter washed ashore, initially a higher frequency of surveys may be necessary in order to identify significant seasonal patterns, which can then be considered when treating raw data for long-term trend analyses.

The survey periods below are suggested:

1) Winter: Mid-December–mid-January
2) Spring: April
3) Summer: Mid-June–mid-July
4) Autumn: Mid-September–mid-October

Monitoring should start about one hour after high tide to prevent surveyors being cut off by incoming tide. If working on remote beaches it is recommended to work with a minimum of two people.

It is very important to document and characterise the survey sites. As surveys should be repeated on exactly the same site the coordinates of the site should be documented. It is strongly recommended to use the Marine Litter Beach Documentation and Characterization Form of the OSPAR Marine Litter Beach Questionnaire (OSPAR Commission 2010b).

Sampling unit

Once a beach is chosen sampling units can be identified. A sampling unit is a fixed section of beach covering the whole area between the water edges (where possible and safe) or from the strandline to the back of the beach.

- At least 2 sections of 100m on the same beach are recommended for monitoring purposes on lightly to moderately littered beaches
- At least 2 sections of 50 m for heavily littered beaches

Coordinates obtained by GPS are useful for identifying the reference beaches especially where easily identifiable landmarks are lacking.

Units (quantification) of litter

The unit in which litter is assessed on the coastline can be number, weight or volume, or a combination of these units. Counts of items are recommended as the standard unit of litter to be assessed on the coastline.

The assessment of weight of litter is problematical because it is dependent on whether litter items are wet or dry and often whether they are covered with or full of sand and gravel. Some items are even too big to be weighed and their weight must be estimated. The results of weight-based surveys and number-of-item-based surveys cannot be compared directly. Estimates of the weight of items counted could be

made if average weights of the litter items assessed are known. However, this would not be possible for all items e.g. nets, which occur on beaches in a wide range of sizes and weights.
The assessment of the volume of litter is also problematical because it depends on the level of compression of the litter involved. Measurements of litter volume are not easily reproducible and only give a rough idea of the amount of litter recorded.

Collection and identification of litter items

All items found on the sampling unit should be entered on the survey forms. Unknown litter or items that are not on the survey form should be noted in the appropriate "other item box". A short description of the item should then be included on the survey form. If possible, digital photos should be taken of unknown items so that they can be identified later and, if necessary, be added to the survey form.
It is strongly recommended to produce regional photo guides including pictures of all litter items on the regional survey protocol.

Size limits and classes of items to be surveyed

There are no upper size limits to litter recorded on beaches. A lower limit of 2.5 cm in the longest dimension is recommended for litter items monitored during beach surveys. This would ensure the inclusion of caps & lids and cigarette butts in any counts.

Removal and disposal of litter

Removal of litter should be carried out at the same time as monitoring the litter. Larger items that cannot be removed by the surveyors should be marked, with for example paint spray so they will not be counted again at the next **survey**.
Many municipalities will have their own cleaning programme, sometimes regularly, sometimes seasonal or incident related. Arrangements should be made with the local municipalities so that they either exclude the reference beach from their cleaning scheme or they provide their cleaning schedule so surveying can be carried out a few days before the municipality will clean the beach.

Microlitter

An upper size bound of 5mm has been widely adopted and for the purpose of MSFD we suggest the upper bound to be taken to as items <5mm in their largest dimension. Current definitions do not explicitly state a lower size limit and lower size limits have seldom been reported for microplastic concentrations in the environment. The lower size limit is perhaps assumed to be the mesh size of the net or sieve through which the sample passed during the sampling, sample preparation or extraction. The size limits of microplastic particles that can be reported are also dependent on the method of detection, in many cases microscope-aided visual inspection. When identifying microparticles there are also size limits imposed by the analytical techniques employed (e.g. minimum sample intake requirements for detection and analysis). Hence an important part of establishing standard methods and protocols within MSFD will first be to define the appropriate size range, and this aspect is considered in the present report.
Microplastics comprise a very heterogeneous assemblage of pieces that vary in size, shape, colour, specific density, polymer type, and other characteristics. For meaningful comparisons and to answer the specific questions and to test hypotheses through monitoring, it is important to define methodological criteria to quantify such metrics as for e.g. the abundance, distribution and composition of microplastics and to ensure sampling effort is sufficient to detect the effects of interest.

General Sampling Methods

In all four compartments (sea surface, water column, sediment and biota) we recommend quantifying microplastics in the size range 20µm to 5mm. Microplastics should be categorised according to their physical characteristics including size, shape and colour. It is also important to obtain information on polymer type, since this can help identify potential sources and pathways, which is potential monitoring goal. Microplastics should be categorised according to size with a minimum level of resolution being to allocate the material found in to size bins of approximately 100 µm (20-100 µm, 101- 200 µm, 201- 300µm etc). Ability to visually distinguish synthetic fragments from other natural and man- made particulates becomes increasingly difficult as the size of the piece under examination decreases, unless IR techniques are used.

We advocate that all particles in the range 20- 100 µm be subjected to further analysis to confirm identity (e.g. using FT-IR). For particles in the size range 0.1 -5mm we recommend that a proportion (for example 10%) of the material in each size class, up to a maximum of 50 items per year or sampling occasion whichever is the least frequent) of the items considered to be microplastics be subjected to further analysis to confirm identity (e.g. using FT-IR). This step is important in order to 1) ensure quality control of visual identification and 2) gain information on the relative abundance of different polymer types which can be used to help identify potential sources and pathways leading to the accumulation of microplastics.

Sampling intertidal sediments

We suggest that separate samples be collected to monitor each of two sizes of debris (1-5mm and 20 µm – 1mm).

Since most work to date has been from the surface of sediments our recommendation is that samples should be collected from the surface 5cm of the sediment.

Most studies have sampled at the strand line, either:

- sampling a linear extension along the strandline with a spoon and/or a trowel or
- sampling an areal extension using quadrats. Sampling units were directly related to the sampling instrument used. Studies that sampled a specific areal extension (from 0.0079 to 5 m^2) employed quadrats and corers. Other sampling units were weight (from 0.15 to 10 kg) and volume of sediment (from 0.1 to 8 L).

Our suggestion) is that a minimum of five replicate samples be collected from the strandline. Each replicate should be separated by at least 5m. Replicates can be distributed in a stratified random manner so as to be representative of an entire beach or a specific section of beach. This ultimately depends on the specific locations and questions of interest at a local scale. We suggest that power analyses be conducted to further guide the most appropriate level of replication.

Microplastics 1 – 5mm –

This should be collected as an additional entirely independent sample at each location and, in order to minimise the risk of contamination form persons undertakin

The sediment can be sampled by collecting with a metal spoon or trowel the top 5cm of sand from the area contained within a metal 50 cm x 50 cm quadrat and passing through a 1 mm metal sieve and then be stored in metal (e.g. foil) or glass containers (i.e. not stored in plastic containers). Record the volume of sediment examined.

Our recommendation ins that these be sampled using an extension of the protocol for meso debris (5-25mm) which uses a 5mm sieve to separate debris from beach sediment. This approach can be extended by including a further metal sieve of 1mm mesh to achieve volume reduction in the field. Preferably the sieves could be stacked together.

Microplastics 20 µm – 1mm –

should be collected from the top 5cm of sand using a metal spoon (suggest 15ml). Because the weight of sediment can vary considerably according to water content we suggest standardising sampling by volume and collecting approximately 250ml of sediment Microplastics can subsequently be extracted in the laboratory by density separation (see later). Sediment should be stored in metal (e.g. foil) or glass containers (i.e. not stored in plastic containers). The sample can be collected by kneeling on the strand line and collecting a series of scoops at arms-length at intervals within an arc shaped area to the front.

<u>Sampling seawater</u>

Seawater samples have mostly taken by nets, the main advantage being that large volumes of water can be sampled quickly, retaining the material of interest. Most studies from surface waters have used Neuston nets and from the water column, zooplankton nets. Another instrument, that is deployed on a global scale and that has also been used for microplastic sampling is the continuous plankton recorder (CPR).

The most relevant characteristics of the sampling nets are mesh size and the opening area of the net. Mesh sizes used for microplastic sampling range from 0.053 to 3 mm, with a majority of the studies ranging from 0.30 to 0.39 mm. The net aperture for rectangular openings of neuston nets (sea surface) ranged from 0.03 to 2.0 m^2. For circular-bongo nets (water column) the net aperture ranged from 0.79 to 1.58 m^2. The length of the net for sea surface samples has varied from 1.0 to 8.5 m, with most nets being 3.0 to 4.5 m long.

With nets it is important to deploy the trawl out of the wake zone.

At present it is not appropriate to recommend manta trawl, CPR or other methods. Each approach has advantages and disadvantages and may be preferable according to local availability / sampling opportunities, the characteristics of the area to be sampled. Our recommendation is to obtain samples from sea water and to ensure the following details are recorded to accompany each sample: type of net, aperture, mesh size (preferably 333 µm mesh, 6m length for greatest inter-comparability among sampling programmes). It is not possible to specify standard haul duration but a duration of 30 min is suggested and the duration of the trawl and the estimated water volume must be recorded. Samples from nets should be stored in glass jars taking care to rinse material as thoroughly as possible from the sides of the net using filtered sea water. Microparticles are recorded as the total quantity of such captured by the net during the period it is deployed

<u>Sampling Subtidal Sediment</u>

Material can be collected using any approach that recovers a sample of relatively undisturbed surface sediment from the sea bed (e.g. Van veen grab, multi corer, box core etc.). Once recovered onto the vessel a small sample of sediment ideally around 250 ml is recovered to best represent the location of the original 5 cm surface to sub surface of the seabed. Because the weight of sediment can vary considerably to water content we suggest standardising sampling by volume. Avoid sampling next to the edge of the apparatus to minimise risk of contamination form the equipment. The sample is transferred to a metal or glass container for subsequent density separation / FT-IR spectroscopy.

