



Protocol for invertebrates and commercial species - Toolkit for monitoring ML and its impacts on biodiversity in Med MPAs

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*Plastic Busters MPAs – Regional Marine Litter Monitoring Protocols Hands-on
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Sampling of invertebrates and commercial/Harvest species

Organisms will be sampled taking into account the different trophic levels and niches that they occupy in order to obtain a complete overview of the threats caused by microplastics on the whole marine food web

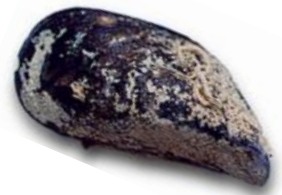
Isopods, Jellyfish and Lanternfish



Crab



Mussels



Sea urchins



Coastal waters

Bogue



Red striped mullet



Fish

Open waters

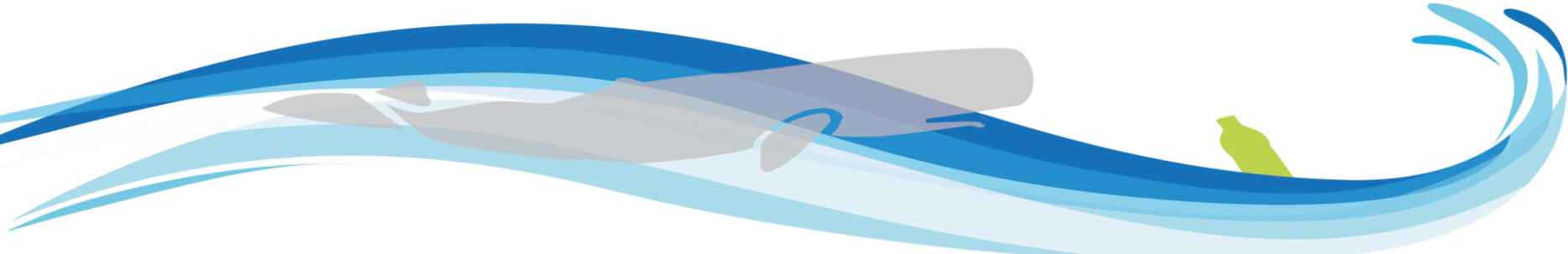
European pilchard



European anchovy

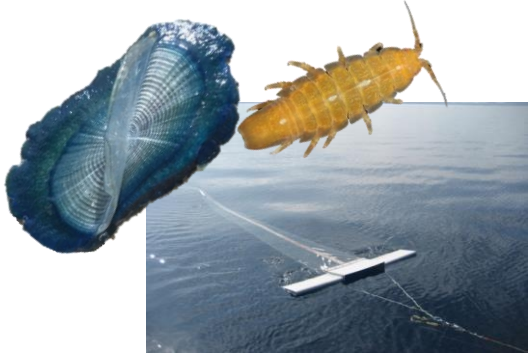


Sea floor



Sampling methodology

Plankton net



Caught by hand or with the help of baits



Bottom trawl



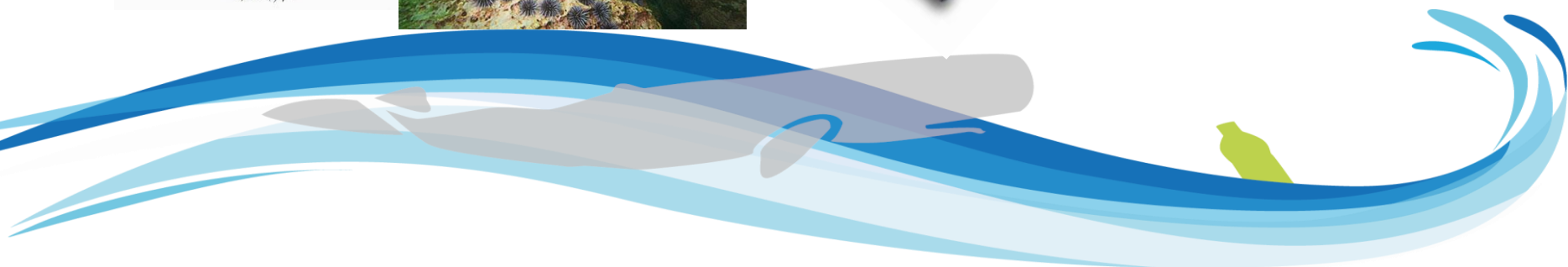
Artisanal fixed net



Scuba divers



ALIVE fish can be collected by researchers on board during the different fishing activities or at landings



