NET4mPLASTIC PROJECT

Activity 3.1

D 3.1.1.f

Project monitoring approach: preliminary version

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CONTRIBUTING PARTNERS	UNIFE AND IZSAM

INDEX

This document describes the methods used within the NET4mPLASTIC project. Subsequent revisions may be introduced on the basis of the project needs.

For the project NET4mPLASTIC, the method that is used for the monitoring and laboratory analyzes is based on the protocol developed within the project DeFishGear for the monitoring of the beach litter and the sea surface microplastic.

1. Beach survey

The monitoring of the beach marine litter regards the macro litter (debris > 2.5 cm to 50 cm and debris > 50 cm), the large micro-litter (1 mm to 2.5 cm) and small micro litter (< 1mm). The beach survey starts with the sampling of the smallest debris - the small microplastics – that should be monitored on the top of the shore, (above the high water mark), in order to reduce the risk of contamination by the persons performing the large microplastic sampling.

1.1 Site description

Before the monitoring activities, information regarding the site will be "collected", eventually by filling a survey form that is provided in Annex 1 or by giving the following information in agreement with the Marine Litter Beach Questionnaire from the OSPAR Commission's Guideline for Monitoring Marine Litter on the Beaches in the OSPAR Maritime Area.

On this form the following information will be reported:

- Name of beach
- Date
- GPS information (GPS coordinate start 100 m, end 100 m, 1 m^2 quadrat position)
- Weather conditions ((for example recent rainfall, wind direction and strength)
- Beach conditions (wet, dry, cleanness…)
- Any factors affecting sampling: for example, disturbance of sand by human activities/beach cleaning, wind blowing sand, water entering quadrat, seaweed or other organic matter on beach

Furthermore, for each pilot site a detailed description of the beach will performed by providing the following information:

- Approximated length of the beach
- Approximated width of beach (from the shoreline to the back of beach)
- What is at the back of the beach: for example, dunes, grass, car park, houses, shops
- Orientation of the beach
- Prevailing currents off the beach (N / E / S / W)
- Prevailing winds (N / E / S / W)
- Type of beach material and percentage coverage (for example sand 80%, pebbles 20%)
- Beach topography (slope)
- Presence of hard defences (like breakwater) that influence the currents
- Major beach usage (local people, swimming/sunbathing, fishing, surfing, sailing etc) specifying the type of usage and the period when it occurs

Information regarding potential sources of microplastic pollution should also be noted by giving the following information:

- What are the nearest population centres to the beach (name, type, population size and distance from beach)
- Is there any development behind the beach (No/yes), and give a small description
- Are there any food/drink outlets on the beach (No/Yes), describe
- What is the distance from the beach to the nearest harbour: Name of harbour, type and size of harbour:
- Position of harbour in relation to survey area (N / E / S / W)
- What is the distance from the beach to the nearest shipping lane if present
- What type of boats use the shipping lane
- Are there any industrial areas near the survey area (No/yes), describe
- What is the distance from the survey area to the industrial area and indicate its position
- What is the distance from the survey area to the nearest river mouth: Name of river
- Position of river mouth in relation to survey area (N / E / S / W)
- Is the beach located near a wastewater discharge (No/Yes), describe
- Distance from the survey area to the discharge point
- Position of wastewater discharge in relation to survey area (N / E / S / W)
- Other kinds of pollution found on the beach (macroplastics, glass, metal)

Beach cleaning activity:

- How often is the beach cleaned:

  All year round: ☐Daily ☐Weekly ☐Monthly ☐Other:
- Seasonal, please specify months: .....

What method is used: Manual or Mechanical

1.2 Sampling

1.2.1 Micropalstic (<1mm)

Five replicate samples, separated by 5 meters, will be collected in a way that they will be representative of the beach. If the beach is narrow, then all the replicates will be sampled on the strand line. If possible, the sampling will be done above the shoreline and in the upper part of the beach (close to the dune foot if present). The person that will perform the sampling will be down the wind, avoiding synthetic clothing.

The sediment will be collected from the top 3 cm of sand, using a metal scoop/spoon. Defishgear method suggest to collect a series of scoops at arms-length at intervals within an arc shaped area to the front (Fig. 1).
About 250 ml of sediment should be collected using a glass becker. Before storing the material in a glass recipient/container, the sediment should be sieve through a 1 mm container.
The material which passed through the sieve can then be put in the glass recipient in order to be transfer to the laboratory for the separation operation.

Fig. 1: Defishgear microplastic sampling methods

<u>Material needed:</u>

- 1 mm sieve
- glass becker
- 5 sample containers (250 ml)
- metallic spoon (scoop)
- GPS

1.2.2 Large microplastic (1mm to 25 mm)

The sampling will allow to collect large microplastics that can be divided in two classes 25 to 5 mm and 5 to 1 mm. Similarly to the small microplastic, five replicate samples will be collected on the beach, and the replicated will be separated by at least 5 m. The samples will be realized between the shoreline (high water mark) and the back of the beach.

As for small microplastic sampling, it is suggested that the person in charge of sampling should be down wind of the sampling area and dressed with no-synthetic clothes to avoid contamination. Obviously plastic containers or instruments should be avoided.

