# Guidelines for Quantifying Plastics in the Marine Environment

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Plastics in the Marine Environment, Trophic System and Aquaculture in the Philippines (PlasMics)
University of the Philippines – Marine Science Institute
National Research Council of the Philippines/Department of Science and Technology

# Guidelines for Quantifying Plastics in the Marine Environment

ONDA Deo Florence L[1], et al.

[1] University of the Philippines Diliman – Marine Science Institute

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For copies of this document, please contact:

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- All sample containers and sampling equipment should be pre-washed with acid and then with filtered distilled water before use. After drying, wrap all metal equipment to be used.
- Avoid using any plastic material, if possible.
- Wear polymer-free clothing or cotton overalls and gloves. The type and color of each person should also be noted.
- Position people down-wind of the sampling apparatus during deployment and recovery.
- Cover sample containers with aluminum foil to prevent sample contamination.
- Use pre-filtered distilled water when washing the equipment in between samples.
- During sediment sampling, place three wet filter papers in glass petri dish in the sampling area to account for contamination from air.
- During water sampling, fill sampling jars with filtered seawater or filtered distilled water to serve as blanks.

## 2.2 Sediments

### 2.2.1 Macro- and Microplastics on/in sediments

(Modified from NOAA [Lippiatt et al. 2013] and the European Commission – Joint Research Centre for the Marine Strategy Framework Directive [2013], Adapted from MicroSEAP)

**Introduction**

Plastics are synthetic hydrocarbon polymers used by humans for its durability and wide range of applications (Thompson et al. 2009). We continually produce plastics because of our dependence on the material, but as synthetic polymers with chemical additives, these take hundreds of years to decompose, accumulating in landfills or in natural environments (Geyer et al. 2017). In 2010, Jambeck et al. (2015) estimated that 4.8 to 12.7 million metric tons of plastics was released into the ocean. Plastic wastes remain afloat at sea or sink down the seafloor. However, majority of these wastes were estimated to accumulate in coastal regions (Lebreton et al. 2019), leaving coastal areas and sediments at risk to plastic pollution (Barnes et al. 2009; Pinnell and Turner 2019). Thus, it is important to quantify plastic debris in coastal areas to measure the extent of pollution.

This document outlines methods for macro-, meso-, and micro- assessment in coastal areas. This also provides a comparison between rapid and comprehensive survey methods, helping develop standardized and optimize methods for shoreline surveys in the Philippines. The supplied methods will determine the debris density (# of plastic pieces/unit area) and the type of debris materials.

**Site selection**

- Sandy or pebble shoreline
- A minimum length of 100 m parallel to the water
- Low to moderate slope (15 to 45 degrees)
- Clear access to the sea (no breakwater or jetties)
- Accessible to survey teams year round
- Must not be part of a clean-up program

**Sampling**

Sampling should be done four times a year, once during the northeast monsoon (Amihan), once during the inter-monsoon from Amihan to southwest monsoon (Habagat), once during the Habagat season, and once during the inter-monsoon from Habagat to Amihan. This is done to look at the possible effects of monsoon on debris loading on shorelines.

Sampling should be started within an hour from the low tide and up to within three hours from the actual low tide to analyze the maximum width of the shoreline.

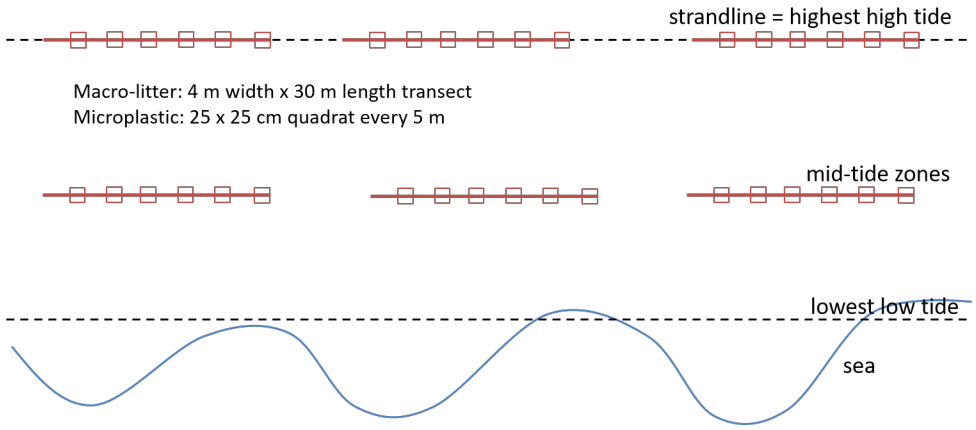
**Materials/Equipment:**

30 m transect tapes (x 3)
25 × 25 cm metal quadrat
8 cm diameter metal ring
250-mL glass jars
Metal shovel / spoon
Metal ruler
Sampling bottles to collect water samples for nutrient analysis
Probes to measure temperature, dissolved oxygen, salinity, etc
Data sheets, pencils
Digital camera

Hand-held GPS unit
Extra batteries (rechargeable batteries)
Flag markers/stakes
10-m measuring tape or surveyor's wheel
First aid kit (sunscreen, bug spray, drinking water)
Work gloves
Clipboard for each surveyor
Data sheets (printed on waterproof paper if possible)
Pencils for data recording
Labels and markers