Sampling methodology: invertebrates

Monitoring Microlitter in biota: mussels

Sampling date and time	Sampling site	GSA	Sampling method	Depth	Coordinates	
					Latitude	Longitude

ID code	Species	Sex	Shell weight (g)	Flesh weight (g)	Digestive gland weight (g)	Soft tissues weight (g)	Hemolymph	Muscle

Notes and remarks:

- Record the name of the species
- Weigh the each individual
- Length and width of each individual
- Record any visible deformations

Monitoring Marine Litter in biota: crabs

Sampling date and time	Sampling site	GSA	Sampling method	Depth	Coordinates	
					Latitude	Longitude

ID code	Species	Sex	Carapace length (cm)	Carapace width (cm)	Total weight (g)	Dig. gland weight (g)	Soft tissues weight (g)	Hemolymph	Hemolymph smears	Pincers	Gills	Muscle

Notes and remarks:

Sampling methodology: fish

Monitoring Marine Litter (Macro-Micro) in biota: dead fish

Sampling date and time	Sampling site	Boat name	GSA	Sampling gear	Depth	Coordinates	
						Latitude	Longitude

ID code	Species	Sex	Total length (cm)	Total weight (g)	GI weight (g)	Muscle	Liver weight (g)

Notes and remarks:

Rack (N₂)

Monitoring Marine Litter (Macro-Micro) in biota: alive fish

Sampling date and time	Sampling location	Boat name	GSA	Sampling gear	Depth	Coordinates	
						Latitude	Longitude

ID code	Species	Sex	Total length (cm)	Fork length (cm)	Total weight (g)	GI weight (g)	Muscle	Liver weight (g)	N°. of liver aliq.	Bile	Brain	Kidney	Gonad weight (g)	Blood smears	Plasma

Notes and remarks:

Rack (N₂)

- Record the name of the species
- Weigh the whole fish
- Measure the total and fork length of the fish

- Record any visible deformations
- Record the gender
- Record the maturity stage

Experimental design: dead species

N= minimum 30 mussel per species

N= minimum 5 crabs per species

N= minimum 5 sea urchins per species

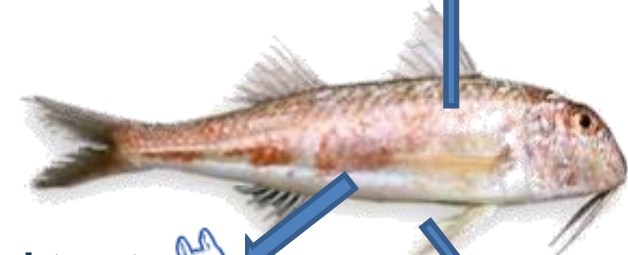
N= minimum 5 pools of isopods per species