Sampling Biota for microplastics

A range of organisms including filter feeders, deposit feeders and detritivores have been shown to ingest microplastic in the laboratory. For biota it is not possible at this time to recommend specific organisms as indicator species of micro plastics. Protocols are provided indicating how biota such as birds, fish, and invertebrates can be sampled. For greatest efficiency we suggest microparticles be quantified as part of routine sampling of macro litter within biota; for example in Birds and Fish.

Laboratory analyses of samples collected in the field

Density Separation for extracting plastics from sediment

The specific density of plastic particles can vary considerably depending on the type of polymer and the manufacturing process. Density values for plastics range from 0.8 to 1.4 g cm^{-3}. These values refer to virgin resins, without taking into account the effect on density of various additives that might be included during product manufacturing or the effects of biofouling on the surface of the plastic. Typical densities for sand or other sediments are 2.65 g cm−3. This difference is exploited to separate the lighter plastic particles from the heavier sediment grains by mixing a sediment sample with a saturated solution of Sodium Chloride and shaking. After mixing, coarse sediment will rapidly settle to the bottom, while low density particles remain in suspension or float to the surface of the solution. Subsequently, the supernatant with the plastic particles can be extracted onto filter paper for further processing. Fine sediments such as silt and organic particulates such as fragments of algae and plants are likely to remain in suspension and will be separated together with any plastic present.

One limitation with this approach is that the density of some plastics (e.g. PVC) is greater than that of saturated NaCl and therefore separation of these denser polymers will be relatively poor. Other solutions of greater density have been applied for example, sodium polytungstate solution with a density of 1.4 g cm−3 tap water, Sodium Iodide solution (NaI) and Zinc Chloride (ZnCl2). Plastics that float in fresh and seawater are polystyrene in foamed form, high and low density polyethylene, and polypropylene. Polystyrene in solid form also floats in a hypersaturated saline solution. The plastics that float in sodium polytungstate solution also include flexible and rigid polyvinyl chloride (PVCs), polyethylene terephthalate (PETs), and nylon. A range of separation devices have also been developed such as the Munich Plastic Sediment Separator.

We therefore recommend extraction with Sodium Chloride as it has been most widely used, extraction apparatus is simple and widely available Sodium chloride is inexpensive and not hazardous. However, in making this recommendation we acknowledge that extraction of denser polymers will be more efficient using some of the other solutions described above.

With the Sodium Chloride separation, a known volume (normally 50 ml) of sediment is added to a separating funnel using a metal spoon and 200 ml of saturated NaCl added. A stopper is added and the mixture agitated by hand for 2 minutes, and then allowed settling for 2 minutes. The supernatant is then transferred to suction filtration via a buckner funnel and passed through 10 µm retention glass fibre filter paper. Filter papers are removed and stored in sealed petri dishes prior to examination under a microscope. The NaCl separation procedure is repeated three times with each sediment sample to ensure a high proportion of buoyant debris is removed data form the three filter papers are added together.

Subtidal sediments are typically finer than those from sandy beaches and so may be likely to clog filter papers and produce a relatively thick layer of fine natural particulates. This problem can be reduced by repeatedly filtering smaller volumes of sediment on and then pooling data form each separation. We recommend using a concentrated saline NaCl solution (1.2 g cm−3) to achieve bulk separation according

to density. This is inexpensive, readily available, non-toxic has been most widely used to date and will achieve good separation for most polymers.
Filter papers can then be examined sealed within the petri dishes under a binocular microscope. The abundance of any pieces of unnatural appearance (due to colour, shape, dimensions) is recorded. Positions can be marked on the top of the petri dish lid to facilitate relocation / removal.
Separation from seawater (e.g. suspended material and seawater retained form plankton nets) –
Samples in seawater can be passed through a 500 µm sieve, and liquid passing through the sieve then filtered through 10 µm retention glass fibre filter paper using a Buckner funnel. Filter papers can then be examined under a dissecting microscope as for intertidal sediment. Sample on CPR silk filter screens can be examined directly under the dissection microscope.

<u>Recommended methods for sampling microplastics</u>
Our recommendation is that microplastics should be monitored on the top of the shore (strand line) and where available on sandy shores (0.1 – 0.0125 mm sediment diameter). Samples should be collected from the surface 5cm of the sediment surface. This will maximise the potential for comparison between regions. Our recommendation is that five replicate samples be collected from the strandline at each site. Each replicate should be separated by at least 5m. Replicates can be distributed in a stratified random manner so as to be representative of an entire beach or a specific section of beach.
Microplastics 1 – 5mm
These should be collected as an additional entirely independent sample at each location sand should be obtained AFTER the sampling the smaller size fraction (<1 mm see below) in order to minimise the risk of contamination form persons undertaking the sampling itself. The sediment can be sampled by collecting with a metal spoon or trowel the top 5cm of sand from the area contained within a metal 50 cm x 50 cm quadrat and passing through a 1 mm metal sieve and then be stored in metal (e.g. foil) or glass containers (i.e. not plastic). Record the volume of sediment examined.
Our recommendation is that these be sampled using an extension of the protocol for meso debris (5-25mm) which uses a 5mm sieve to separate debris from sediment. This protocol can easily be extended by including a second metal sieve of 1mm mesh to achieve volume reduction of the sediment sample in the field. Preferably these sieves could be stacked together.
Microplastics 20 µm – 1mm
need to be collected as a bulk sample of sediment and subsequently extracted in the laboratory by density separation (see later). Sediment should be collected from the top 5 cm of sand using a metal spoon (suggest 15 ml) and then be stored in metal (e.g. foil) or glass containers (i.e. not plastic). Because the weight of sediment can vary considerably according to water content and type of sediment we suggest standardising sampling by volume. Approximately 250 ml of sediment should be collected of 50 ml will normally be sufficient for density separation. The weight used for the density separation should also be recorded so that the quantity of debris per gram can be determined approximately if required. The sample can be collected by kneeling on the strand line and collecting a series of scoops at arms-length at intervals within an arc shaped area to the front.

<u>Recommendations for sampling surface waters</u>
Determine number of microplastics per m^3 of seawater?
Deploy the net from the vessel out of the wake zone. Maintain a steady linear course at a constant speed. The hi-speed trawl can be deployed up to 8 knots, build up the speed slowly towards maximum speed. Higher speeds reduce the ability to sieve seawater, creating a bow wake in front of the trawl. The net can

jerk forcefully as it surfs and ploughs through the waves, so watch the net while you trawl to observe its performance and adjust speed accordingly. Begin with a half hour trawl. Use your judgment on duration based on your field observations and allowed trawling time e.g.: deploy the trawl when leaving a station and trawl up to the next station. Recover and secure trawl on the deck. Record STOP immediately and note down the values on the flow meter.

In order to process the sample for storage - rinse the net from the outside with a hose or bucket to concentrate the sample in the cod end. Never rinse the sample through the opening of the net.

- You will need a large bowl, squirt bottles, sample container, spoon, tweezers, and a preservative (isopropyl alcohol or formalin).
- Remove the cod end over a bucket, as a precaution to catch any spillage
- Transfer sample into a large bowl.
- Invert the cod end and wash it out from the outside using very little water, scrape left over
- sample into the large bowl using the spoon. Rinse the spoon into the bowl.
- Pour entire sample into the sample container and add preservative. A sample may consist out

of several containers.

Label the lid and outside of the sample container with the trawl number, date and time. Use waterproof marker for labels. Include a waterproof label in the sample. This label contains the same information as the external labels.

Sample Preparation:

- Drain sample through 5 mm sieve into one large bowl.
- Use fresh water wash bottle to rinse off plastic particles adhering to the inside of the sample
- jar.
- Rinse sample inside sieve in order to separate plastics thoroughly.
- Transfer each size class to a different large Petri dish.
- Rinse equipment gently with the wash bottle so that no plastic particles are left behind.
- If the process above does not result in adequate liquid in the Petri dishes for sorting, then add sufficient water to float all plastic bits – do not overfill

NOTE: If the sample is too large to perform the procedure above for the entire sample, then split carefully, sort separately, and combine the data later.

Separating sample into size classes >5mm and <5mm:

- Place each Petri dish under a microscope.
- Using forceps, remove all recognizable pieces of floating plastic.
- Rinse off plastic bits with fresh water wash bottle to make sure smaller particles or plankton
- are not sticking to them.
- Place rinsed bits of plastic in a separate labelled empty vial and set aside for later drying, typing, counting and weighing.

For size class <5mm, use a spoon to remove all remaining plastic. There may be more there, so start looking at centre of Petri dish and move out to the sides. Use a dissecting microscope to conduct a more thorough check of the sample. Once the plastic, plankton and organic debris are separated, the plastic is size classed and dried. The wet weight of the plankton and organic debris are measured and then dried.

Drying of separated plastic:

- Set your drying oven at 20°C.
- Sieve sample and spread onto Petri dishes or leave in sieves.
- Place sample in oven or a secure dry location.

- Dry samples at 20° for about 30 minutes. If the samples are still wet after 30 minutes, leave them in the oven and check regularly. If they are left in a dry location, then check every few hours.

When the sample comes out of the oven it is placed in a dissector to cool, then weighed.

Sorting plastic to determine type, count and weight:

- With each size class dried in its own Petri dish or sieve, use forceps to sort sample into
- different types of plastic as categorized on the data sheet (see below).
- Count number of plastics for each type for each size category.
- Tare the scale with Petri dish and weigh sample on a gram scale.
- Record weight and count on the data sheet
- Transfer sorted and weighed plastic to labelled vials.