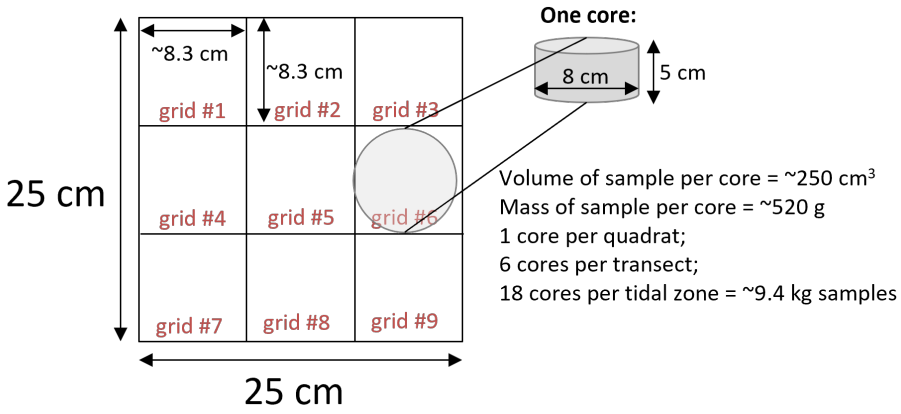
## Field Survey

1. Depending on the type of habitat, lay the following number of transects:
    a. For mangrove: lay three 30 m transects in the mid-tide zones and additional three 30 m transects at the strandline.
    b. For beach: lay three 30 m transects at the mid-tide zones and additional three 30 m transects in the strandline.
    c. For seagrass: lay three 30 m transects in the mid-tide zones.
    d. For coral reef: lay three 30 m transects on the reef crest.
    e. The transects are laid parallel to the shoreline (see illustration below). The minimum distance between two consecutive transects should be at least 2 m apart or wider. For larger study sites, the distance between two consecutive transects can be larger.




    f. For smaller sites that cannot fit 3 x 30 m transects, shorter transect length (e.g., 15 or 20 m) may also be adopted, but the number of transects at each tidal zone should still be three.

2. For sampling macro-litter (> 25 mm in size), record the following information for each litter item found within 2 m to the left and 2 m to the right of the transects (i.e., 4 m total width x 30 m total length belt transect).
3. Place a 25 × 25 cm quadrat every 5 m (i.e., at 5, 10, 15, 20, 25, and 30-m mark). Take a top-down view photo of the entire quadrat (as vertical as possible). Include the entire quadrat frame and tape measure.
4. For sampling mesoplastics (5 – 25 mm) and microplastics (<5 mm):
    a. Divide each quadrat into 3 × 3 grids, and randomly select one grid (use random number generator to generate one number from 1 to 9) for coring (8 cm diameter, 5 cm depth).




   b. If there is an obstacle (e.g., plant, rock, coral) that prevents quadrat to be placed at the transect mark, scan clockwise at a radius of 5 m looking for the first substratum free to place the quadrat.
   c. If the surface of the sediment within the core is uneven, use a scraper to flatten the surface. This is to keep sample depth equal for each core.
   d. Use a scoop and/or scraper to take out sediment samples from the top 5 cm of the surface within the core. Use metal ruler to control the depth.
   e. Store each core sample in glass jars.
   f. Field blanks: place three wetted filter papers on metal trays during sediment collection in the field. Store the filter papers in glass petri dishes and bring back to lab for checking under microscope.
5. Collect the following environmental parameters at each site:
   a. Tidal distance (maximum horizontal distance between the low- and high-tide line) and the distance between strandline and transect.
   b. Depth of transects (for coral reefs site; using dive watch)
   c. Wind speed and direction (determined using anemometer / phone app, or simply leeward / windward)
   d. Three water samples (250 ml for nitrate, phosphate analyses)
   e. Temperature, dissolved oxygen, salinity, light levels, total suspended solids (using YSI probes or other available equipment; 3 point samplings per site)
   f. Note: See datasheet template for other site characteristics that can be filled before / after the field survey.
   g. Note: Other environmental data related to seasons (e.g. average monthly rainfall) will be extracted from nearby meteorological stations.
6. Store sediment samples at 4 ° C (or lower) for short-term storage (< 2 weeks) or -20 ° C (or lower) for long-term storage until further lab processing.

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

$$C = (n)/wl$$

Where $C$ = concentration of debris items (# of debris items/square meter)
$n$ = # of macro-debris items observed
$w$ = width (m) of shoreline section recorded during sampling (i.e. transect width)
$l$ = length (m) of the shoreline sampled = 30

For a given sampling event

1. The debris concentration for each transect is surveyed (a minimum of four per survey).
2. Take the mean concentration at each transect to calculate an overall site concentration (± standard deviation) for that date.

## 2.3 Water

### 2.3.1 Sea surface

(Adapted from Lippiatt et al. 2013, Green et al. 2018, Michida et al. 2019, GESAMP 2019)

Manta net tows and grab sampling should be done in tandem since net tows alone can underestimate smaller microplastics, those particles smaller than the size of the mesh. Since obtaining grab samples does not initially involve pre-filtering of samples prior to analysis in the laboratory, this sampling technique can collect microplastics smaller than the mesh size of the net.

Manta net tows should be done under calm sea conditions and no algal blooms. Wave height should be under 0.5 meters and Beaufort wind force should be under three (GESAMP 2019). A constant immersion depth should be maintained all throughout the sampling.