Whole organisms

N= minimum 30 fish per species

Muscle



Gastro-intestinal tract



Liver



Interreg
Mediterranean

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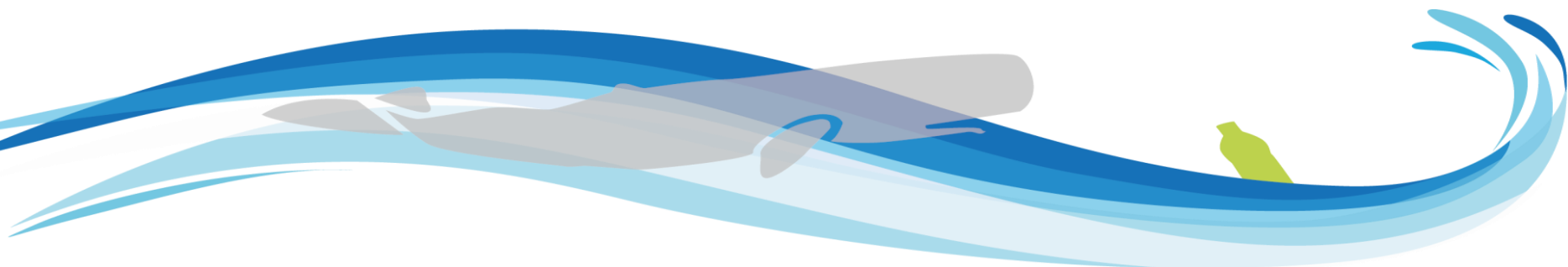
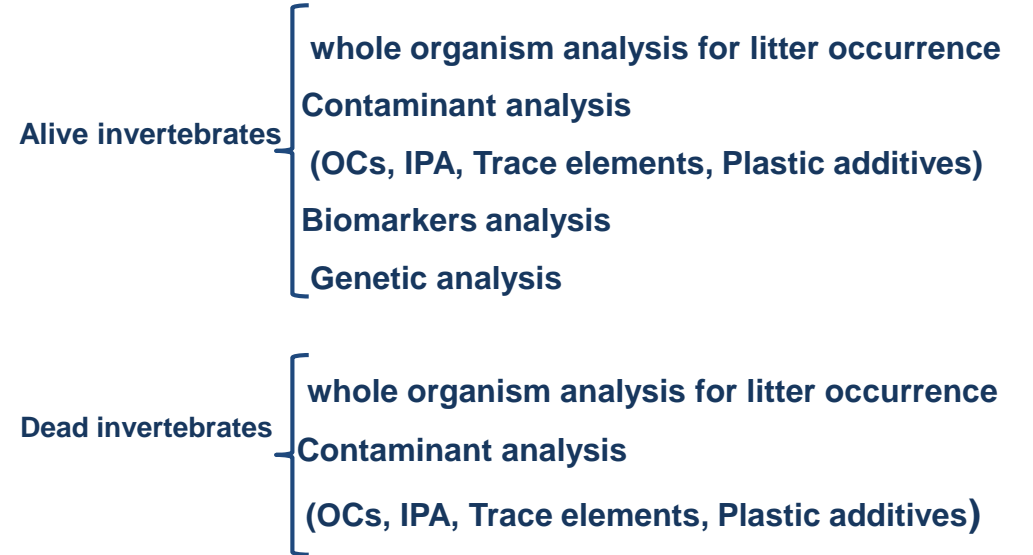
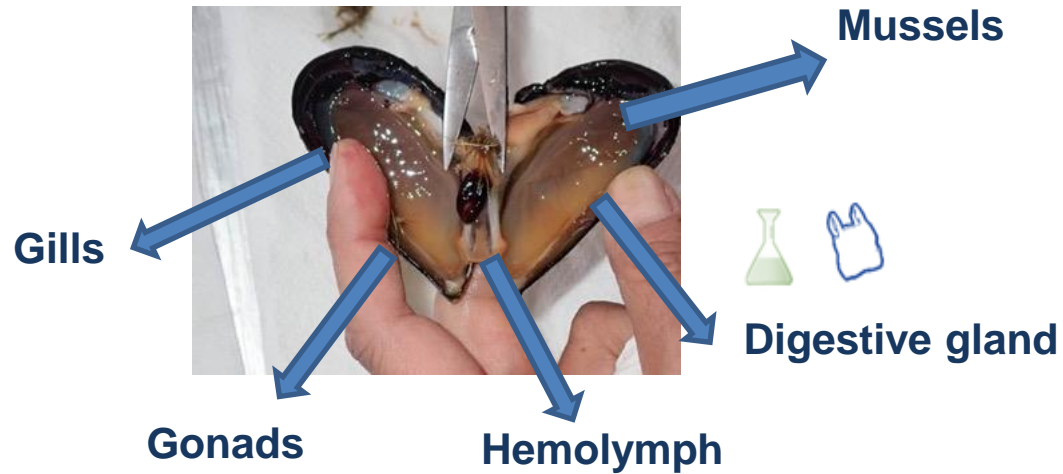
Experimental design: alive species