The sampling will be done next to the previous sampling, e.g. the small microplastic locations. It is also recommended that small and large microplastics samplings spots should not be mixed, unless the beach is not long enough.

The sampling procedure first requires to place a quadrat (1 X 1 m, metallic or wood) on the sand surface, on the top of the shore (Fig. 2). With a metal scoop, the superficial sediment (top 3 cm) will be sampled within the quadrat and will be put in a 2l glass becker in order to calculate the volume of sediment sampled. Then the sediment will be sieved at 1 mm and 5 mm. This operation will be repeat as many times as necessary until the sampling of the 1 m^2 quadrat is completed (Fig. 3).

The total volume of the sediment sampled will be written and the material retained on the sieve (1mm and 5 mm) will be stored in metal or glass recipients or eventually in paper bags, before being transferred to the laboratory.

Material needed:

- GPS
- 1 m^2 quadrat
- 5 Sample containers (metallic or glass jars or paper bags)
- 2 Metal sieves with collecting pan (1 mm and 5 mm mesh size; preferably stacked together)
- 1 Glass beaker (2 l)
- 1 Metal scoop

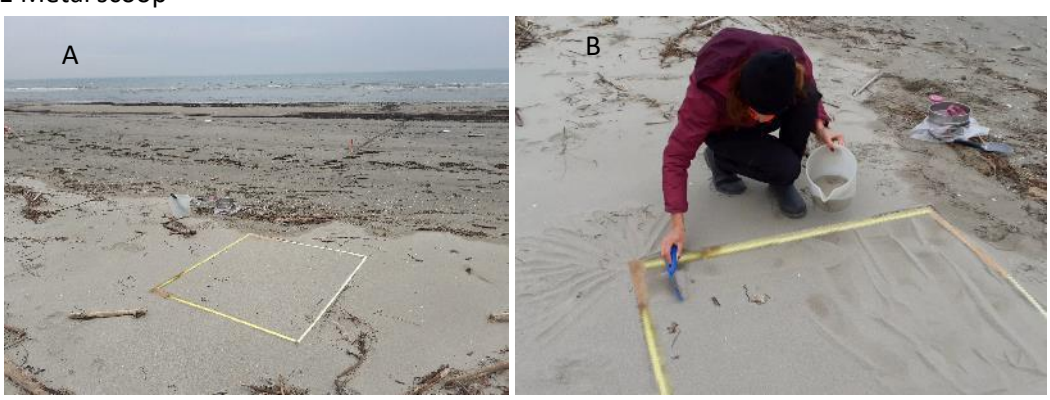

Fig. 2: Microplastic (2.5 cm to 1mm) *sampling: A)* Quadrat on the sand surface, B) Collecting the top 3 cm of sand with a metal scoop

Fig. 3: A)Sieving sediments B) Material retained on the 5 and 1 mm sieves

Fig. 4: Blue square indicates appropriate location for beach sediment microplastic

1.2.3 Beach litter's sampling >25 mm

The investigation area's minimum length must be 100 meters and must cover the entire beach width, from shore to dunes, or vegetation limit. The position of the area should be taken with a GPS.

The 100 m beach will be divided in different stretches/paths (5 or 10 m wide) perpendicular to the shoreline that will be further divided in quadrats in order to consider the different units of the beach (beach-face, low backshore, upper backshore, foot of the dune if present). The sampling will then be done by walking through the beach methodically, orthogonally from the shoreline, along 5-10 meters distance paths (Fig. 5) and collecting all the items >2.5 cm found on the beach.

All the litter standing on the beach surface, visible on the strand, even partially covered by the sand, will be collected, except those that you found digging. All the items will be stored in specific containers (correctly targeted). During the sampling, take pictures of the objects that are difficult to classify, and also of the "curious''.

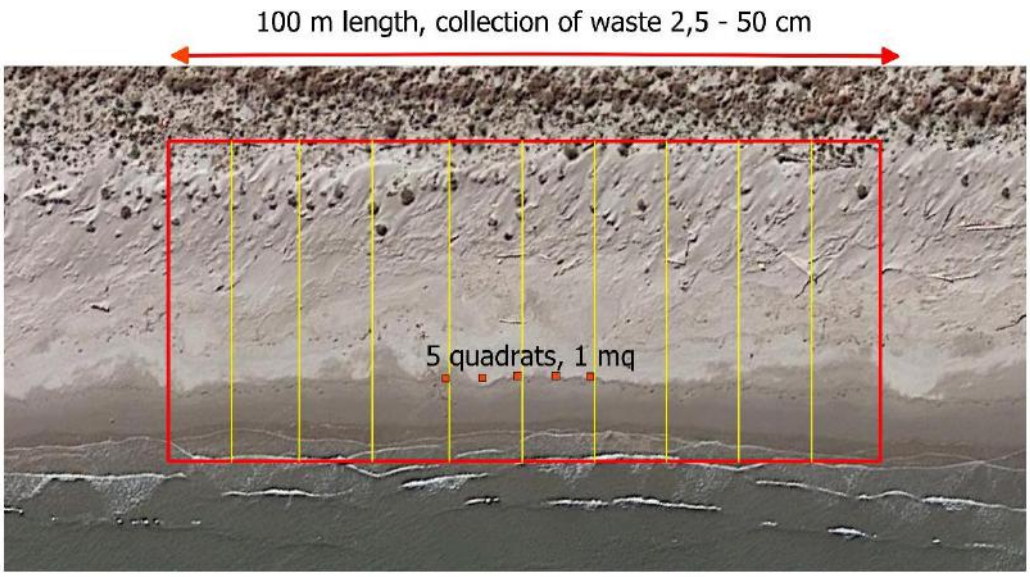

Fig. 5: 100 m investigation area; the paths to be done during the sampling are drawn in yellow, perpendicularly from the coast line and separated from a 10 meters distance from each other