**Materials/Equipment:**

Manta net
Rope for manta
Weights
Pre-filtered distilled water
Flow meter
Anemometer
Current meter
Multiparameter meter
1000 mL sampling jars
1000 mL HPDE bottles for chlorophyll-a
1000 mL HDPE bottles for total suspended solids
250 mL HDPE bottles for nutrients
Labels and markers
Spray bottle or hose for washing the net
Glass petri dishes
GF/F filter papers
Metal forceps for GF/F filter papers
Foil for closing sample jars
Long gloves for sampling
Cooler with ice

**Chemicals:**

Isopropanol for preservation

**Procedure:**

1) Before going out to sea, wash manta net from the outside with pre-filtered distilled water (PFDW). Store in a natural fiber bag.
2) At sea and before deployment, wash net from the outside using PFDW using a garden sprayer. If there is water on the ship available, wash using the hose. Concentrate the contents to the cod end and label as one of your blanks. Store together with samples.
   MNLBAY_04May2021_Blank_Manta_S1
   [cruise_date_Blank_Manta_site/station#]
3) Set up other blanks: petri dish with PFDW and petri dish with wet GF/F (accomplish in triplicates).
   MNLBAY_04May2021_Blank_PD_S1a
   MNLBAY_04May2021_Blank_GFF_S1a
   [cruise_date_Blank_PD or GFF_site/station#]
   where PD = petri dish

GFF = wet filter paper
1a = station or site 1, replicate 1 (b and c for second and third replicates)

4) Before sampling, fill three 1000-mL sampling jar with 500 mL pre-filtered distilled water. These three will also serve as your blanks.
   MNLBAY_04May2021_Blank_Grab_S1a
   [cruise_date_Blank_Grab_site/station#]
   where Grab = blank for grab
   1a = station or site 1, replicate 1 (b and c for second and third replicates)
5) Attach flowmeter and weights to net.
6) Wash net twice and deploy net at the side of the boat.
7) Collect metadata before starting.
8) Obtain 1000 mL of seawater for grab sample of sub-surface water (~50 cm) and cap while under water to prevent airborne contamination.
9) then seawater for nutrients, chlorophyll-a, total suspended solids, and environmental parameters using a multiparameter meter.
10) Tow for 20 min at 1-2 knots.
11) At the halfway mark or after 10 minutes, obtain again 1000 mL of seawater for grab sample, then seawater for nutrients, chlorophyll-a, total suspended solids, and environmental parameters using a multiparameter meter.
12) After 20 minutes, retrieve and hang net.
13) While allowing the net to hang, obtain 1000 mL of seawater for grab sample, then seawater for nutrients, chlorophyll-a, total suspended solids, and environmental parameters. Also record required metadata.
14) Wash net from the outside using hose or garden sprayer, concentrating contents to cod end.
15) Drain cod end contents to labelled glass jars:
    MNLBAY_04May2021_Manta_S1
    [cruise_date_Manta_site/station#]
16) Add isopropanol (10% of the total sample volume).
17) Cover petri dishes and glass jars (with foil before replacing lids) and store with samples.
18) Storage: -20° C to 4° C (may be best to store in 4° C as the glass may break).

### 2.3.2 Water column

**Materials/Equipment:**

Plankton net
Rope for plankton net
Weights
Pre-filtered distilled water
250 mL or 300 mL glass sampling jars
Labels and markers
Spray bottle
Glass petri dishes
GF/F filter papers
Metal forceps for GF/F filter papers
Foil for closing sample jars
Gloves

**Chemicals:**

Isopropanol for preservation

**Procedure:**

1) Before going out to sea, wash plankton net from the outside with pre-filtered distilled water (PFDW). Store in a natural fiber bag.

2) At sea and before deployment, wash net from the outside using PFDW using a garden sprayer. If there is water on the ship available, wash using the hose. Concentrate the contents to the cod end and label as one of your blanks. Store together with samples.
    WPS_04May2021_Blank_net_S1
    [cruise_date_Blank_net_site/station#]
3) Set up other blanks: petri dish with PFDW and petri dish with wet GF/F (accomplish in triplicates).
    WPS_04May2021_Blank_PD_S1a
    WPS_04May2021_Blank_GFF_S1a
    [cruise_date_Blank_PD or GFF_site/station#]
        where PD = petri dish
        GFF = wet filter paper
        1a = station or site 1, replicate 1 (b and c for second and third replicates)
4) Attach weights to net.
5) Wash net twice and deploy net at the surface (or whichever depth is preferred).
6) Retrieve net and hang, allow contents to concentrate to cod end.
7) Wash net from the outside using hose or garden sprayer, concentrating contents to cod end.
8) Drain cod end contents to labelled glass jars:
    WPS_04May2021_Net_S1
    [cruise_date_Net_site/station#]
9) Add isopropanol (10% of the total sample volume).
10) Cover petri dishes and store with samples.
11) Storage: -20° C to 4° C (may be best to store in 4° C as the glass may break).

Note: Seawater samples from the water column should have accompanying metadata.

## 2.4 Biota

**Introduction**

Plastic debris as emerging pollutants in the environment are found to degrade into microplastics which are characterized as fibers, fragments, and pellets that are less than 5 mm in size (Andrady et al. 2011). The ubiquity of microplastics, which have been detected in a variety of habitats, has become a threat to marine species, ecosystems, and human health. The ingestion of microplastics by marine biota across different trophic levels, including organisms significant in mariculture, has been reported by various studies (Lushar et al. 2015; Argamino and Janairo 2016; Karlsson et al. 2017; Naidoo et al. 2020). Microplastics ingestion was found to cause internal blockages and injury to the digestive tract of organisms, and has been speculated as the initial route for associated toxic chemicals to enter and spread through the food web with microplastics as vectors, culminating in human consumption (Nadal et al. 2016; Diepens and Koelmanns 2018). However, several processes that contribute to possible biomagnification of species remain little understood. Therefore, quantification of ingested and accumulated microplastics in biota is a significant step in understanding such processes and assessing the risk of such hazards, as a baseline data for setting rules and guidelines for the trade and consumption of mariculture species.