The plastic is removed from the sieves and each of the six size classes is sorted into shape type (fragment, pellet, line, film, and foam). The colour of each piece of plastic is also recorded (by size class) on a separate sheet. During this process each container is labelled and all data sheets are updated.

Precautions to minimise contamination (field) - Since the majority of microparticles is plastic care should be taken to avoid use of plastic during the protocol. Metal equipment should be used and should be cleaned prior to sampling and wrapped. Samples should be collected and stored in metal or glass containers. People undertaking the sampling should minimise any synthetic clothing and avoid wearing garments that are likely to shed synthetic fibres (such as fleece). Position for those undertaking sampling down-wind of the sampling apparatus during deployment and recovery. Prior to use equipment can be swabbed with damp filter papers which are sealed in petri dishes and checked for contamination.

Meta data –record: date, mesh size, aperture size, type of net, depth (preferably either at the sea surface or within surface 10m for greatest inter-comparability among sampling programmes) distance towed, location of tow (in / out of water) volume of water filtered (this is best obtained from a current meter as this will allow for tidal movement as well as ship speed). Also prevailing weather conditions and sea state, together with any relevant information on the volume of plankton or other particulates sampled, for example if there is concern that the net may have become clogged due to high concentration of plankton, this must be recorded.

Required reporting units – items/ m^3 of water, size, colour and shape, etc. If FT-IR or Raman is used then polymer type should also be recorded. Microplastics should be categorised according to size with a minimum level of resolution being to allocate the material found in to size bins of 100 µm (20-100 µm, 101-200 µm, 201- 300µm etc.).

<u>Recommendations for sampling Subtidal Sediments</u>

Determine number of microplastics per cm3 of sediment from the seabed

Material can be collected using any approach that recovers a sample of relatively undisturbed surface sediment from the sea bed (e.g. van veen grab, multi corer, box core etc.). Once recovered onto the vessel a small sample of sediment ideally around 250ml is recovered to best represent the location of the original 5cm surface to sub surface of the seabed. Because the weight of sediment can vary considerably to water content we suggest standardising sampling by volume. Avoid sampling next to the edge of the apparatus to minimise risk of contamination form the equipment (e.g. pain flakes other contamination on the grab / core). The sample is transferred to a metal or glass container for subsequent density separation / spectroscopy.

Meta data – Date, location, depth, sea state, type of equipment used, volume of sample collected, any relevant information e.g. complete quantitative sample, or some material lost during recovery etc. nature of sea bed sediment including particle size, organic matter, any available data on biota present.

4.5 UNEP, 2016. Marine plastic debris and microplastics – Global lessons and research to inspire action and guide policy change

The following document "**Marine plastic debris and microplastics – Global lessons and research to inspire action and guide policy change" (UNEP, United Nations Environment Programme, Nairobi 2016**) reports a synthesis of some methods used form sampling marine plastic debris and microplastics in different environments,

For **rivers,** they indicate that surface sampling of microplastics stationary or towed nets have been used. An underwater pump has also been used to collect water which is then passed through a net (van der Wal et al. 2015). Bottom nets designed for fishing (Mirrit et al. 2014) has been used for river bed sampling. In addition, floating booms may also serve as litter traps.

Shorelines
Sampling for microplastics on shorelines usually consists of passing sediment samples through a sieve, either in-situ or in a laboratory. A wide range of sampling techniques are used for monitoring microplastics in sediments (reviewed in (Hidalgo-Ruz et al. 2012, van Cauwenberghe et al. 2015, Rocha-Santos and Duarte 2015). These methods include density separation, filtration and/or sieving Hidalgo-Ruz et al. 2012, Rocha-Santos & Duarte, 2015). To facilitate the plastic extraction among organic components such as organic debris (shell fragments, small organ-isms, algae or sea grasses, etc.), solutions can be applied to selectively digest and remove the organic material (Galgani et al. 2011, Hidalgo-Ruz et al. 2012, Cole et al. 2014) such as for water samples.

Upper ocean

Sampling microplastics

Microplastics are usually sampled using towed nets, originally designed for sampling plankton. Manta trawls are commonly used for surface sampling and Bongo nets for mid-water. Mesh sizes may vary (0.053 – 3 mm) but most surveys use a 330 µm mesh. Particles below this size are captured but are under-represented. Net apertures vary from 0.03 to 2 m^2, depending on the type and shape. Smaller mesh sizes result in increased net resistance and clogging, resulting in under-sampling and potentially ripping. Results are usually reported in number of items or mass of items m^{-2} or m^{-3}. More recently some researchers have started to use on-board filtration of seawater (Desforges et al. 2014).

Long-term data from Continuous Plankton Recorders (CPRs), sampling on regular and fixed routes, have also been used to determine relative microplastic abundance. The CPR samples the water column at about 10m depth, using 280 µm mesh (not directly comparable with net data).

4.6 Frias et al., (2018). Standardised protocol for monitoring microplastics in sediments. JPI-Oceans BASEMAN project.

The protocol proposed by Frias et al. (2018) regards the presence of microplastics in sediments and in particular in intertidal and subtidal sediments. Microplastic is defined as any synthetic, solid particle or polymeric matrix which is insoluble in water, with a size range from 1 µm to 5 mm3, of either primary or secondary origin. But for monitoring purposes the lower size limit has been set at 100 µm.

Intertidal sediments

Because the beaches are dynamic sysetms, the authors suggest that monitoring surveys should be realized once per season (spring, summer, autumn and winter). The area of sampling is 100 m wide, parallel to the shoreline and record the GPS position. The sampling area (Fig. 16) extends from the shoreline (low tide) to the above the strand line (accumulation zone).

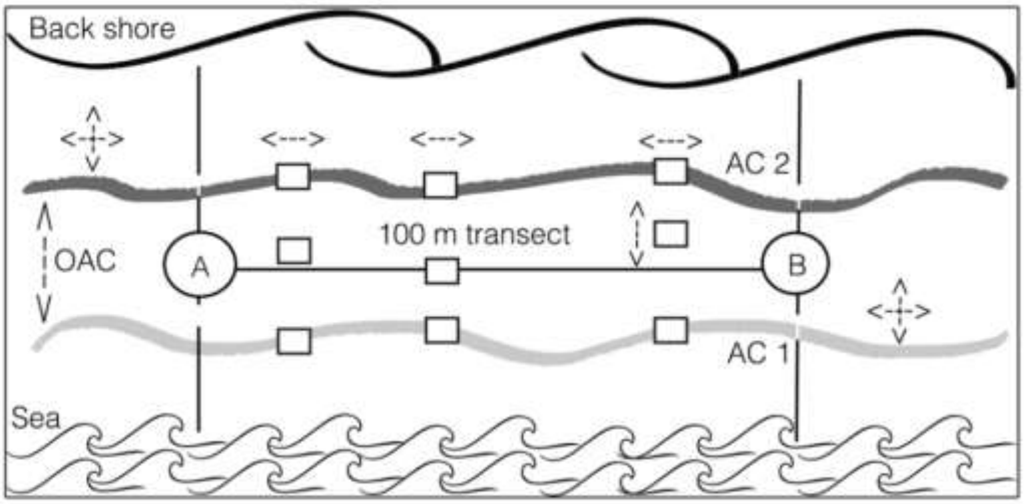


Fig. 16: beach JPI: Example of 100m transect (adapted from OSPAR, 2010 and NOAA, 2013) (AC – accumulation area, OAC – outside accumulation area)

A minimum of 3 samples (represented by square areas in Figure beachJPI) will be collected along a transect in each high tide line. The sampling unit is 30 x 30 cm and the top 5 cm (total volume of approximately 4,500 cm3 = 4.5 L) of sediment using a metal shovel or similar is collected and stored for laboratory analysis. This method will allow to estimate concentration of microplastics both horizontally and vertically, allowing collected data to be compared with a wide range of studies. During the field survey, a specific sheet describing the beach should be filled including information on beach cleaning operations.

Subtidal sediment

The sampling of sediment for the analysis of microplastic in subtidal sediments needs specific tools, which are: grab samplers, dredge samplers, core samplers and remotely operated vehicles.

The two most common benthic samplers are Grabs and Corers, and the most common tools are, respectively the van Veen grab and the Box Corer. The authors recommend the use of a box corer sampler (e.g. Reineck box corer), as this sampling tool has minimal impact in surface deformation and maintains the sediment integrity. In addition, this method allows an easier calculation of the volume of sediment collected.

The sampling requires to collect at least 6 samples per site from different depths and sediment types, to allow comparison among studies. On retrieval of the benthic sampler it is possible to subsample the van Veen grab or box core using a metal corer or the surface sediments to 5 cm depth is collected. The samples or subsamples need then to store into labelled glass jars.If samples are not being processed immediately it is suggested that they are frozen and stored at -20ºC until further processing. It is advised to collect associated environmental data to the sediment sampling by compiling a specific sheet.

5 Microplastic in water

5.1 **Protocol for Microplastics Sampling on the Sea Surface and Sample Analysis** (Kovač Viršek et al., 2016)

The authors present the following methodology for

1. Sampling of microplastics on the sea surface

Deploy the manta net from the side of the vessel using a spinnaker boom or »A-frame« using lines and karabiners.

Deploy the manta net out of the wake zone (approx. 3 - 4 m distance from the boat) in order to prevent collecting water affected by turbulence inside the wake zone.

Write down the initial GPS coordinates and initial time in the data sheet.