Fig. 6: Images of marine litter collection (>25 mm)

Material needed:

- GPS
- Container
- Camera

1.2.4 Beach litter > 50 cm

Finally, if the beach is long enough (more than 1 km) the beach litter > 50 cm will be noted, described and photographed. The object found in the 1 km beach should be marked in order to signalize that they have been observed. Their position should be taken with the GPS.

1.4 Laboratory

1.4.1 Small microplastic (<1mm)

Each sample collected in order to determine the presence of SMP (Small microplastic), about 250 ml, will be divided into 5 parts (about 50 ml) that should be weighted using a centigram technical balance. We decide to use a concentrated saline NaCl solution (1.2 g/cm^3) to achieve bulk separation according to density because this solution is inexpensive, readily available, non-toxic. This solution has been most widely used to date and achieve good separation for most polymers. The saline solution is prepared by dissolving 360 g of NaCl in 1 liter of distilled water within a glass beaker, taking care to keep stirring the solution (mechanical stirrer equipped with a steel blade) for 20 - 25 minutes. Successively, the solution is filtered using a glass fiber filter (Wathman GF / D) to eliminate the presence of any impurities. The density of some solutions will be checked by using a He pycnometer (or similar instrument).

Fig. 7: Laboratory instruments

Therefore, 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 or using a magnetic stirrer, then allowed to settle for 4 minutes. However, is the sediment is rich of silt, then the settlement will be longer (about 10 min) or different filters should be used.

The supernatant is then transferred to suction filtration via a Buckner funnel and passed through a 10µm retention filter paper. Filter papers are removed and stored in sealed petri dishes prior to examination under a microscope (stereomicroscope with a 80X objective). The NaCl separation procedure is repeated three times with each sediment sample to ensure a high recovery of buoyant debris. Data from the three filter papers are added together. Finally, the suction filtration is cleaned with 200 to 500 ml of distilled water to remove the residues of the saline solution before being reused for the next sample. Extreme care will be taken to ensure the processing area is meticulously clean and in particular free from dust or particles.

Prior to microplastic identification, the filters in Petri dishes will be dried at the room temperature for 24 h in a desiccator or under a hood, so that no contamination occurs from the atmosphere. Alternatively, the petri dishes will be put in an oven at 40 °C overnight.

Fig. 8: Petri's dishes containing the filters to be analyzed

Fig. 9: Zeiss Stemi 508 stereoscopic microscope with camera used for the detection of small microplastics (SMP)

Any plastic objects observed on the filters will be counted and subsequently, if possible, picked up by using micro tweezers. Each individual observed object will be classified using Table 1 and Table 2 and will be photographed (for the subsequent determination of the dimensions, diagonal length measurements except for the filaments and writing the color).

Table 1: Categories of micro litter Items taken from EU TG ML Master List

Micro litter categories
Pellets (G107, G108, G109, G110, G111)
Granules (G116)
Filaments (G113)
Films (G114)
Foam (G115, G117)
Uncategorized plastic pieces*

Table 2: Master List of Colours and Transparency of Micro Litter Item

Colourof plastic items
White
Clear-white-cream
Red
Orange
Blue
Black
Grey
Brown
Green
Pink
Tan
Yellow

Transparency of plastic items
Transparent
Opaque

According to Hidalgo-Ruz et al. (2012) microplastic has the following characteristics:

1. Small size (largest dimension ≤5mm)
2. No cellular or organic structures visible
3. Fibers should be equally thick throughout their entire length
4. Particles should exhibit clear and homogeneous color throughout

1.4.2 Large microplastic (1 mm to 25 mm)

The procedure for the laboratory separation of LMP (1 mm – 5 mm) is the following:
1. Put the material retained on the 5 mm and 1 mm sieves into two or more (depending on the quantity) plastic trays.

2. Separate all artificial items size 5 – 25 mm (meso litter) (collected on the sieve with 5 mm mesh size) with tweezers in one glass vial. Categorize each found particle according to the Annex 3. Note the weight of all particles together.

3. Separate all artificial items (litter) size 1 – 5 mm (collected on the sieve with 1 mm mesh size), categorize each particle according to the Table 1, describe each particle with the color according to the Table 2, measure the length of each particle (measure the longest diagonal) and store them in the glass vials for each category separately. For the smaller items you may use a stereomicroscope.

4. Weigh the particles in each category. If the weight of the particles of each category is too small for weighing, then weigh the particles from all categories together.

1.4.3 Beach litter >25 mm

Back to the laboratory, classify all the items found on the field using the master list reported in Annex 2 and weight all items from the same category. The results will be put on a specific form (Annex 3).