N= minimum 30 mussel per species

N= minimum 5 crabs per species

N= minimum 5 sea urchins per species

N= minimum 5 pools of isopods per species



Experimental design: *alive species*

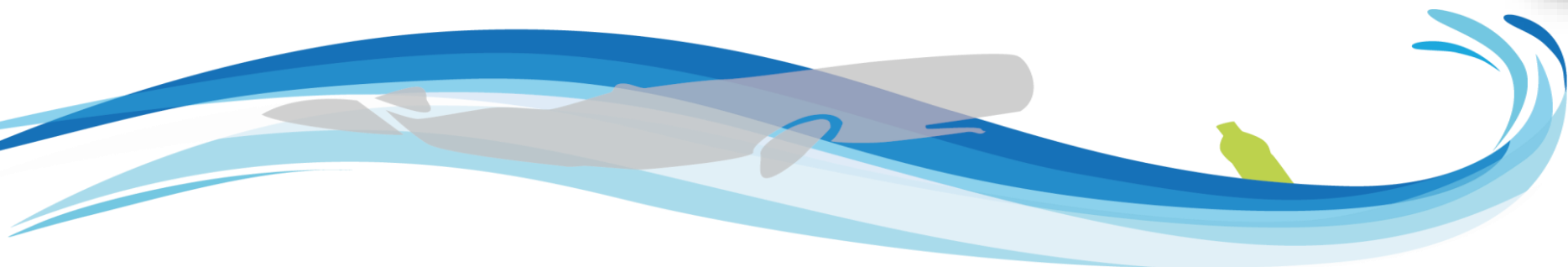
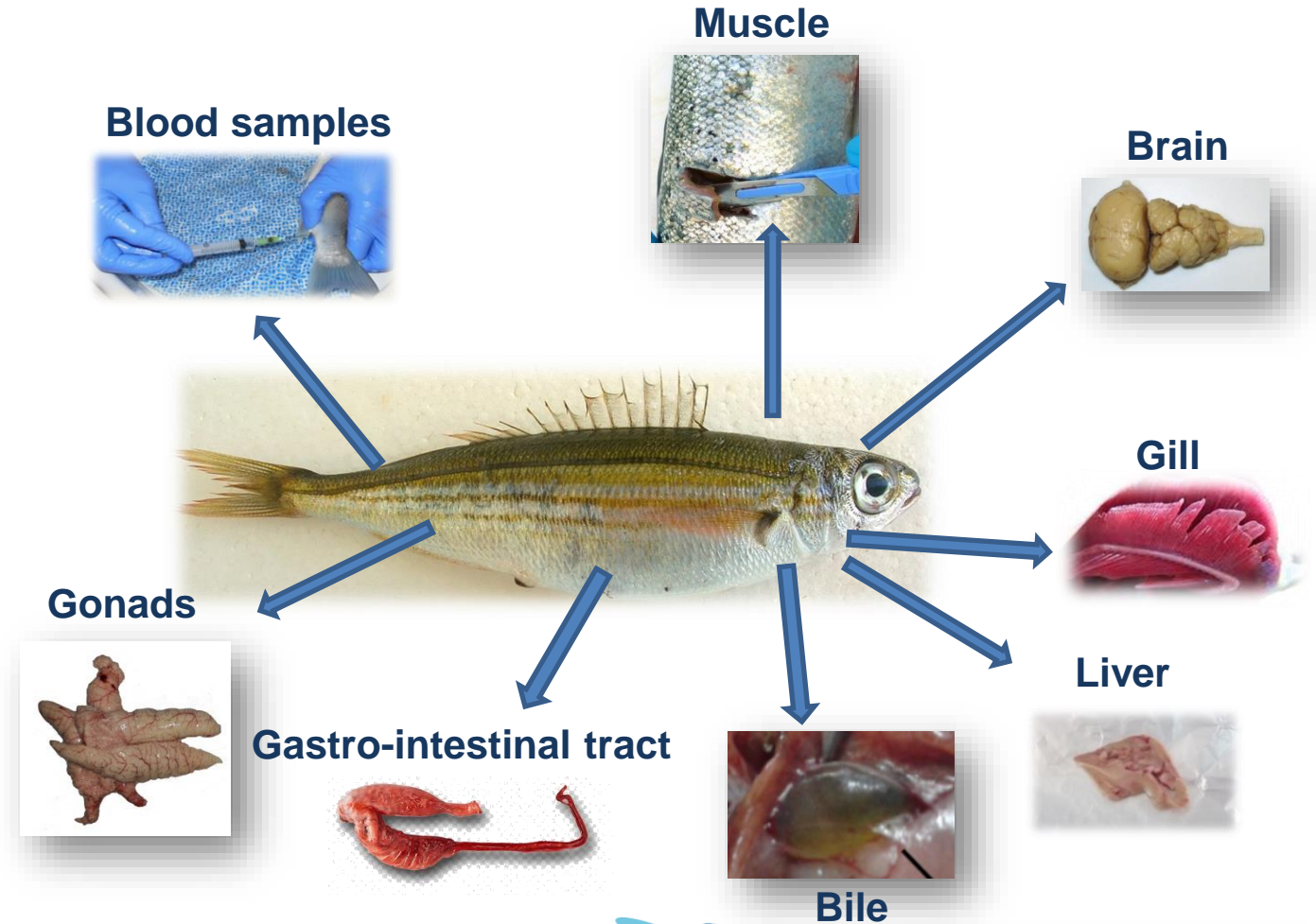
N= 20 fish per species