This document provides methods for quantification of microplastics in biota, specifically in bivalves (mussels and oysters) and fish. The methods listed will outline protocols from sampling, quality controls, sample processing, until microplastics identification, characterization, and quantification. The protocols will determine microplastics type and abundance as number of plastic particles per gram of wet weight.

**Materials and Equipment**

Aluminum foil
Ice box
Ziplock bags
Rubber bands
Dissecting pans

**Reagents**

Sterile filtered distilled water

**Procedure:**

1. Collect 50 individuals from each sampling site. Wash the samples with pre-filtered water then wrap with aluminum foil and rubber bands to prevent contamination. Store in an ice box and transfer to a -20° C freezer until lab processing.

# 3 Laboratory Analyses

## 3.1 Contamination Control

- All materials, equipment, and laboratory surfaces to be used should be thoroughly rinsed and cleaned with pre-filtered water and stored under clean air conditions.
- Reagents and solutions should be pre-filtered before treatment.
- Sample handling and processing should be conducted in clean air facilities. If clean air conditions cannot be fully achieved, use of negative controls needs to be established.
- Triplicate negative controls need to be included in the treatments for each batch of sample processed.
- For additional controls, clean Petri dishes or glass slides should be placed around the area for sample processing to check for airborne contamination.
- Samples should be covered with aluminum foil before and after processing. Polymer-free gloves and laboratory coat made of 100% cotton material should be worn during analysis to prevent contamination.

## 3.2 Preparation of spiked samples

### 3.2.1 Preparation of microplastics

(Methods from Nuelle et al. 2014; Kuhn et al. 2018; Selonen et al. 2020)

**Materials/Equipment:**

Grinder or mill
Liquid nitrogen
Sieves of varying sizes
Fleece blanket – brightly-colored
Dog brush
Cutter
Cutting mat
Plastics:
- PET – water bottle
- PP – straw
- LDPE – ice bag
- HDPE – shampoo container
- PVC – pipe
- PS – yogurt cup

Fibers:
- Nylon, PET, and PP

**Procedure:**

1. Cut plastics into smaller pieces, less than 25mm. Soak in liquid nitrogen for 30 minutes to increase brittleness. Grind or mill plastic pieces. Sieve plastic pieces through 5mm, 1mm, and 100 $\mu$m. Sort plastics per size and per type and store in glass containers.
2. Plastics can also be cut using a cutter to produce larger MPs (1mm to 5mm). Sieve cut plastics through through 5mm, 1mm, and 100 $\mu$m, sort per size and per type and store in glass containers.
3. To obtain long fibers, brush a fleece blanket using a dog brush with bent wires. Store in glass containers. For short fibers, cut fleece blanket into small pieces, 0.5 square centimeter. Soak cut blanket in liquid nitrogen for 5 min before milling for 2.5 minutes. Store milled fibers in a sealed paper container or glass container.

### 3.2.2 Organic matter

(Methods from Isobe et al. 2019)

**Materials/Equipment:**

Glass containers
Wood chips
Bivalve shells
Crab shells
Eggshells
Zooplankton (optional)
Grinder
Sieves (5mm, 1mm, and 0.3mm)

**Procedure:**

4. Mechanically fragment organic matter using a grinder. Sieve fragmented organic matter through 5mm, 1mm, and 0.3mm sieves, sort according to type and size, and store in glass containers.

### 3.2.3 Sediments

(Modified from Shim et al. 2016)

**Materials/Equipment:**

Beach sand
Sieves (1000 $\mu$m, 63 $\mu$m)
Density separation set-up
Furnace
Furnace-friendly container for sand combustion
Prepared microplastics (MPs) for spiking

**Chemicals:**

Zinc chloride for density separation

**Procedure:**

1. Collect natural sand from a beach.
2. Remove light particles and plastics by density separation. Repeat until no more particles are visible.
3. Pre-combust cleaned sand overnight at 450 ° C to remove the remaining plastics and organic matter.
4. Add known amount of MPs to spike 50 g of the pre-combusted sand in a beaker. Also add organic matter into the mixture. Mix.
5. Analyze this sand and plastic mixture along with samples to test for extraction efficiency.

### 3.2.4 Seawater

(Modified from Isobe et al. 2019)

**Materials/Equipment:**

Seawater
Filters: 10 $\mu$m and 1 $\mu$m
1 L glass bottle container
Prepared microplastics (MPs) for spiking

**Procedure:**

1. Filter seawater with 10 $\mu$m filter twice and with 1 $\mu$m filter before filling sample bottles. Make sure there are no plastic fragments in the water and in the sample bottles.
2. Add known amount of MPs per type and size to the sample bottle containing filtered seawater.
3. Add organic material to the sample bottle. Mix.
4. Analyze this mixture along with samples to test for extraction efficiency.

## 3.3 Extraction of MPs from environmental samples

### 3.3.1 Sediments

**Materials/Equipment:**

Stainless steel sieves, each measuring 8 in (diameter) and 2 in (depth)
- o 5.6 mm mesh (Number 3.5)
- o 1 mm mesh (Number 18)
- o 0.3 mm mesh (Number 50)
- o 0.037 mm mesh (Number 400) – or smallest limit of detection

Squirt bottle containing distilled water
500-mL glass beaker
Analytical balance (precise to 0.1 mg)
Metal spatula
Drying oven (90oC)
Stir bar
Laboratory hot plate
Watch glass
Standard Metal Forceps
Density separator, which is assembled using the following method:
- o A glass funnel (122-mm in diameter) is fitted with a 50-mm segment of latex tubing on the bottom of the stem and a pinch clamp is attached to control liquid flow from the funnel.