2 Drone Survey

The detection of macroplastics can be performed using imaging systems on Unmanned Aerial Vehicles (UAVs). Remotely piloted platforms have the advantage of flexibility and versatility, especially considering lightweight aircrafts. The pixel size, also known as Ground sample Distance (GSD), depends on the actual flight altitude of the UAV, as well as it depends on the technical specifications of the camera used for the aerial survey. Parameters such as the field of view and the focal length of the imaging system impact on the actual GSD. However, generally speaking, at lower flight altitudes with the same camera it is possible to increase the spatial resolution (lower GSD).

Consequently, the flight planning should consider a minimum size of macroplastic litter that has to be detected through the aerial survey. Largest items can be recognized at high altitudes, but smaller ones, such as bottle stoppers, are usually more difficult to be detected on images. Another important aspect to consider is the possibility that part of an even larger item may be covered by the sand. This is quite common when detecting and classifying beach litter and makes the macroplastic identification harder.

Fig. 10: From the left to the right: ground survey picture; low-altitude aerial image; high-altitude aerial image.

Using aerial images it is possible not only to detect items, but also to describe their properties such as the shape or the color, as well as to measure the object size. All the information about an item can therefore help operators to classify the object properly, discerning plastics from other type of litter.

Within this process, it is also the experience of the operator that may impact on the final detection of small items.

While a general mapping of beach litter can be performed with a low spatial resolution to detect main macroplastics (i.e. large items), the quantification and characterization of a wider range of items should be carried out with a small GSD (low flight altitude) in order to detect the highest number of plastics as possible. This task is generally applied to the mapping of small beach areas that are assumed as test-sites and that can be representative of the overall beach litter.

The introduction of Ground Control Points (GCPs) for the georeferencing of the aerial orthomosaics enables the user to assess also the spatial distribution of macroplastic litter with a centimeter-level accuracy. In this way, it is possible to identify zones in which the litter accumulates as well as to monitoring the migration of single items along the beach.

RTK drones take advantage from their own on-board GNSS receiver and make the need for GCPs unnecessary: orthomosaics can be generated at centimeter-level accuracy on the basis of aerial images only. In this way, both the mapping operations and the monitoring activities are less time-consuming.

In addition to traditional RGB imagery, UAVs can be equipped with multispectral sensors providing information in the near infrared wavelengths. Vegetation is easily recognizable and the different response of items can improve the decision making process of litter detection and classification.

The efficiency of macroplastic litter identification by aerial images can be assessed through a comparison with ground surveys. As previously highlighted, many are the aspects that affect the final rate of detection and the accuracy of classification.

However, the use of imaging systems on UAV, especially when combined with RTK receivers on-board, is a powerful tool for the monitoring of beach litter and can identify potential microplastic sources. Largest accumulation areas can be detected with wide aerial missions at high altitudes, while detailed characterizations can be performed in a particular zone of interest.

Fig. 11: Drones during field campaign

3 Seabed survey

Sampling is performed on the pilot sites along at least 2 predefined transects following a linear course using a GPS. The wind speed should not be more than 2 Beaufort and/or the wave high should not be more than 0.3 m.

Samples are collected using Van Veen grab (200 × 300 mm; 0.1 m2), at the following depths: -2, -4, -6 m. Van Veen sampler is attached with the rope (Φ6 mm) onto the boom part of the boat and manually or automatically retrieved once in the water. The sampler is opened very carefully to keep the sediment sample intact. Sediment samples are removed directly from inside the grab after each individual deployment, placed in double plastic bags, labelled and stored until processed for the analysis. Each sampling is deployed 2 times.

Similarly, a sediment sample is taken at the same depths for granulometry analysis.

See paragraphs 1.4.1 and 1.4.2 for laboratory separation and analysis.

Fig. 12: Sampling with Van Veen grab

4 Sea surface survey

4.1 Sampling

Sampling will be performed on the pilot sites and eventually on the river outflow or near the river mouth. The sampling will be done by deploying a manta from the vessel that will travel at a speed ranging from 2 to 3 knots. Therefore, the start and stop point of transect will be recorded using a GPS. During trawls, a steady linear course at a constant speed will be maintained. Furthermore, as suggested by the Defishgar protocol (http://mio-ecsde.org/wp-content/uploads/2014/12/Protocols-sea-surfacebeach-sediments-Feb15.pdf) regarding the weather conditions, the wind speed should not be more than 2 Beaufort and/or the wave high should not be more than 0.5 m. The manta net collects material >300 µm. The manta net will be deployed from the side of the vessel using for instance spinnaker boom and attention will be taken to ensure that half part of manta net opening will be submersed during the sampling.