Start to move in one straight direction with a speed of approx. 2 - 3 knots for 30 min and begin the time measurement.

After 30 min stop the boat and write down final GPS coordinates, the length of the route (the most correct way is to calculate the length from the GPS coordinates) and the average boat speed into the data sheet provided and lift the manta net out of the water.

Rinse the manta net thoroughly from the outside of the net with seawater using a submersible pump or water from the boat water reservoir. Rinse in the direction from the manta mouth to the cod end in order to concentrate all particles adhered to the net into the cod end. Note: Never rinse the sample through the opening of the net in order to prevent contamination.

Safely remove the cod end and sieve the sample in the cod end through a 300 µm mesh size sieve or less.

Rinse the cod end thoroughly from the outside and pour the rest of the sample through the sieve. Repeat this step until there are no longer any particles inside the cod end.

Concentrate all material on the sieve in one part of the sieve.

With the use of a funnel, rinse the sieve into a glass jar or plastic bottle using 70 % ethanol.

Close the bottle, wipe it with paper towels and label the lid and outside of the jar with the sample name and date with waterproof marker (you should also put a second label written with a pencil on velum paper in a jar to avoid the possible loss of the sample name due to the erased label on the jar). Transfer labeled plastic bottle into the cool box.Note to general sampling conditions: The wind speed should not be more than 2 Beaufort, since the waves are too high and the net is not stable on the sea surface. It is important to maintain a steady linear course at a constant speed during the trawls. Half of the manta net opening should be submersed during sampling. Duration of sampling should be 30 min (in cases where there is a large amount of natural material, e.g. plankton bloom, the duration of sampling can be shorter). Avoid the use of plastic tools and containers. Avoid synthetic clothing (e.g. fleece), ropes and contact of manta net with vessel to prevent contamination of the sample. Be very careful not to damage the manta net or the boat hull while deploying and capturing the net.

5.2 Gago et al., (2018). Standardised protocol for monitoring microplastics in seawater. JPI-Oceans BASEMANproject.

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.

The authors report that polymers have different buoyancies and some microplastics are positively buoyant, which allows them to float and travel large distances from their origin. The method presented focus to microplastics sampling at sea either in the sea surface, sub-surface or water column.

<u>nets</u>

Different nets can be used for sampling microplastics but the most common devices 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: first 15-25 cm for the manta while the Neuston net is generally larger (generally slightly less than 50 cm). Another sampling method is the AVANI trawl 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.

With the net, large volume of waster can be sampled and filtered according the net mesh size that ranges from 53 to 3000 µm (commonly 300 to 390 µm, Hidalgo-Ruz et al., 2012). Ideally, the device should be deployed from the side of the vessel during 20 minutes (between 10 and 60 minutes due to different in-situ factors, as well as mesh size of the net). GPS position needs to be recordied

After each sampling event, the whole net must be rinsed carefully.

Larger plastic debris and items are picked out and rinsed in the same way. 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 -20°C until subsequent analysis.

<u>Surface and sub-surface bulk water sampling</u>

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, Anodiscs2 or glass microfiber membranes using a vacuum pump.
- Filters are then placed into labelled Petri dishes until further processing.

Barrows et al., 2017 have described alternative methods such as bulk water sampling. This method is suitable for sampling microfibers from the water surface. Samples are taken on the downwind side of the boat in the top 45 cm of the water. 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. The disadvantage of this method is the low volume of water collected.

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.

5.3 UNEP/IOC 2009 Guidelines on Survey and Monitoring of Marine Litter (Cheshire et al., 2009)

The guidelines of UNEP aim to:

- Quantify and characterize marine litter to understand the effectiveness of management/mitigation strategies;
- Understand the level of threat posed by marine litter to biota and ecosystems;

➢ Provide comparable datasets to support national, regional and global assessments of marine litter.

UNEP reports that two fundamentally different approaches to floating litter sampling are available:

➢ Trawl surveys where litter floating at the surface is collected; and
➢ Remote observation surveys where floating litter is assessed without being colleced.

5.3.1 Floating litter trawl survey operational guidelines

UNEP highlight that floating litter protocols need to include the definition and specification of the survey location, choice of sampling units, methodology for collection, classification and quantification of litter and a process for data integration, analysis and reporting of results. Furthermore, particular attention should be taken when using trawl equipment that may impact the marine environment

Litter collected during trawl operations should be categorised using the standardized litter classification system, while for remote visual assessments UNEP proposed a simplified list.

Site selection for trawl operation should be selected in an area where marine litter accumulate without damadging enadegered or protected species.

Sampling units should be stratified relative to sources within a region such that samples are obtained from:

➢ Urban coasts (terrestrial inputs).
➢ Rural coasts (oceanic inputs).
➢ Within close distance to major riverine inputs.
➢ Offshore areas (major currents, shipping lanes, fisheries areas, etc.).

The sampling unit is 5 km × 5 km survey area (Fig. 17), which should be divided into twentyfive sub-blocks of 1 km × 1 km. To ensure an unbiased sample a group of 3 sub-blocks should be randomly selected for trawling. Then the trawl operations should be conducted at least once a year eventually at the same time to beach survey sites such that:

➢ Ship speed should be restricted to 3-4 knots;
➢ Each sub-block should be trawled using five parallel trawl shots up to 800 m long;
➢ Trawl shots should be separated by a minimum of 200 m.

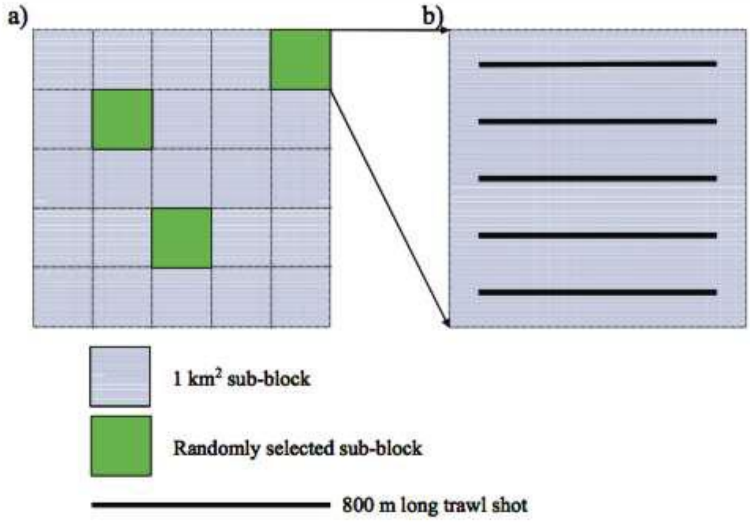


Fig. 17: Floating UNEP, (2009)

Data should be reported per unit length trawled and the width of the trawl net needs to be incorporated to provide a measurement of area of sea surface trawled. The data are expressed in kg of litter per square metre of sea surface. Furhtermore, during the operations different sheets should be filled to characterize the site (Fig. 18, UNEP), the litter (Fig. 19, UNEP). A specific sheet is used for describing the items that cannot be removed (Fig. 20, UNEP).

FLOATING LITTER Site Data Sheet FL01	Organization		Name of the organization responsible for collecting the data
	Surveyor Name		Name of the surveyor (person responsible for filling in this sheet
	Contact		Phone contact for surveyor
Complete **ONCE** at each site	Date		Collection date for this data
SAMPLING AREA			
LocationID			Unique code for the location (office use only)
Site name			Name by which the site is commonly known
Region name			Name for the region (office use only)
LME			Name for the LME in which the Site is located (office use only)
Latitude/Longitude corner 1			As nnn.nnnnn degrees NSEW at one corner of the site
Latitude/Longitude corner opp.			As nnn.nnnnn degrees NSEW at the diagonally opposite corner of site
Co-ordinate system			Datum and coordinate system used to record latitude and longitude
SITE CHARACERISTICS			
Prevailing wind			Direction of prevailing wind (degrees)
Depth			Average depth of the site (metres)
SOURCE CHARACTERISTICS – POTENIAL DEBRIS INPUTS			
Nearest river name			Name of nearest river (if relevant) – null value means no inputs
Nearest river distance			Distance to the nearest natural input (river or stream) (kilometres)
Nearest river direction			Direction to the nearest river or stream (degrees)
Nearest major fishery			Name of the nearest major fishery (named by type)
Nearest major fishery distance			Distance to the nearest major fishery (kilometres)
Nearest major fishery direction			Direction to the nearest major fishery (degrees)
Nearest town			Name of nearest town
Nearest town distance			Distance to the nearest town (kilometres)
Nearest town direction			Direction to the nearest town (degrees)
Distance to nearest coast			Distance to the closest coastline (kilometres)
Direction to nearest coast			Direction to the closest coastline (degrees)
Notes			

Fig. 18: UNEP 2009: Floating litter assessment – site characterization data sheet

FLOATING LITTER **Trawl Litter Data Sheet** **FL02**	Organization		Name of the organization responsible for collecting the data
	Surveyor Name		
	Contact		Phone contact for surveyor
To be completed once for **EACH** trawl	Date		
	LocationID		Unique code for the location (office use only)

VESSEL AND GEAR CHARACTERISTICS

Vessel name			Name of the vessel
Vessel Length and tonnage			Length of the vessel (metres) Gross tonnage of the vessel (tonnes)
Trawl gear/net details			Grapple, net mesh, net dimensions, etc
Gear anchoring point			Stern or beam/height above water
Distance behind vessel			Distance behind vessel the trawl operates (m)
Depth			Maximum depth at the site (m)