Retort stand
O-ring
Spring clamp (2-inch)
Aluminum foil
4-mL glass vials
Dissecting microscope (40X magnification)

**Reagents:**

$ZnCl_2$ salt solution with density of 1.5 g $cm^{-3}$
a) Mix 972 g of $ZnCl_2$ in 1 l of DI / Milli-Q water, stirred for at least 24 h at 600 rpm. Adjust the density to 1.5 g $cm^{-3}$ by checking the mass and volume of the solution.
b) If white precipitate is observed, add concentrated HCl until the solution turns clear (pH of solution should be <5)
c) Filter using 0.45 um filter papers to remove impurities.

Iron (Fe(II)) solution (0.05 M)
a) Prepared by adding 7.5 g of FeSO4° 7H20 (= 278.02 g/mol) to 500 mL of water and 3mL of concentrated sulfuric acid
b) Filter using 0.45 um filter papers to remove impurities.

30% Hydrogen peroxide

**Procedure:**

1. Dry sediment at 50 ° C for at least 48 h or until sample dryness. Record the dry mass of the sediment sample.
2. Sieve the sediment sample through a 5 mm metal sieve (and any other smaller sieve sizes if you are looking at sediment size grains) on top of a metal tray. Record the dry mass of sediment for each size category (< 5 mm, > 5 mm).
3. For sediment samples > 5 mm, visually select plastic-like particles using forceps and record the number and mass.

4. For sediment samples < 5 mm, conduct density-separation method:
    a. Prepare $ZnCl_2$ salt solution
    b. Add salt solution at a ratio of 100 g dried sediment to 400 ml salt solution.
    c. Mix sediment and salt solution thoroughly for 2 min. Remove the magnet from the mixture solution.
    d. Cover the beaker with aluminium foil and let the particles settle >8 h or overnight.
    e. Gently pour only the supernatant from the beaker and filter through 20 um under vacuum.
        i. Filter 5% of the resultant filtrate through 0.22 um filter for analysis by microRaman (to be sent to the UK team).
        ii. Collect used salt solution and filter them through 0.22 um filter to recycle for future use (up to 5x).
    f. Rinse the filter paper with enough amount of DI / Milli-Q water to remove the salt solution
    g. Transfer the particles on filter paper into a 250 ml beaker with as little DI / Milli-Q water as possible. Cover the glass beaker with aluminium foil, but one edge is slightly opened.
    h. Dry the beaker at 50° C in the oven for 24 h or longer until sample dryness.
5. Conduct oxidative digestion:
    a. Prepare 0.05M Fe (II) solution.
    b. Add magnetic stirrer to the 250 ml beaker (with microplastics obtained from Step i in density-separation method).
    c. Add 20 ml of Fe(II) solution and 20 ml of 30% H2O2 solution into the beaker.
    d. Stir the solution at room temperature.
        i. Monitor the temperature and keep the solution between 20–40 ° C. Put solution in ice bath intermittently when temperature is >40 ° C. Temperature should not fall <15 ° C as yellow precipitate will form. If yellow precipitate is formed, add concentrated H2SO4 to dissolve it.
    e. If the solution is still brownish color, add more Fe(II) solution and H2O2 (repeat step c and d).
    f. Weigh clean filter paper.
    g. Let the solution cool down. Gently pour the solution over the filter paper.
    h. Rinse the filter paper with enough amount of DI / Milli-Q water to remove any chemical.
    i. Transfer the filter paper into the glass petri dish / crucibles. Cover the sample container with aluminium foil, but one edge is slightly opened.
    j. Dry samples at 50° C in an oven for 1¬–3 days (until no change in mass).
    k. Weigh the filter paper.

6. Repeat density-separation and/or digestion steps if filter paper in Step 5k is still dirty.
7. Store filter papers in sealed glass containers until further processing.

### 3.3.2 Water

**Materials/Equipment:**

Stainless steel sieves, each measuring 8 in (diameter) and 2 in (depth)
- o 5.6 mm mesh (Number 3.5)
- o 1 mm mesh (Number 18)
- o 0.3 mm mesh (Number 50)
- o 0.037 mm mesh (Number 400) – or smallest limit of detection

Squirt bottle containing distilled water
250-mL glass beaker
Analytical balance (precise to 0.1 mg)
Metal spatula
Drying oven (90oC)
Stir bar
Laboratory hot plate
Watch glass
Standard Metal Forceps
Density separator, which is assembled using the following method:
- o A glass funnel (122-mm in diameter) is fitted with a 50-mm segment of latex tubing on the bottom of the stem and a pinch clamp is attached to control liquid flow from the funnel.

Retort stand
O-ring
Spring clamp (2-inch)
Aluminum foil
4-mL glass vials
Dissecting microscope (40X magnification)

**Reagents:**

$ZnCl_2$ salt solution with density of 1.5 g $cm^{-3}$

d) Mix 972 g of $ZnCl_2$ in 1 l of DI / Milli-Q water, stirred for at least 24 h at 600 rpm. Adjust the density to 1.5 g $cm^{-3}$ by checking the mass and volume of the solution.
e) If white precipitate is observed, add concentrated HCl until the solution turns clear (pH of solution should be <5)
f) Filter using 0.45 um filter papers to remove impurities.