The Defishgear protocol reports the following operations:

- Deploy the manta net out of the wake zone (at about 4 m from the vessel) in order to the effect of the turbulence inside the wake zone and note the GPS coordinate of the vessel and the initial time
- Travel in one straight direction (2 - 3 knots) and collect the debris on the sea surface for 30 min (or less in case in case of huge amount of natural material, e. g. plankton bloom).
- After 30 min stop the boat and record the final GPS coordinates. Consequently, the length of the route and average boat speed will be calculated and recorded as well using eventually a specific data form that will be used by the partners (Annex 4)
- Pick up the manta net from the water and rinse the manta net thoroughly from the outside of the net with sea water using a pump or water from the boast water. The rinsing operation should be done from the manta mouth to the cod end in order to concentrate all particles sampled into the cod end. Attention: the rinsing operation should not be done through the opening of the net to prevent contamination.
- Safely remove the cod end over a bucket, from the net using screw driver and invert the cod end and sieve the sample in the cod end through 300 µm mesh size sieve (or less).
- Rinse cod end thoroughly from the outside and pour the rest of the sample through the sieve (repeat this step until no particles are present inside the cod end).
- Concentrate all material on the sieve in one part of the sieve.
- With the use of funnel, rinse the sieve into glass jar or plastic bottle by using 70 % ethanol (one sample max. 250 ml in the end).
- Close the bottle, wipe it with paper towels and label the lid and outside of the jar with the sample name, date, location and eventually GPS coordinates. The sample should be then transfer into the cool box.

Fig. 13: Images of sampling campaign

4.2 Laboratory analysis

The methodology proposed within the Defishgear project reports the following steps:

- Pour sample through the sieve (≤ 300 µm mesh size) and remove all natural or artificial litter objects of size > 5 mm (macro and mezzo litter) from the sample, using visual identification and tweezers.
- Rinse each removed object carefully with distilled water in order to remove microplastic litter adhered to it. Store all natural and artificial litter objects in separate containers. Dry all natural and artificial litter objects in a desiccator (or on the air, but in closed dish) and weigh them.
- Collect and identify according to the WP4 list of Marine Litter (Annex 3) all items > 25 mm and weight them
- After removing all larger objects concentrate all remaining pieces in one part of the sieve using squirt bottles or tap water. Pour sample into glass container using minimum amount of alcohol with the help of funnel.
- Take small amount of sample (subsample) and pour it into glass Petri dish. Analyze the sample with the use of stereomicroscope (20 - 80x zoom) in order to identify microlitter particles.
- The microplastic particles identified will be then categorized according to Table 1 and Table 2.
- Each subsample should be reviewed by another person. Be careful to rinse the glass container with the sample so that all particles adhered to glass walls are washed into Petri dish.
- When all of subsamples of one general sample are checked by two persons, weigh the microplastic particles of each category separately. Microplastic particles need to be previously dried.
- Put Petri dish under the microscope with measuring equipment and measure the size of each particle (measure the longest diagonal), except filaments and note its colour (Table 2).

5 Biota survey

5.1 Sampling

Biota can be sampled from the environment in many ways including trawling, nets, cages and hand collection from shore. When collecting mussels for microplastic analysis, it is important to carefully remove the byssus threads from the substrate to avoid stressing them. The time between collection and preservation should be as short as possible to minimize stress. Furthermore, to avoid the loss of microplastics possibly present inside the mussels it is necessary to freeze them immediately after sampling, as when mussels are stressed they close.

Mussels (*Mytilus galloprovincialis*) will be collected, for both Italy and Croatia, from 2 macro-areas.
For each sampling station, mussels will be taken from both farms and natural banks. Specifically, the sampling will be spread over the years 2019-2020 and 2020-2021, proceeding to carry out an autumnal and a spring sampling for each station for each year, in order to assess seasonal changes in plastic pollution.

Natural banks

In natural banks the mussels will be harvested using different techniques depending on their position in the water column:

- ✓ Mussels on the surface (0-1 m) will be harvested by hand picking;
- ✓ Mussels submerged will be collected by snorkeling.

In this case it is necessary to create the global sample consisting of 3 elementary samples taken at the surface, in the middle and at the bottom of the natural banks.

Mussel farms

In a long line type mussel farms, sampling involves the creation of a global sample composed of 3 primary samples which will be performed on three different depth levels:

- greater depth
- in the middle
- at 50 cm from the surface

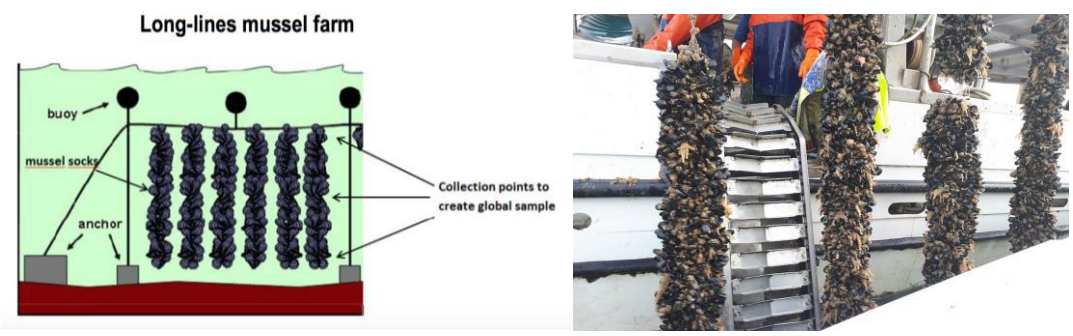

Fig. 14: Long-lines mussel farm

You will need to collect for each sampling station a required amount of about 10 kg of mussels (size 4-7 cm in length). Only individuals who are alive and obviously not damaged will be collected.

From the starting sample 3 kg must be frozen immediately after collection as they will be used for the detection and quantification of MP and for chemical analyzes related to the determination of the presence of PCBS / Dioxins / PAHs and heavy metals (lead, cadmium, mercury).

