2014 - 2020 Interreg V-A
Italy - Croatia CBC Programme
Call for proposal 2019 Strategic

MARLESS - MARine Litter cross-border awarenESS and innovation actions

Priority Axis: Environment and cultural heritage
Specific objective: Improve the environmental quality conditions of the sea and coastal area by use of sustainable and innovative technologies and approaches

PROTOCOL FOR MONITORING MICROPLASTICS LITTER IN BIOTA

PROJECT MARLESS

11.10.2021

Work Package:	**3. Monitoring optimization**
Activity:	**3.1 Harmonization of the monitoring plan and methodology**
Phase Leader:	**ARFVG – Autonomous Region of Friuli-Venezia Giulia**
Deliverable:	**3.1.3 Agreement on a method to use in the following activity**

Version:	**Final**	**Date:**	**11/10/2021**
Type:	**Methodology**		
Availability:	**Public**		
Responsible Partner:	**PP2 ARFVG – Autonomous Region of Friuli-Venezia Giulia**		
Involved Partners	**LP ARPAV, PP5 UNIDU, PP6 UNIBO, PP7 CIM-IRB, PP10 Puglia Region**		
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1. INTRODUCTION

Microplastics significantly vary in size, shape, color, specific density, polymer type, and other characteristics. In order to have comparable analysis results of microplastic in biota it is important to define criteria for quantifying parameters such as abundance, distribution and composition of microplastics by forming protocols.

2. EQUIPMENT

Equipment needed for sampling is listed below:

- GPS
- Cooler containers (ice boxes)
- Aluminum foil or aluminum trays for fish packaging
- Latex gloves without powder

Equipment needed for sample separation is listed below:

- Measuring ruler
- Analytical Balance
- Dissection scissors
- Scalpels and blades
- Dissection forceps
- Pinpoint tweezers
- Petri dishes
- Filter paper
- Stereomicroscope (min. 80x zoom; also recommended: transmitting light with dark field, polarization contrast and ring light)
- Glass flasks or beakers
- H2O2 30%
- Hot plate
- Concentrated saline solution (250 g NaCl/L H2O)
- Magnetic stirrer
- Glass pipettes (e.g. 10ml)
- 0.2µ membrane filters (e.g. Nucleopore Track-Etch Membrane Whatman)
- Filtered or distilled water
- Büchner funnel or a vacuum filtering device
- Laboratory oven
- Fume hood
- FT-IR spectrometer

- Lab coat
- Latex gloves without powder

3. MUSSELS SAMPLING

There are three options for doing biota sampling, where the first option has the highest priority and the last option has the lowest priority:

- sampling by the research team

sampling in collaboration with fishermen

- sampling done on the fish market

Sampling location should always be reported. It is recommended to have a sample size of at least 30 specimens per species and age group. Four different fish species should be sampled.
Directly after sampling the following parameters should be recorded:

- sampling location
- trawl/fishery type
- species
- date and time of capture
- depth

Biota samples should be frozen immediately after sampling and then transported to laboratory.

4. LABORATORY SEPARATION OF MICROPLASTICS

1. Biota samples should be defrosted before starting an analysis and assigned with an ID.

2. Their length, weight, visible deformations and skin condition (e.g. ulcers), gender and maturity stage should be recorded.

3. Whole tissue should be carefully removed, rinsed in deionized water, placed in separate petri dishes and weighted.

4. The whole mussel tissue is examined using a stereomicroscope in order to identify plastics.

5. In case unidentified items are found, those should be removed with forceps, placed on clean filter papers in petri dishes and sealed for further examination by FT-IR Spectroscopy. Number of items found, their size (longest diagonal), color and shape should be recorded (see Table 1 and Table 2).

6. When the items seen under stereomicroscope are removed, a digestion procedure using 30% H_2O_2 is suggested for degrading natural organic matter to ease the detection of small microplastic particles. After the H_2O_2 treatment 1 mm sieve should be used to isolate the fraction smaller than 1 mm.

7. The whole mussels tissue are placed in glass flasks and 30% H_2O_2 is added (approximately 50 ml of 30% H_2O_2 per gram wet weight).

8. Flasks are placed on a hot plate at 55 to 65 ˚C until all H_2O_2 is evaporated (fume hood). The flasks should be covered with aluminum foil while digesting to avoid contamination from air.

9. In order to separate microplastics by flotation, 100 mL of concentrated saline solution (250g NaCl/L dH_2O) is added, stirred at high intensity for 1–2 min using a magnetic stirrer, left to settle for 1-2 min. Supernatant is then transferred by a glass pipette onto 0.2 µ membrane filters using a vacuum filtering device. The microplastic separation is repeated three times.

10. Filters should be oven dried at 60 ˚C.

11. Filters should be examined for microplastics using a stereomicroscope. Filters are examined for microplastics under a stereomicroscope. Number of items found, their size (longest diagonal), color and shape should be recorded (see Table 1 and Table 2). Filters are then placed in petri dishes and sealed for further analysis by FT-IR Spectroscopy.

12. Clean filters should be air exposed in the working area during the microplastic separation procedure as a contamination control.

Table 1. Master list of categories of micro litter items

General code	General name
G103	Plastic fragments rounded <5 mm
G104	Plastic fragments subrounded <5 mm
G105	Plastic fragments subangular <5 mm
G106	Plastic fragments angular <5 mm
G107	Cyllindrical pellets <5 mm
G108	Disks pellets <5 mm
G109	Flat pellets <5 mm
G110	Ovoid pellets <5 mm
G111	Spheruloids pellets <5 mm
G113	Filament <5 mm
G114	Films <5 mm
G115	Foamed plastic <5 mm
G116	Granules <5 mm
G117	Styrofoam <5 mm
G217	Other (glass, metal, tar) < 5 mm

Table 2. Master list of colors of micro litter items

Color Name
Transparent
Crystalline
White
Clear-white-cream
Red
Orange
Blue
Opaque
Black
Grey
Brown
Green
Pink
Tan
Yellow

5. META DATA

The following information should be noted:

- Sampling date,
- Location coordinates,
- Trawl/fishery type,
- Species,
- Length and weight,
- Gender,
- Visible deformations and skin condition,
- Reproductive state,
- Weight of gut and contents, presence of parasites etc.

6. REQUIRED REPORTING INFORMATION

Items found per gram of intestine should be reported, as well as their size, color, shape etc. Species and standard dimensions should also be reported. In case FT-IR is used for the analysis then the polymer type is also reported.

Microplastics should be categorized by their size where the minimal resolution level is represented by the material found in size bins of 100 µm (e.g. 20-100 µm, 101-200 µm, 201- 300µm etc.).

7. REFERENCES

[1] IPA DeFishGear 2014.