TRAWL SHOT DETAILS

Site sub-block (numbered 1-25)			Sub-blocks are numbered from 1-25 starting at the NE corner and running E-W and progressing N-S
Latitude/Longitude start			Recorded as nnn.nnnnn degrees at the start of the sample unit
Latitude/Longitude end			Recorded as nnn.nnnnn degrees at the end of the sample unit
Co-ordinate system			Datum and coordinate system employed
Distance covered			Total distance covered by the trawl shot (m)

OBSERVATION DETAILS

Time start/end			Time over which the survey was undertaken
Current seas			Wave and swell height (metres)
Current wind			Estimate wind speed & direction at sample start (km/hr & degrees)

LITTER DATA (continues over page)

Item code (standard	Description	Count (# items)	Weight (kg)	Item code (standard list)	Description	Count (# items)	Weight (kg)

Fig. 19: UNEP. Floating litter assessment – trawl litter data sheet

MARINE LITTER **Large Items Data Sheet** **ML01**	Organization		Name of the organization responsible for collecting the data
	Surveyor Name		Name of the surveyor (person responsible for filling in this sheet
	Contact		Phone contact for surveyor
Use only for items that were not collected. Complete survey data at top of form and then ONE row for EACH ITEM. Use additional forms if required.	Date		Collection date for this data
	Region name		Name for the region
	LocationID		Unique code for the location
	Coordinate system		Used for all GPS data on this page – provide datum and format

LARGE ITEM DESCRIPTION

Item type (If possible use standard codes)	Status (floating, sunken, stranded, buried)	Latitude (nnn.nnnnn NS)	Longitude (nnn.nnnnn EW)	Description

Fig. 20: Floating litter assessment – large items not removed data sheet

Equipment needs and operational logistics for trawl surveys are extensive and highly specialised but generally requires:

- Configuration of trawl equipment - typically nets may be 2-4 cm mesh size and up to 6 m wide;
- Rope length depending on the size of the trawl net and net spread;
- Floating litter should be taken on board with proper equipment;

Litter can be sorted directly on board and after collection and sorting, facilities are required to safely count and weigh litter.

5.3.2 Floating litter visual survey operational guidelines

Visual survey sites are chosen in the same way as for trawl survey (area where marine litter accumulate, no impacy on species and in specific zones):

The basic sampling unit for a visual survey will comprise a transect represented by an imaginary line over the surface of the ocean which is either travelled by a vessel or aircraft. The observer will record all litter within a fixed distance on one or both sides of the line. The width of the field of view should be recorded along with data about the distance travelled and the litter observed. Visual Transects should be established by monitoring the time employed by observers rather than attempting to identify fixed length units.

A minimum distance between transects of 1 km should prevent overlap and the minimum sampling frequency for any site should be annually. Similarly two sheets should be filled (Fig. 21).

FLOATING LITTER **Visual Observation Data Sheet** **FL03**	Organization		Name of the organization responsible for collecting the data
	Surveyor Name		Name of the surveyor (person responsible for filling in this sheet
	Contact		Phone contact for surveyor
To be completed once for **EACH** trawl	Date		Collection date for this data
	LocationID		Unique code for the location (office use only)

VESSEL AND GEAR CHARACTERISTICS

Vessel name			Name of the vessel (or plane registration)
Vessel length and tonnage			Length of the vessel (metres) Gross tonnage of the vessel (tonnes)
Depth			Maximum depth at the site (m)

VISUAL SURVEY TRANSECT DETAILS

Latitude/Longitude start			Recorded as nnn.nnnnn degrees at the start of the sample unit
Latitude/Longitude end			Recorded as nnn.nnnnn degrees at the end of the sample unit
Width of assessment			Total width of transect (including both sides of transect line) (m)
Co-ordinate system			Datum and coordinate system employed
Distance covered			Total distance covered by the trawl shot (m)

OBSERVATION DETAILS

Time start/end			Time over which the survey was undertaken
Current seas			Wave and swell height (metres)
Current wind			Estimate wind speed & direction at sample start (km/hr & degrees)

LITTER DATA (continues over page)

Item code (standard list)	Description	Count (# items)	Item code (standard list)	Description	Count (# items)

Fig. 21: UNEP 2009 Floating litter assessment – large items data sheet

5.4 NOAA, 2013 – Marine Debris Program - Marine Debris Monitoring and Assessment: Recommendations for Monitoring Debris Trends in the Marine Environment.

5.4.1 Floating debris survey techniques

The method proposed by NOAA is based from published literature and was tested in a pilot sampling effort.

5.4.1.1 Site selection

The authors report that the method follows a regional perspective and additional considerations for offshore sampling include oceanographic conditions; known currents, eddies, convergence patterns, mixing, and seasonal fluctuations; known or potential sources of marine debris; shipping lanes; and the bathymetry and geomorphic structures that may influence the generation and eventual fate of floating debris.

To provide a statistically robust dataset, selected sites for coastal surface water sampling should be stratified based on appropriate parameters, for example land use associated with nearby shorelines, fishing activities, or storm water or sewage outfalls. Random site selection from each stratum is a useful tool to assess temporal and spatial variability while controlling for some of the expected variability and reducing sampling error.

5.4.1.2 Sample Number and Frequency

Once location is determined, at least ten transects are identified, mapped and randomly numbered. Three numbers are selected from a random number table to determine which transects are evaluated on a sampling event. At least three transects should be completed within two nautical miles parallel to the adjacent shoreline site and within one nautical mile perpendicular to the shore (Fig. 22). It is suggested that surveyors pair the surface water sampling frequency with adjacent shoreline assessments. And, where possible, groups are encouraged to conduct surveys in conjunction with ongoing marine research and/or water quality assessments.

Before completing floating debris surveys, shoreline characterization is completed for each 100 m site. For surveys of coastal waters adjacent to shoreline sites, current bathymetric maps should be obtained for the area within two nautical miles of the chosen shoreline site. Several potential sites for trawls are chosen based on ease of access and strata described in the survey design section.

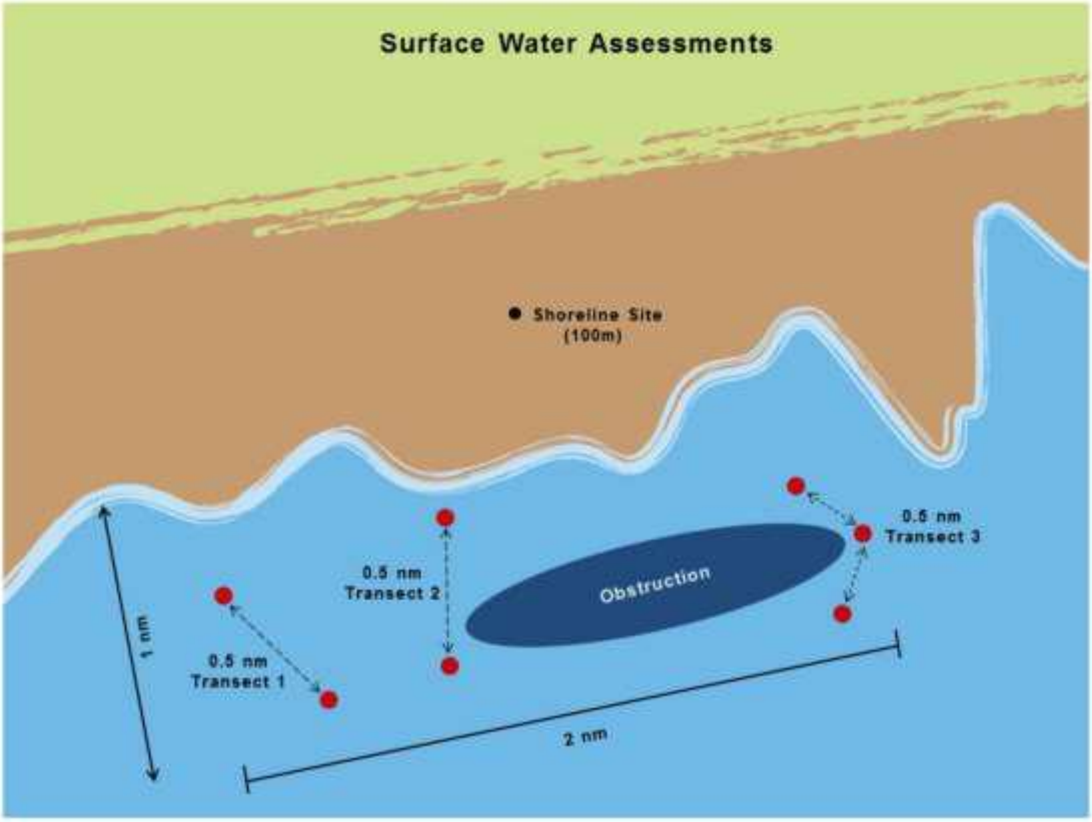


Fig. 22: Shoreline and pelagic sampling should be coordinated so that the pelagic trawl transects occur within two nautical miles of the shoreline assessment sites. Three trawls, each approximately 0.5 nm, will be conducted at each site.

5.4.1.3 Surface Water Trawl Survey Methodology (> 0.30 mm)

Trawling technique

All transects follow the same trawling technique. A manta net, with a body composed of 0.330 mm nylon mesh and measuring approximately 3 m in length, is towed horizontally at the surface (Fig. 23). Depending on sea state, weights are added to the bridle to ensure balanced positioning and coverage of the surface waters, or weights may be added to a tow line that connects the bridle to the winch line. A digital or analog flowmeter is attached to the net frame and suspended in the center of the net mouth.

The net is deployed from the back or the side of the vessel, with enough slack to allow the net to smoothly skim the surface of the water and avoid the vessel's wake. The trawl is deployed for approximately 0.5 nautical miles at a speed of 1-3 knots, an approximately 15 minuts duration. Information on the survey should be detailed on a large debris data sheet as well as GPS coordinates. If obstructions are present in the area and require alteration of the original transect, GPS coordinates should be recorded when the vessel changes heading.