The remaining part of the sample will be kept at a refrigerated temperature of 4-6 °C and will be sent within 24 hours to the laboratory that will perform the analyzes for the assessment of the intestinal clearance rate in bivalves at the following address:

Viale Marinai d'Italia
86039 Termoli (CB)
Resp. Dr. Nadia Barile
Tel. 0875 81343

At the time of sampling it will be advisable to record a series of parameters, present in the appropriate sampling sheet (see ANNEX 5), in order to have more information on the sample taken.

Measures for mussel collection

- Handling stress and physical movement may cause loss of microplastics through gut evacuation or inversion prior to preservation.
- When collecting mussels for microplastic analysis, it is important to carefully remove the byssus threads from the substrate to avoid stressing them. The time between collection and preservation should be as short as possible to minimise stress.
- Only alive and not obviously damaged individuals were collected. Individuals for chemical and MP detection analysis will be frozen (- 20 °C) as soon as possible after collection while those used for depuration analysis will be stored at 4 C° and will be sent to IZS within 24 hours of collection.

5.2 Laboratory analysis

Initial sample preparation

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

- A sample size of at least 30-50 specimens is recommended
- Defrost mussel samples
- Individuals will be measured with calipers before opening. Soft tissue will be excised from the shells and weighed (g, w.w.)

The method used for the microplastic separation is based on a digestion procedure using 30% H_2O_2 solution to degrade natural organic matter in order to facilitate detection of small microplastic particles, and consists in the following steps:

- Add 20 ml of 30% H_2O_2 per 1 g of soft tissue
- Incubate for 24 h at 55 - 65°C (cover with aluminum foil to avoid air contamination)
- After samples will be removed from the incubator and cooled, the homogenate was filtered under vacuum into membrane filter (Whatman, Glass Microfiber filters GF/D, 2.7 µ)
- Rinse the conical flask 3 times with 50 ml of distilled water and filter this water.
- Dry the filter paper at room temperature overnight (cover e.g. in petri dishes).
- Check the filter paper for microplastic particles by the use of stereo microscope (LEICA MZ6) with image analysis software (Image management systems-IM LITE).

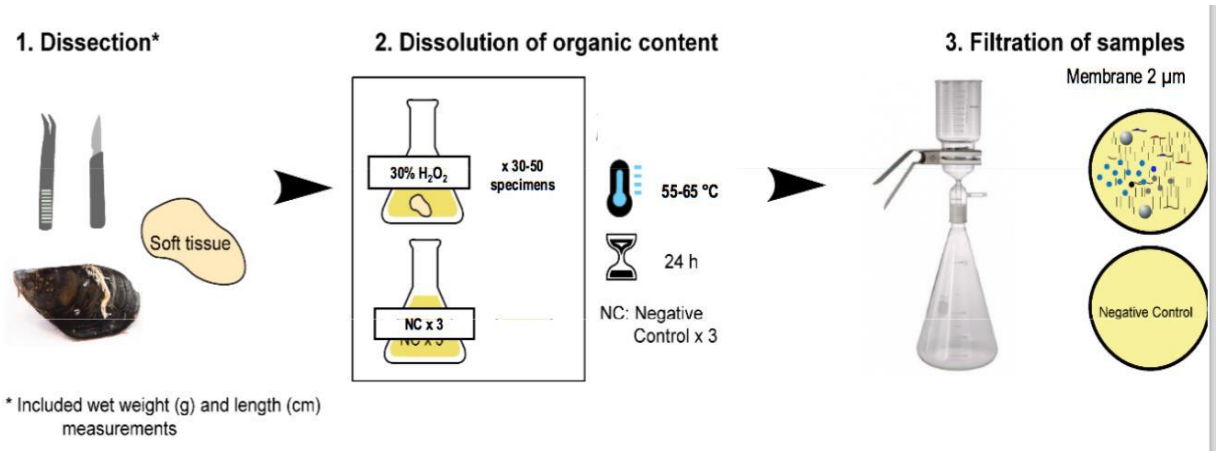

Fig. 15: Laboratory procedure

Note: It possibile to use other reagents for digestion procedure: Alkaline digestion: 10% KOH solution mixed digestion: 10% KOH: 30% H_2O_2 acid digestion: HNO_3 69% solution

The microplastic items that will be found inside the mussels will be categorized for:

- colour (white, clear, red, blue, green, yellow, black, other colours);
- type (fragments, pellets, filaments, film, foam, granules, not categorized);
- size class (15 µm, 15-50 µm, 50-100 µm, 100 -500 µm, > 500 µm) (Fig 16: MP classification table).

Furthermore, the University of Trieste/Ferrara on this MP items will carry out Raman spectroscopy analysis for identification of the different polymers.

Colour								Type							Size class				
white	clear	red	blue	green	yellow	black	other colours	fragments	pellets	filaments	film	foam	granulues	not categorized	< 15 µm	15-50 µm	50-100 µm	100 -500 µm	> 500 µm

Fig. 16: MP classification table

Negative controls and blanks

In the process of degradation, the negative control should be included (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)

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). Most probably contamination with fibers from the air will occur.