Iron (Fe(II)) solution (0.05 M)

c) Prepared by adding 7.5 g of FeSO4° 7H20 (= 278.02 g/mol) to 500 mL of water and 3mL of concentrated sulfuric acid
d) Filter using 0.45 um filter papers to remove impurities.

30% Hydrogen peroxide

**Procedure:**

1. Filter water samples through 20 um filter (if it is not done in the field).
   a. Transfer the particles on 20 um filter into a 250 ml beaker with as little DI / Milli-Q water as possible. Cover the glass beaker with aluminium foil, but one edge is slightly opened.
   b. Filter 5% of the resultant filtrate through 0.22 um filter for analysis by microRaman (to be sent to the UK team).
2. Dry the beaker at 50° C in the oven for 24 h or longer until sample dryness.
3. If the water samples are clear of sediment and contain mainly planktons, conduct oxidative digestion first followed by density-separation.

If the water samples are turbid and contain mainly fine silt, conduct density-separation first followed by oxidative digestion.

4. Oxidative digestion:
    a. Prepare 0.05 M Fe(II) solution:
    b. Add magnetic stirrer to the 250 ml beaker (from step 2 above).
    c. Add 20 ml of Fe(II) solution and 20 ml of 30% $H_2O_2$ solution into the beaker.
    d. Stir the solution at room temperature.
        i. Monitor the temperature and keep the solution between 20–40 ° C. Put solution in ice bath intermittently when temperature is >40 ° C. Temperature should not fall <15 ° C as yellow precipitate will form. If yellow precipitate is formed, add concentrated $H_2SO_4$ to dissolve it.
    e. If the solution is still brownish color, add another Fe(II) solution and $H_2O_2$ (repeat step c and d).
    f. Weigh clean filter paper.
    g. Let the solution cool down. Gently pour the solution over the filter paper.
    h. Rinse the filter paper with enough amount of DI / Milli-Q water to remove any chemical.
5. Density-separation method:
    a. Prepare $ZnCl_2$ salt solution with density of 1.5 g $cm^{-3}$
    b. Transfer the particles found on filter paper from step 4h into a beaker using the $ZnCl_2$ salt solution. Add 400 ml salt solution for every 100 g of particles.
    c. Mix sediment and salt solution thoroughly for 2 min. Remove the magnet from the mixture solution.
    d. Cover the beaker with aluminium foil and leave the beaker overnight
    e. Weigh clean filter paper.
    f. Gently pour the supernatant from the beaker and filter through filter paper.
        i. Collect used salt solution and filter them through 0.22 um filter to recycle for future use (up to 5x?).
    g. Rinse the filter paper with enough amount of DI / Milli-Q water to remove the salt solution.
6. Transfer the particles on filter paper (from Step 4h or 5g) into a glass petri dish / crucible with as little DI / Milli-Q water as possible. Cover the sample container with aluminium foil, but one edge is slightly opened.
7. Dry samples at 50° C in an oven for 1–3 days (until no change in mass).
8. Weigh the filter paper.

### 3.3.4 Biota

**Materials and Equipment**

Aluminum foil
Ice box
Ziplock bags
Rubber bands
Dissecting kit (scalpel, forceps, needle, scissors)
Dissecting pans
Stainless steel caliper
Stainless steel shucking knife
Metal spatula
Beakers (250 ml, 600 ml)
Petri dishes (60 mm, 100 mm)
Glass microfiber filters (1 um, 47 mm)
Buchner funnel
Buchner flask
Stopper and rubber tubing
Vacuum pump
Stereomicroscope
Blue LED lamp (450 nm)
Top loading balance

**Reagents**

Potassium hydroxide
Hydrogen peroxide
Sodium chloride
Nile red
Sterile filtered distilled water

**Procedure**

**A. Bivalves**

1. Using a stainless-steel shucking knife, open the shells and empty the contents of 6 individuals into a clean 1L beaker.
2. Add 10% KOH 4 times more than the tissue volume, cover the beaker opening with foil and glass cover, and stand at 60° C for 48 hours. Agitate the set-up manually every 4 hours.
3. Group the beakers into 2 set-ups: Set-up A and Set-up B. Set-up A will follow the protocol established by Bendell et al. 2020, while Set-up B will proceed according to the methods conducted by Li et al. 2015.
4. For Set-up A, add 2.5 mL of Dye More, and vacuum filter the digested solution through #1 GF/F filter paper. Wash the filter paper with 30% H2O2, and afterwards stain the filter paper with Nile red.
5. For Set-up B, add 800 mL saturated NaCl into the digested solution and mix. Allow to settle overnight. Repeat this step for 3 more times, then vacuum filter through 1.0 um GF/F. Stain the filter paper with Nile red.
6. Store the filter paper in a sterile petri dish, with the lid wrapped by foil to prevent contamination, until MP quantification.

**B. Fish**

1. Rinse the exterior of the fish thoroughly before placing in the dissection area to reduce particle contamination during processing.
2. Using a sterile scalpel blade, make an incision 1 mm in front of the rectum and cut towards the anterior part of the fish.