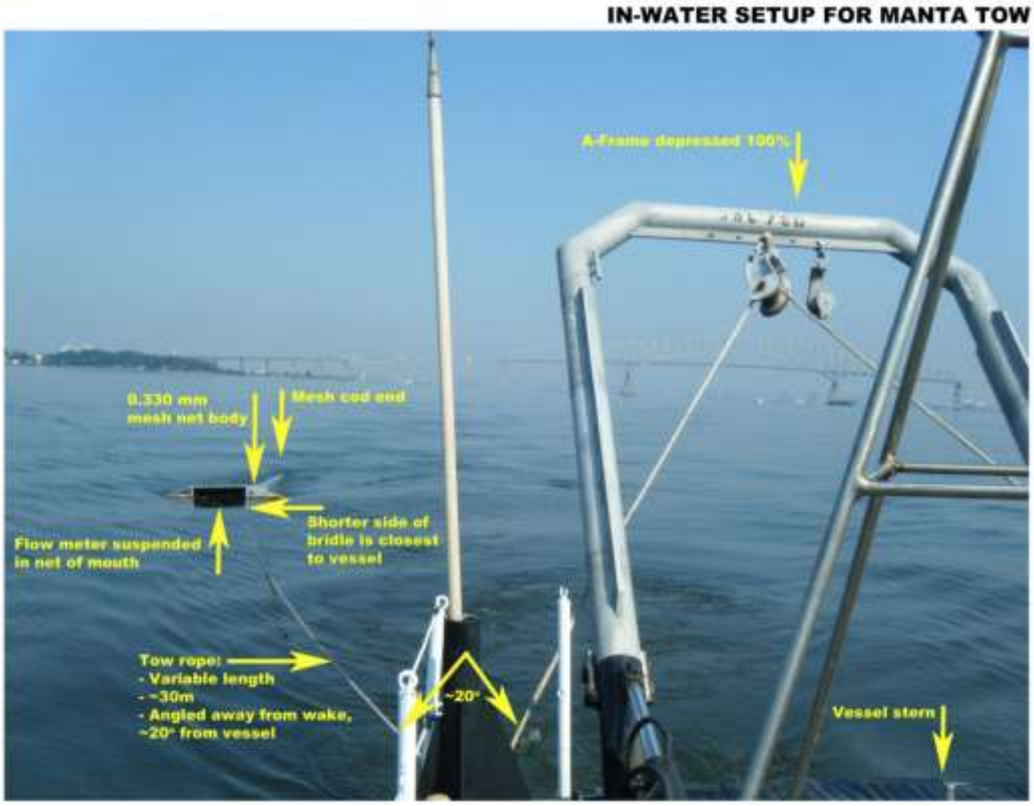


Fig. 23: In-water setup for a manta tow

Contents of the net are gently washed with natural seawater from the outside, into the cod end. If possible, ambient seawater is filtered through a 0.333 mm mesh sieve to remove particles that could bias the sample. The cod end is detached and its entire contents are rinsed with seawater. Samples may be processed on the vessel or transferred to the lab. Any obvious large debris items, >30 cm, are counted on a separate large debris data sheet, rinsed to collect any small attached particles, photographed, and then stored in bags or discarded appropriately.

When processing samples on the vessel, the remaining sample from the cod end is rinsed into stacked stainless steel sieves (5 mm and 0.333 mm) to separate debris items into two size fractions, (x > 5 mm) and (5 mm > x > 0.333 mm). Debris items larger than 5 mm are sorted by material category and tallied on debris data sheets. Macro-debris may then be discarded appropriately or archived depending on study objectives. The size fraction smaller than 5 mm, composed of micro-debris, is carefully rinsed into glass sample bottles and stored frozen to prevent any sample degradation.

When applicable, archiving frozen samples for further analyses is suggested.

Ship-based visual surveys

Ship-based visual surveys are a relatively easy, cost-effective method for crowd-sourcing open ocean marine debris sightings and can provide useful information on the types of debris commonly encountered and spatial and temporal variability of floating debris. The quality of the data depends on environmental factors (e.g., weather conditions, sea state), observers and vessel size and speed. To account for the likelihood of surveyors missing some debris items located on a transect apply a correction factor to measured debris counts based on item size and distance. It must be noted that visual surveys only account for debris that is visible at the surface. Visual survey data should be interpreted as a low-end estimate of the total concentration of floating debris.

Visual surveys may complement surface water trawl surveys and shoreline surveys. A survey design that includes visual surveys of floating debris conducted in conjunction with other survey types will lead to a more robust data set. Where possible, groups are encouraged to conduct surveys in conjunction with ongoing marine research and/or water quality assessments.

Visual surveys should be conducted along strip transects. If possible, two surveyors should conduct surveys from the bow of the vessel and each surveyor is responsible for visually scanning the sea surface and recording all debris > 2.5 cm (Fig. 24).

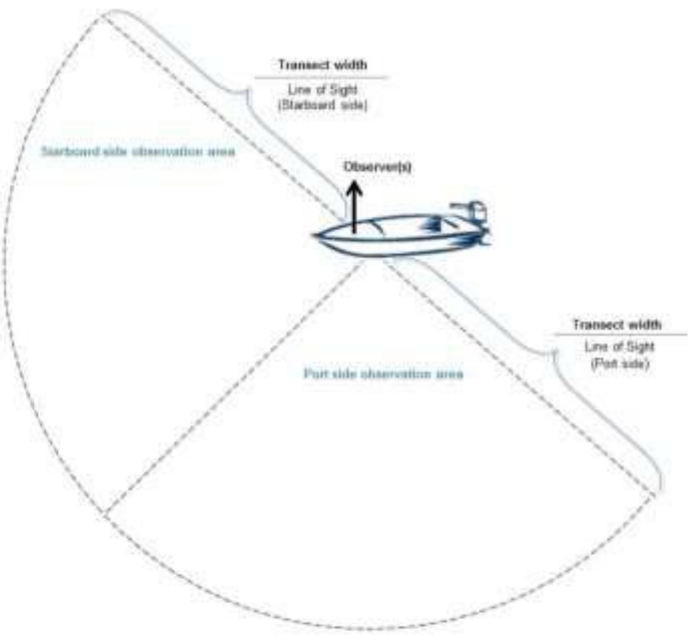


Fig. 24: During visual surveys, observers are responsible for visually scanning the sea surface on either the port or starboard side of the vessel, within a defined transect width.

Visual survey data should be reported in terms of # items/km2, based on the transect width and length.

5.5 DEFISHGEAR, 2014 Methodology for Monitoring Marine Litter on the Sea Surface, 2014

5.5.1 Visual observation

Prepared by: Thomais Vlachogianni (MIO-ECSDE, DeFishGear WPL) With contributions from: Francesca Ronchi, Tiziana Chieruzzi & Tomaso Fortibuoni (ISPRA), Vicky Paraskevopoulou & Vaggelis Kalampokis (MIO-ECSDE), Sabina Cepuš & Uros Robic & Andreja Palatinus (IWRS)
The method developed within the DEFISHGEAR project wsa based on the methodology developed by EU MSFD TG10 and NOOA "considering also the "UNEP/MAP MEDPOL Monitoring Guidance Document on Ecological Objective 10: Marine Litter (2014)".
The monitoring of floating marine litter by human observers is a methodology indicated for short transects in selected areas. The transect width is of 10m, however depending on the observation level of the surveyor for the predefined ship speed of 3.7km/h the transect widths reported in Table 8 might be used:

Table 8: Observation width from different observation levels above the sea for a ship speed of 3.7 km/h.

Observation level of the surveyor above the sea	Observation width (ship speed= 3.7 km/h)
1m	6m
3m	8m
6m	10m
10m	15m

Defishgear suggests to realize at least two surveys 1hour along transect by year (autumn, spring) after a minimum duration of calm sea, and the wind speed should be less than 2 Beaufort. The observation from boats should ensure the detection of litter items in the size range of 2.5cm to 50cm, therefore along with the observation transect width of 10m, the speed of the boat should not be higher than 3knots. Fig. 25: Location for observationsshows the best location for observation.

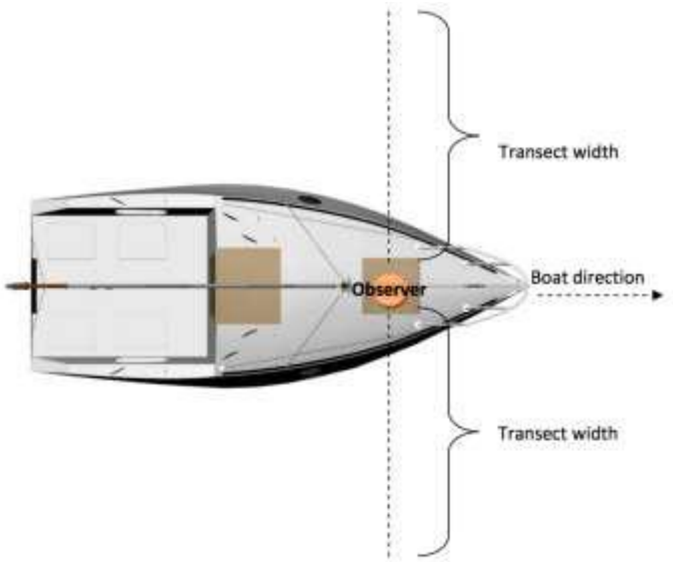


Fig. 25: Location for observations

Litter items in the size range of a 2.5cm (in the longest dimension) to 50cm should be monitored and reported, but they also suggest to record items larger than 50cm. For each items collected the following size should be used: 2.5 to 5 cm – 5 to 10 cm – 10 to 20 cm – 20 to 30 cm – 30 to 50 cm and >50 cm. A specific sheed is used to record all items observed, on the sheet, each type of item is given a unique identification number. A short description and location of the items should be provided.