In order to reduce contamination, the following principles should be observed:

- keep your filter covered whenever possible. If you are not looking at it under the microscope, it should be covered,
- Store filters in glass petri dishes.
- Wear cotton or natural fiber clothes

In order to assist the visual identification, the *hot needle test* could be use, especially to distinguish plastic pieces from organic matter or other anthropogenic debris.

In the presence of a very hot needle, plastic pieces will melt or curl, while biological and other non-plastic materials will not. It is important that the needle is sufficiently hot (e.g., >200 °C) or plastics may not react. However, some particle types (e.g., microbeads) may not exhibit a clear reaction based on their form. Semi-synthetic fibres such as rayon will not react based on their chemical composition (typically produced from organic material).

6 Chemical analysis

The chemical analysis is reported in the reports of activity 3.4. but the DEFISGEAR protocol indicates:

1. ATR-FTIR spectroscopy
    1. Prior to the analysis clean the detection system with alcohol and a lint free cloth.
    2. Record a background spectrum. Place the sample on the sample holder and collect the spectra. Identify the obtained ATR- FTIR spectra using an automated comparison of the obtained spectrum with spectra in a database.

2. Micro ATR-FTIR spectroscopy
    1. Prior to the analysis clean the detection system with alcohol and a lint free cloth.
    2. Place the sample on a glass filter. Note: Other filters can be used but their polymer nature can interfere with the characterization.
    3. Place the filter with the sample on the automatic scanning table and use the joystick to locate the sample.
    4. Record an optical image and mark an area (e.g. 20 by 20 µm) where the sample will be characterized.
    5. Record a background spectrum.
    6. Place the sample on the sample holder and collect the spectra at the predefined location.
    7. Identify the obtained micro ATR-FTIR spectra using an automated comparison of the obtained spectrum with spectra in a database.

Annex 1

BEACH IDENTIFICATION FORM				
Organization (Who?): **Email:** **State, region:** **Telephone number:**				
City		**Date** **Responsible**		
Beach name				
Investigation area's coordinates (gg,ggg°)				
Start		End		
LAT:	LONG:	LAT:	LONG:	
Investigation area's effective width (m)		100 m X ….width		
Beach description				
Are there urban areas?			Yes	No
City/Town		City	City	
Distance from the investigation areas (km)				
Are there rivers' estuaries or water sewers? Yes No			Yes	No
Rivers/sewers name		River	River	
Distance from the investigation areas (km)				
Are there harbours nearby? Yes No			Yes	No
Name of the closer harbours		Harbour	Harbour	
Distance from the investigation areas (km)				
Are there industrial sites nearby? Yes No			Yes	No
Name of the closer sites Site		Site	Site	
Distance from the investigation areas (km)				
Are there beach resorts/kiosks nearby? Yes No				
Is it a bathing beach? Yes No				
Access to the beach Vehicles Yes No Pedestrian Yes No Only by the sea Yes No				
Other				
Beach type				
SILT - SAND – PEBBLES				
NOTES (events that can influence the sampling)				
i.e wind, rain, etc…				

Annex 2

BEACH LITTER SAMPLING FORM (100 m)				
Beach name:				
Date:				
CODE (annex)	**Number of objects**	**Category**	**More than 25 cm size object (Yes/No)**	**Weight**

Annex 3

CODES FOR MACRO LITTER

Code	Items name	Item counts	Total
ARTIFICIAL POLYMER MATERIALS			
G1	4/6-pack yokes, six-pack rings		
G3	Shopping Bags		
G4	Small plastic bags, e.g. freezer bags, including pieces		
G5	Plastic bag collective role; what remainsfrom rip-off plastic bags		
G7	Drink bottles <=0.5l		
G8	Drink bottles >0.5l		
G9	Cleaner bottles & containers		
G10	Food containers incl. fast food containers		
G11	Beach use related cosmetic bottles and containers, eg. 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 drinks		
G22	Plastic caps/lids 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		
G32	Toys and party poppers		
G33	Cups and cup lids		
G34	Cutlery and trays		
G35	Straws and stirrers		
G36	Fertiliser/animal feed bags		
G37	Mesh vegetable bags		
G40	Gloves (washing up)		
G41	Gloves (industrial/professional rubber gloves)		
G42	Crab/lobster pots and tops		
G43	Tags (fishing and industry)		
G44	Octopus pots		
G45	Mussels nets, Oyster nets		
G46	Oyster trays (round from oyster cultures)		
G47	Plastic sheeting from mussel culture (Tahitians)		
G49	Rope (diameter more than 1cm)		
G50	String and cord (diameter less than 1cm)		
G51	Fishing net		