3. In order to estimate ingested microplastics accurately, the entire gastrointestinal tract should be examined. Cut through the esophagus while keeping the entire stomach intact, and cut through the gut approximately 2-3 mm before the anus. Record the weight of the stomach and gut.
4. Place the excised stomach and gut in a clean 1L beaker. Add 10% KOH solution 3 times more than the tissue volume. Cover the beaker opening with foil and glass cover, and stand at 60° C for 48 hours. Agitate the set-up manually every 4 hours.
5. After digestion, add 800 mL saturated NaCl to separate plastics from digested tissue via floatation. Allow the solution to stand overnight until clearance is observed. Filter solution through 1.0 um GF/F filter.
6. Stain the filter paper with Nile red. Place filter paper in a sterile petri dish then wrap with foil for storage until MP quantification.

## 3.4 Counting, visual identification, and sorting

**Materials and Equipment**

Stereomicroscope with camera
Fluorescent blue lamp
Computer
Extracted microplastics on filter papers

**Reagents**

Ethanol

**Procedure**

1. Subject MPs in filter papers to initial visual inspection and quantification under a stereomicroscope. Observe stained filter paper under fluorescent blue lamp (420-495 nm) to detect fluorescing MPs.
2. Characterize fluorescent MPs according to shape (fibers, spherules, fragments, or sheets), colors, and sizes. Record colors as dominant visual property and categorize sizes into five groups: 0.05-0.1 mm, 0.1-0.5 mm, 0.5-1 mm, 1-1.5 mm, 1.5-2 mm.
3. The abundance of microplastics in the samples will be calculated as the number of plastic particles per gram of wet weight.

## 3.5 Characterization

(Adapted from Harshvardhan and Jha (2013), Jung et al. (2018)

To check for the formation of new functional groups, which can be attributed to polymer degradation, Fourier Transform Infrared Spectroscopy with Attenuated Total Reflection (ATR) (Bruker, Massachusetts, United States) is done.

**Materials and Equipment**

Oven
Fourier Transform infrared spectrophotometer with attenuated total reflectance
Computer
Forceps

**Reagents**

70% Ethanol

**Procedure**

1. Among the identified MPs, random samples will be selected from each size category and will be further verified through FT-IR spectroscopy.
2. Dry samples overnight in oven at 60° C.
3. Collect spectra from plastic samples from 4,000 $cm^{-1}$ to 450 $cm^{-1}$ with a data interval of 1 $cm^{-1}$, with the resolution set to 2 $cm^{-1}$.
4. Clean the ATR diamond with 70% ethanol before and in-between samples.
5. Perform background scans between samples.
6. Each sample is compressed against the diamond with the minimum force recommended by the manufacturer to ensure good contact between the sample and the ATR crystal.
7. The resultant spectra will be identified using the spectra library in the software and will be use to determine polymer types.

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# 5 Appendices

## MACRO- AND MICROPLASTIC DATASHEET – SITE CHARACTERISTICS

| | |
|---|---|
| Surveyor name: | Survey date: |
| Site name: | Country: |
| GPS start: | GPS end: |
| SHORELINE CHARACTERISTICS | |
| Habitat type: beach mangrove seagrass coral reef | |
| Backshore type: cliff seawall urban building forest/tree shrub mangrove other: | |
| Shore exposure: cove/bay straight headland | |
| Tidal distance (m): | |
| ENVIRONMENTAL CONDITIONS (three readings per site) | |
| Weather: clear rain/storm overcast drizzle | |
| Wind speed (m/s): | Wind direction: |
| Sea surface temp (°C): | Salinity (psu): |
| Dissolved $O_2$ (mg/L): | Total suspended solids (mg/L): |
| Nitrate (mg/L): | Phosphate (mg/L): |
| LAND-USE CHARACTERISTICS | |
| Access: vehicular trail isolated | |
| Major site usage: tourism fishing protected isolated other: | |
| Nearest town distance (km): | Nearest river distance (km): |
| River input: yes no | Pipe/drain input: yes no |
| Evidence of dumping: none construction household other: | |
| Evidence of recent activities: none clean-up/rubbish removal apparent spilled trash storm/flood strong winds public event other: | |
| Notes (*include descriptions on landmarks, coastal hydrography, etc*): | |

Notes:
GPS start: GPS coordinates of the start of the first transect
GPS end: GPS coordinates of the end of the third transect
Tidal distance: the maximum horizontal distance between the low- and high-tide line
Access: vehicular (you can drive to the site), trail (you must walk), isolated (you need a boat/plane)
Nearest town distance: nearest distance to villages / residences / towns / human populations

## MACRO- AND MICROPLASTIC DATASHEET

| Surveyor name: | | Survey date: | | |
|---|---|---|---|---|
| Site name: | | Country: | | |
| Transect number: out of | | Transect size (length × width): | | |
| Distance from strandline (m): | | Depth (m): | | |
| Sampling zone: strandline mid-tide reef crest | | | | |
| Substratum type: sand mud boulder seagrass seaweed coral other: | | | | |
| Main category | Specific litter category | Count | Mass (g) | Note* |
| **Plastic** | Beverage bottles < 1 L | | | |
| | Beverage bottles ≥ 1 L | | | |
| | Buckets / jerry cans / drums | | | |
| | Caps / lids / covers | | | |
| | 6-packs rings / drink package rings | | | |
| | Straws / pipettes | | | |
| | Clear cups / bowls / food containers | | | |
| | Foamed cups / bowls / food containers | | | |
| | Knives / forks / spoons | | | |
| | Bags | | | |
| | Thin plastic wraps / labels / packagings | | | |
| | Thick plastic wraps / sacks | | | |
| | Lighters / matches | | | |
| | Cigarette tips / butts / filters | | | |
| | Ropes / strings / strapping bands | | | |
| | Pipes / hoses | | | |
| | Fishing lines / nets / rods | | | |
| | Buoys / floats | | | |
| | Shampoos / shower gels / toothbrushes | | | |
| | Fragments (hard plastic) | | | |
| | Fragments (soft plastic / films / sheets) | | | |
| | Fragments (foamed) | | | |
| | Other: | | | |
| | | | | |
| | | | | |
| **Rubber** | Slippers / flip-flops / shoes / gloves | | | |
| | Tires | | | |
| | Balloons, balls | | | |
| | Rubber bands | | | |
| | Other: | | | |
| | | | | |
| | | | | |
| **Metal** | Aluminium / tin / aerosol cans | | | |
| | Bottle caps | | | |
| | Buckets / drums | | | |
| | Nails / irons | | | |
| | Fishing related (lures, hooks, sinkers) | | | |
| | Other: | | | |
| | | | | |