5.6 MFSD JCR, 2013. Guidance on Monitoring of Marine Litter in European Seas.

The methodology has been prepared for the floating items in the water column close to the surface and reports that litter in the deeper water column is not recommended for routine monitoring. The monitoring of floating marine litter by human observers is a methodology indicated for short transects in selected areas, for instance to understand the variability of litter distribution in area where no information is available. The observation of floating marine litter mainly depends on the sea state and wind speed.
The items observed should be categorized according to the materiel, type and size of the litter; the categories is similar to the categories for beach litter, seaflorr litter and others. Furthermore, it should be noted that since floating litter items will only be observed, the size (longest dimension, width or length) is only an indicative parameter. The size should enclose the following classes: 2.5 to 5cm; 5 to 10 cm; 10 to 20 cm; 20 to 30 cm; ;30 to 50 cm.
The protocol for visual monitoring of floating litter. Covering short transect, is similar to the method described for UNEP. It focuses on litter from 2.5 to 50 cm and is based on transect sampling. The observation transect width (tipically 10 m) depends on the elevation above the sea, the ship speed and the observation conditions. The observation is generally realized in the bow area of the ships The observation, quantification and identification of floating litter items should be made by a dedicated observer who does not have other duties contemporaneously. Observation errors are generally due to intense surveys of small items. Generally, the survey is about 1 h representing a few kilometres.

For floating marine litter, the unit of reporting will be: items/km^2. The data will be available for the different categories and size classes. They can then be aggregated at different levels for providing overview data.

6 Seabed observations

As more studies are completed it has come apparent that significant quantities of plastic debris are lying on the seabed in parts of the global ocean. Different methods have been used like direct observation by cameras (Pham et al.), scuba, bottom trawls, grab, remote operated vehicle, side-scan sonar.

6.1 NOAA, 2013 – Marine Debris Program - Marine Debris Monitoring and Assessment: Recommendations for Monitoring Debris Trends in the Marine Environment.

NOAA describes different methods in their review and reports that the survey of sea floor debris vary according to vessel capabilities and available equipment, target debris type and size, location, personnel and environmental conditions. They are often cost-prohibitive and more logistically challenging than some other types of marine debris monitoring. However, these recommendations are based on the MSFD (2013) method and differenciate the methods by dividing shallow and deep sea floor enviroments. They further indicate that there is no single technique that will work across survey efforts in diverse environments and with different objectives and available resources.
The guideline suggests that benthic debris items should be catalogued according to the same classification system used for other environmental compartments and consequently according to the material types and item categories indicated for shoreline and surface waterr. Furthermore, they indicate that it may be useful to sample micro-debris (< 5 mm) during macro-debris assessment that requires the use of sediment grabs or trawls with a fine mesh size (e.g., Cole et al., 2011).
NOAA suggests that survey locations are dependent on accessibility, study objectives, and available resources and equipment. Sensitive habitats or species and underwater hazards should be avoided. Sampling should focus on areas where debris is suspected to accumulate and may be stratified by factors such as land use, proximity to river mouths, substrate, tourism, fishing pressure, or oceanic current patterns. Bathymetry and hydrodynamics should be considered during site selection as there is growing evidence of their influence on benthic debris accumulations. Furthermore, they report that salinity fronts associated with river mouths tend to trap debris and may be common accumulation areas Acha et al. (2003). The Sample frequency depends on study objectives, available resources, and expected seasonal or annual variability.

6.1.1 Shallow Environments (< 20 m)

Dive surveys along line or strip transects are often the preferred method for assessment of seafloor debris in shallow or coastal environments. The guideline also suggests that a minimum debris size must be identified prior to any survey activities. The minimum debris size should be based on study objectives but should not be smaller than the lower limit of detection (Donohue et al., 2001, Timmers and Kistner, 2005); ideally all items > 2.5 cm are detectable. Selecting a smaller minimum debris size cut-off will require more time and resources.

To ensure that all of the appropriately sized debris items within a transect are recorded, quality control estimates should be conducted by a second surveyor on 20% of the total number of transects sampled per site over the course of the study. Both SCUBA and snorkel free-dive techniques have been used for shallow water benthic debris assessments (e.g., Donohue et al., 2001, Bauer et al., 2008).

6.1.2 Continental Shelves (up to 800 m)

In locations where it is too deep for dive surveys, debris assessments can be combined with ongoing trawl surveys that can provide an idea of the relative types and abundances of benthic marine debris, which is informative at a local or regional level. The guideline suggests to follow the indication of UNEP (Cheshire et al., 2009) that provides a benthic trawl survey design. The suggested approach is to select a 5 km by 5 km survey area, create a grid of 25 km^2, randomly select three sub-blocks of 1 km^2, and conduct five parallel trawls of 800 m each within each selected sub-block. Trawls should be separated by at least 200 m and data from all transects should be aggregated to report an overall debris concentration. Trawl equipment should have a fixed mouth width (e.g., otter trawls) such that debris concentrations can be reported in units of #items/km^2 based on the distance trawled.

6.1.3 Deep Sea Floor

In regions of the seafloor with varying topography submersibles are the only viable option for marine debris surveys. Remotely operated vehicles (ROVs) and manned submersibles have previously been used for debris surveys, but are restrictively expensive in many cases.

6.2 MSFD JCR, 2013. Guidance on Monitoring of Marine Litter in European Seas.

The guideline reports that the most common approaches to evaluate sea-floor litter distributions use opportunistic sampling because it is usually coupled with regular fisheries surveys and programs on biodiversity, since methods for determining seafloor litter distributions (e.g. trawling, diving, video) are similar to those used for benthic and biodiversity assessments. Other methods can be used like submersibles or Remotely Operated Vehicles (ROVs) for deep sea areas, but because litter degradate slowly in deep sea waters, a multiyear evaluation will be sufficient

The guidelines further report that priority should concern (i) the localisation of accumulation areas and supporting tools to identify possible priority areas and to enable backtracking transportation schemes and sources, (ii) an analysis of existing data to characterise the most important sources, and (iii) the improvement of imaging tools for the deep sea video protocol. In addition, due to the persistence of litter materials, the monitoring of litter on the sea floor must consider accumulation processes for past decades. Timescales of observation should therefore be adapted, requiring multiannual frequencies for deep sea floor surveys. The guidelines reports methods for

(i) Shallow coastal waters
(ii) Margin / continental plate (<800m)
(iii) deep sea floor

6.2.1 Shallow coastal areas

The most commonly used method to estimate marine litter density is to conduct underwater visual surveys with SCUBA; snorkelling has been used for very shallow waters (usually < 10 m depth) and for larger forms of marine litter (nets/fishing gear). To overcome an underestimation of abundance, Distance Sampling is more often applied (line transect sampling, Thomas et al., 2006). The field protocols for line transect surveys of litter on the sea-floor are the same as those for benthic sessile fauna, described in detail in Katsanevakis (2009). Only litter items above 2.5 cm are considered, between 0 and 20 m (to 40 meters with skilled divers).

6.2.1.1 Technical requirements

Frequency: at least once a year, but it is recommended one every three monts

Surveys are conducted through 2 line transects for each site. Line transect are defined with a nylon line, marked every 5 meters with resistant paints, that is deployed using a diving reel while SCUBA diving.

Individual litter within 4 m of the line (half of the width –Wt - of the line transects) are recorded. For each observed litter item, when possible, the corresponding line segment of occurrence and its perpendicular distance from the line (yi - for the estimation of detection probability, measured with the use of a 2 m plastic rod), and litter size category (wi) are recorded. The nature of the bottom/habitat is also recorded. The length of the line transects vary between 20 and 200 m (Table 9) and results are expressed in litter density (items/m2 or items/ 100 m2). Finally, in distance sampling surveys, detectability is used to correct abundance estimations (Katsavenakis, 2009).

Table 9: Spatial sampling units for litter evaluation on the sea floor (shallow waters) depending on density of items and sea conditions (Katsanevakis, 2009)

Litter density	Conditions	Method	Sampling Unit (strips: length x width)
0.1-1 items/m2	Low turbidity - high habitat complexity	distance sampling	20 m x 4 m
0.1-1 items/m2	high turbidity	distance sampling	20 m x 4 m
0.01-0.1 items/m2	for every case	distance sampling	100 m x 8 m
<0.01 items/m2	for every case	distance sampling	200 m x 8 m

6.2.2 Deep Sea-floor

For monitoring, the use of trawls in deep-sea areas is restricted to flat and smooth bottoms. For slopes and rocky bottoms, more specialised equipment is necessary. ROVs, which are less complicated than submersibles and generally cheaper, are recommended for litter surveys of deep see-floor.

Benthic litter assessments need to be planned with defined protocols, including the definition and specification of the survey location, choice of sampling units, methodology for collection, classification and quantification of litter and a process for data integration, analysis and reporting of results.

6.2.2.1 Protocol for Sea-floor (20-800m)

From all the methods assessed, trawling (otter trawl) has been shown to be the most suitable for large scale evaluation and monitoring (Galgani et al., 2000). The guideline indicate that as recommended by UNEP (Cheshire, 2009), sites selection should nsure that they (i) Comprise areas with uniform substrate); (ii) consider areas generating/accumulating litter, (iii) avoid areas of risk (presence of munitions), sensitive or protected areas; (iv) do not impact on any endangered or protected species. Sites should be related to sources and impacted offshore areas.