G53	Nets and pieces of net < 50 cm		
G54	Nets and pieces of net > 50 cm		
G56	Tangled nets/cord		
G57	Fish boxes - plastic		
G58	Fish boxes - expanded polystyrene		
G59	Fishing line/monofilament (angling)		
G60	Light sticks (tubes with fluid) incl. packaging		
G62	Floats for fishing nets		
G63	Buoys		
G64	Fenders		
G65	Buckets		
G66	Strapping bands		
G67	Sheets, industrial packaging, plastic sheeting		
G68	Fibre glass/fragments		
G69	Hard hats/Helmets		
G70	Shotgun cartridges		
G71	Shoes/sandals		
G72	Traffic cones		
G73	Foam sponge		
G75	Plastic/polystyrene pieces 0 - 2.5 cm		
G76	Plastic/polystyrene pieces 2.5 cm >< 50cm		
G77	Plastic/polystyrene pieces > 50 cm		
G78	Plastic pieces 0 - 2.5 cm		
G79	Plastic pieces 2.5 cm >< 50cm		
G80	Plastic pieces> 50 cm		
G81	Polystyrene pieces 0 - 2.5 cm		
G82	Polystyrene pieces 2.5 cm >< 50cm		
G83	Polystyrene pieces > 50 cm		
G84	CD, CD-box		
G85	Salt packaging		
G86	Fin trees (from fins for scubadiving)		
G87	Masking tape		
G88	Telephone (incl. parts)		
G89	Plastic construction waste		
G90	Plastic flower pots		
G91	Biomass holder from sewage treatment plants		
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 bag		
G102	Flip-flops		
G108	Industrial pellets		
G124	Other plastic/polystyrene items (identifiable)		
RUBBER			
G125	Balloons and balloon sticks		
G126	Balls		

G127	Rubber boots		
G128	Tyres and belts		
G129	Inner-tubes and rubber sheet		
G130	Wheels		
G131	Rubber bands (small, for kitchen/household/post use)		
G132	Bobbins (fishing)		
G133	Condoms (incl. packaging)		
G134	Other rubber pieces		
CLOTH/TEXTILE			
G137	Clothing / rags (clothing, 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)		
PAPER/CARDBOARD			
G147	Paper bags		
G148	Cardboard (boxes & fragments)		
G150	Cartons/Tetrapack Milk		
G151	Cartons/Tetrapack (others)		
G152	Cigarette packets		
G153	Cups, food trays, food wrappers, drink containers		
G154	Newspapers & magazines		
G155	Tubes for fireworks		
G156	Paper fragments		
G158	Other paper items		
PROCESSED/WORKED WOOD			
G159	Corks		
G160	Pallets		
G161	Processed timber		
G162	Crates		
G163	Crab/lobster pots		
G164	Fish boxes		
G165	Ice-cream sticks, chip forks, chopsticks, toothpicks		
G166	Paint brushes		
G167	Matches & fireworks		
G171	Other wood < 50 cm		
G172	Other wood > 50 cm		
METAL			
G174	Aerosol/Spray cans industry		
G175	Cans (beverage)		
G176	Cans (food)		
G177	Foil wrappers, aluminum foil		
G178	Bottle caps, lids & pull tabs		
G179	Disposable BBQ's		
G180	Appliances (refrigerators, washers, etc.)		
G181	Tableware (plates, cups & cutlery)		
G182	Fishing related (weights, sinkers, lures, hooks)		
G184	Lobster/crab pots		

G186	Industrial scrap		
G187	Drums, e.g. oil		
G188	Other cans (< 4 L)		
G189	Gas bottles, drums & buckets (> 4 L)		
G190	Paint tins		
G191	Wire, wire mesh, barbed wire		
G193	Car parts / batteries		
G194	Cables		
G195	Household Batteries		
G198	Other metal pieces < 50 cm		
G199	Other metal pieces > 50 cm		
GLASS/CERAMICS			
G200	Bottles, including pieces		
G201	Jars, including pieces		
G201	Light bulbs		
G203	Tableware (plates & cups)		
G204	Construction material (brick, cement, pipes)		
G205	Fluorescent light tubes		
G206	Glass buoys		
G207	Octopus pots		
G208	Glass or ceramic fragments >2.5cm		
G210	Other glass items		
UNIDENTIFIED AND/OR CHEMICALS			
G211	Other medical items (swabs, bandaging, adhesive plaster, etc.)		
G213	Paraffin/Wax		

Annex 4

SEA SURFACE SURVEY FORM	
Place: **Harbor name**:	
Date:	
Wind speed	
Mean boat speed	
1.GPS coordinates starting point Time:	
1.GPS coordinates ending point Time:	
NOTE 1	
Length transect 1	
2.GPS coordinates starting point Time:	
2.GPS coordinates ending point Time:	
Length transect 2	
NOTE 3	
3.GPS coordinates starting point Time:	
3.GPS coordinates ending point Time:	
NOTE 2	
Length transect 3	

Annex 5

NET4mPLASTIC

New Technologies for macro and Microplastic Detection and Analysis in the Adriatic Basin

Sampling BIOTA form

Partner/Organizzation	Date of Sampling	Country - Macroarea	Species sampled	Weather condition (rainy –sunny – cloudy)	*Remark*

ID campione	
Start/end time	
GPS latitude	
GPS longitude	
Habitat (natural bank or mussel farm)	
Depth	
Collection method	
Average T° water	
Wind (direction and speed)	
Date and time of arrival in the laboratory	

Water column parameters		
Surface	T (°C)	
	Ph	
	Salinity (ppt)	
	O_2 (mg/l)	
Intermediate	T (°C)	
	Ph	
	Salinity (ppt)	
	O_2 (mg/l)	
Bottom	T (°C)	
	Ph	
	Salinity (ppt)	
	O_2 (mg/l)	

Quantity for each sampling:
8-10 kg of mussels (size 4-7 cm):

☐ For MP quantification: about 2 kg

☐ For Chemical analyses: about 4-5 kg

☐ For Clearance experiment: about 2 kg