| | | | | |
|---|---|---|---|---|
| | | | | |

Notes:
Distance from strandline: the horizontal distance between the strandline and and transects
Depth: Depth of transects; for coral reef sites only
If the macro-debris cannot be weighed because it is too big / heavily encrusted / soaked, record its size (length, width, height) for mass estimation.

| Main category | Specific litter category | Count | Mass (g) | Note* |
|---|---|---|---|---|
| **Glass** | Bottles / jars | | | |
| | Light bulbs / tubes / globes | | | |
| | Fragments | | | |
| | Other: | | | |
| | | | | |
| | | | | |
| **Wood** | Cigarette packs | | | |
| | Lighters / matches | | | |
| | Paper / newspaper / pieces of papers | | | |
| | Crates / boxes / cardboards | | | |
| | Fishing traps / pots | | | |
| | Ice cream sticks / chopsticks / toothpicks | | | |
| | Fragments | | | |
| | Other: | | | |
| | | | | |
| | | | | |
| **Cloth** | Clothes / towels / rags | | | |
| | Sacking / gunny sacks / canvas | | | |
| | Diapers / sanitary pads | | | |
| | Fabric pieces | | | |
| | Other: | | | |
| | | | | |
| | | | | |
| **Other** | Batteries | | | |
| | Appliances, electronics | | | |
| | Furniture | | | |
| | Contraceptions / condoms | | | |
| | Masks / gloves / face shields | | | |
| | Syringes | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

*Note:
If the macro-debris cannot be weighed because it is too big / heavily encrusted / soaked, record its size (length, width, height) for mass estimation.

## PLANKTON TOW DATASHEET

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Surveyor name(s): | | | | | | Tow date: | | |
| Site name: | | | | | | Country: | | |
| Net type: | | | Mesh size: | | | Net mouth diameter: | | |
| ENVIRONMENTAL CONDITIONS (three readings per site) | | | | | | | | |
| Weather: clear rain/storm overcast drizzle | | | | | | | | |
| Sea surface temp (°C): | | | | | | Salinity (psu): | | |
| Dissolved $O_2$ (mg/L): | | | | | | Total suspended solids (mg/L): | | |
| Nitrate (mg/L): | | | | | | Phosphate (mg/L): | | |
| LAND-USE CHARACTERISTICS | | | | | | | | |
| Major site usage: tourism fishing protected isolated other: | | | | | | | | |
| Nearest town distance (km): | | | | | | Nearest river distance (km): | | |
| River input: yes no | | | | | | Pipe/drain input: yes no | | |
| Evidence of dumping: none construction household other: | | | | | | | | |
| Evidence of recent activities: none clean-up/rubbish removal apparent spilled trash storm/flood strong winds public event other: | | | | | | | | |
| Notes (*include descriptions on landmarks, coastal hydrography, etc*): | | | | | | | | |

| Tow details | #1 | #2 | #3 |
|---|---|---|---|
| Wind speed (m/s) | | | |
| Wind direction | | | |
| Start latitude | | | |
| Start longitude | | | |
| Start time | | | |
| Start flow meter count | | | |
| End latitude | | | |
| End longitude | | | |
| End time | | | |
| End flow meter count | | | |
| Average boat direction | | | |
| Average depth (m) | | | |
| Note | | | |

## WATER GRAB SAMPLING DATASHEET

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Surveyor name(s): | | | | | Sample date: | | | |
| Site name: | | | | | Country: | | | |
| Water sampler: | | | Volume (L): | | | Sampling depth (m): | | |
| ENVIRONMENTAL CONDITIONS (three readings per site) | | | | | | | | |
| Weather: clear rain/storm overcast drizzle | | | | | | | | |
| Sea surface temp (°C): | | | | | Salinity (psu): | | | |
| Dissolved $O_2$ (mg/L): | | | | | Total suspended solids (mg/L): | | | |
| Nitrate (mg/L): | | | | | Phosphate (mg/L): | | | |
| LAND-USE CHARACTERISTICS | | | | | | | | |
| Major site usage: tourism fishing protected isolated other: | | | | | | | | |
| Nearest town distance (km): | | | | | Nearest river distance (km): | | | |
| River input: yes no | | | | | Pipe/drain input: yes no | | | |
| Evidence of dumping: none construction household other: | | | | | | | | |
| Evidence of recent activities: none clean-up/rubbish removal apparent spilled trash storm/flood strong winds public event other: | | | | | | | | |
| Notes (*include descriptions on landmarks, coastal hydrography, etc*): | | | | | | | | |

| Sample details | #1 | #2 | #3 |
|---|---|---|---|
| Wind speed (m/s) | | | |
| Wind direction | | | |
| Latitude | | | |
| Longitude | | | |
| Time | | | |
| Note | | | |