The protocol for sampling and trawling margins (20-800m) has been standardized for each region: For the Mediterranean Region, the protocol is derived from the MEDITS protocol. The hauls are positioned following a depth stratified sampling scheme with random drawing of the positions within each stratum. The number of positions in each stratum is proportional to the surface of these strata and the hauls are made in the same position from year to year. The following depths (10 – 50; 50 – 100; 100 – 200; 200 – 500; 500 - 800 m) are fixed in all areas as strata limits. The haul duration is fixed at 30 minutes on depths less than 200m and at 60 minutes at depths over 200m.

Litter categories from MEDITS litter for Mediterranean and Black Sea

A. Plastic	B. Rubber	C. Metals	D: Glass/ Ceramics	E. textiles / natural fibers	F. Wood (processed)	G. Paper / cardboard	H. Other (specify)	I. Unspecified
A1.Bags	B1. Tyres	C1. Beverage cans	D1. Bottles	E1. Clothing (clothes, shoes)				
A2. Bottles	B2. Other (gloves, shoes, etc.)	C2. Other food cans/wrappers	D2. Pieces of glass	E2. Large pieces (carpets, etc)				
A3. Food wrappers		C3. Middle size containers	D3. Ceramic jars	E3. Natural ropes				
A4. Sheets		C4. Large metallic objects	D4. Large objects (specify)					
A5. Other plastic objects		C5. Cables						
A6. Fishing nets		C6. Fishing related						
A7. Fishing lines								
A8. Other fishing related								
A9. Ropes/strapping bands								
A10. Sanitaries (diapers, etc.)								

Related size category

A: <5*5 cm= 25 cm^2
B: <10*10 cm= 100 cm^2
C: <20*20 cm= 400 cm^2
D: <50*50 cm= 2500 cm^2
E: <100*100 cm= 10000 cm^2= 1 m^2
F: >100*100 cm = 10000 cm2= 1 m2

Fig. 26: Litter categories from MEDITS litter

A standardized litter classification system has been defined before monitoring the sea floor in accordance with types of litter found at regional level. The main categories have a hierarchical system including sub categories. It considers 5 main categories of material (Plastics, metal, rubber, glass/ceramics, natural products) and additional ones: 1 for NE Atlantic (miscellaneous) or 4 for Mediterranean (wood, paper/cardboard, other, unspecific).

6.2.2.2 Complementary protocol for sea-floor – Video camera

Large-scale evaluations of marine litter in the deep sea-floor are scarce because of available resources to collect data. Special equipment is necessary including ROVs and/or submersibles that may be very expensive to operate, especially in deep sea areas.

Towed video camera for shallow waters (Lundqvist, 2013) or ROVs for deeper areas are simpler and generally cheaper and must be recommended for litter surveys. There are some available protocols where litter is counted on routes and expressed as item/km, especially when using submersibles/ROVs at variable depths above the deep sea floor (Galgani et al., 1996) but technology enables the evaluation of densities trough video-imagery using a standardized approach especially for shallow waters.

Shallow sea-floor using towed video

The principles for monitoring with towed video are essentially the same as for the diving protocol, but transects are filmed and analysed either immediately during the filming or afterwards in the lab/office.

The method is based on the protocol developed by Lundqvist (2013), as tested for recording the number of litter objects on shallow (<20m) seafloor biotopes (soft, hard and sand/stone bottoms). The equipment used consisted of a steel rig with two consumer type video cameras (mounted for filming obliquely forward and straight down.

The width of the transect is estimated using a line placed perpendicular to the tow direction and marked at every 0.2 m. The types of litter must be then recorded using the categories defined for the sea-floor but whenever possible, a more detailed description of the item should be added. In turbid waters, cameras could be used down to approximately 20 m depth without any additional light source (Lundqvist, 2013). In total, it takes approximately 60 minutes to perform one transect in the field and then analyse it on land, including the preparation and disassembly of the system (camera and sleigh). The total area monitored during one workday (8h) (including boat transport, analysis, etc.) can be on average 2900 m^2/day.

Deep sea-floor using video

For deep sea-floor, data collection is to be performed on irregular basis, using mainly opportunistic circumstances, considering and counting only litter larger than 2.5 cm, along submersibles/ROVs routes of minimum 0.5 km. Priority must then be given to coastal canyons, or on other areas that are known to generate or accumulate marine litter. For shallow waters and deep sea floor (range 200-4000m), results are expressed as items/ha or km^2 when the measure of the surface is possible or items/100 m or items/km when length based measures are necessary.

7 Seabed

Grøsvik, B. E., Prokhorova, T., Eriksen, E., Krivosheya, P., Horneland, P. A., & Prozorkevich, D., 2018: Assessment of marine litter in the Barents Sea, a part of the joint Norwegian-Russian ecosystem survey. Frontiers in Marine Science, 5(MAR).

The distribution and abundance of marine litter near the bottom are based on trawling with the standard research bottom trawl "Campelen 1800 shrimp trawl" with 80mm (stretched) mesh size in the front, cod-ends of 22mm mesh size and a cover net of 116mm meshes. The trawl was equipped with a rockhopper ground gear and sweep wire length of 40m, plus 12m wire for connection to the doors. Standard tow duration was 15min at three knots. Trawl performance was constantly monitored by Scanmar trawl sensors, i.e., distance between the doors, vertical opening of the trawl and bottom contact control. From trawl catches, marine litter were sorted and classified according to material type and weight. When starting up registration of marine litter as bycatch from the Barents Sea ecosystem surveys IMR and PINRO decided to use a simple classification of marine litter: plastic, wood, metal, rubber, glass, paper, and textile (some years). The data were recorded (category and numbers) in standard data base on board and later transferred to the IMR/PINRO data base. The data of marine litter do not include information about sources of (e.g., from fisheries, human consumables, or other). (Grøsvik et al., 2018)

8 Biota

Initial sample preparation

Mussels will procesed in a clean laboratory environment to reduce sources of contamination.

In the laboratory, the first operation to be performed is to defrost the mussels at room temperature before dissection.

For every individual is record total length and subsequently the soft tissue is remove from the shell and weighed (g, w.w. - wet weight).

Each sample of the 4 sampling macro-areas consists of a range of replicates of 30-50 individuals.

Microplastic extraction

In brief, the soft tissues of each individual is place into glass beakers in in 1:20 (w/v) H_2O_2 30% and incubate for 24 h at 55-56 °C covering with aluminum foil to avoid air contamination. Samples are then will remove from the incubator and cooled.

After the homogenate is dilute with 100-150 ml of purified water (Milli-Q), stirred and filtered under vacuum onto membrane filter (Whatman, Nucleopore Track-Etch Membrane pore size 2 µm, diameter of 45 mm). Each filter is place in glass Petri dishes, covered and dried at room temperature overnight.

Microplastic observation and quantification

The filters will analyze to verify the presence of microplastics, with precaution and always covered to prevent their contamination by airborne fibres, under a stereomicroscope with a camera attached to it.
Microplastic particles will be visually identified according to Hidalgo-Ruz's et al. (2012) protocol: (1) the colour of the particles is homogeneously distributed; (2) no tissue or cell structures are visible; and (3) if the particle is a fiber, it should be equally thick, not taper towards the ends and have a three-dimensional bending. If necessary, tweezers can be used to check whether a particle is a microplastic. If it breaks, it is not a microplastic.
Using a digital camera and the specific software, MP will be photographed, counted and categorized according to maximum length, color (white, clear, blue, black, red, green and yellow), and type (fragments, pellets, filaments, films, foam, granules and uncategorized plastic pieces) following guidelines produced by the MSFD technical group on marine litter (Galgani et al., 2013). Every particle will assigne to one of the distinct class sizes in accord to Hermabessiere et al. (2019): < 15 µm; 15-50 µm; 50-100 µm; 100-500 µm and > 500 µm.
Synthetic polymer types were not identified. Each plastic fragment was verified as plastic with a hot needle (De Witte et al., 2014; Devriese et al., 2015; Naji et al., 2018). This test is useful in cases where you are not able to distinguish between plastic pieces and organic matter. In the presence of a very hot needle, plastic pieces will melt or curl. When using this technique, be sure your needle is very hot and held as close as possible to the piece in question (without blocking your view). If the needle is not hot enough, you will see no movement, even if the piece is plastic.
Guide to Microplastic Identidication developed at Shaw Institute, ex Marine & Environmental Research Institute (MERI, 2015) will be an support to microplastic identification.
In order to facilitate comparisons to data present in literature, the possible proposals to express the microplastics abundance for each individual are:

a) average number of microplastic items per individual in all individuals examined from a macro-area
b) average number of microplastic items per gram wet weight of mussel tissue, in individuals containing microplastics.

Contamination precautions and quality control

To avoid contamination, all laboratory material used during sample preparation and extraction will be rinse twice with bidistelled water and all liquids will be filter with 1 µm pore size filters, prior to use.
Mussel samples will be covered by foil paper during digestion and when not in use.
Furthermore, simultaneously one blank extraction control will be also run without tissues containing only the digestion solutions (the conical flask with 20 ml of 30% H_2O_2 and 180 ml of distilled water is incubated with the other samples and after incubation the solution is filtered and filter paper checked under the stereomicroscope)
At last, in the process of separation of microplastic particles under the stereomicroscope the blank sample should be included (the clean filter paper is exposed to air in the working area). The items similar to those will find in blank samples will be exclud, as they were considered airborne contamination. Filters will be covered with glass lids during observation under a stereomicroscope (Galgani et al., 2013).

9 Bibliography

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