Alive fish

- GIT analysis for litter occurrence
- Contaminant analysis
(OCs, IPA, Trace elements, Plastic additives)
- Biomarkers analysis
- Genetic analysis

Dead fish

- GIT analysis for litter occurrence
- Contaminant analysis
(OCs, IPA, Trace elements, Plastic additives)



Threefold approach

DEAD-SAMPLED ORGANISM



LIVE-SAMPLED ORGANISM



i) Plastic detection



- Analysis of the ingested marine litter/microplastics:
 - Occurrence (%)
 - Abundance (n°)
 - Weight (g)
 - Polymer analysis

ii) Plastic tracers detection



- Analysis of plastic additives:
 - Phthalates
 - PBDEs
 - Bisphenol A
- Analysis of PBT compounds:
 - PCBs
 - DDTs
 - PAHs
 - Mercury

iii) Biomarkers detection



- Effects at molecular level:
 - Measure of DNA damage
 - Alterations of gene expression
 - Alteration of proteins
- Effects at cellular level:
 - Alteration of cell functions
- Effects at tissue level:
 - Hystological and hystopathological alterations

Each one of the three investigation tools (plastic detection, plastic tracer detection and biomarkers) that compose the threefold approach, can be applied independently or simultaneously in the bioindicator species selected.

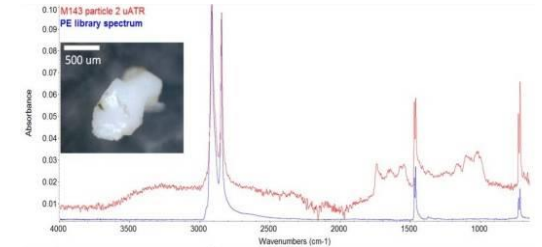
Microplastics analysis: ingestion

DIGESTION

FILTRATION

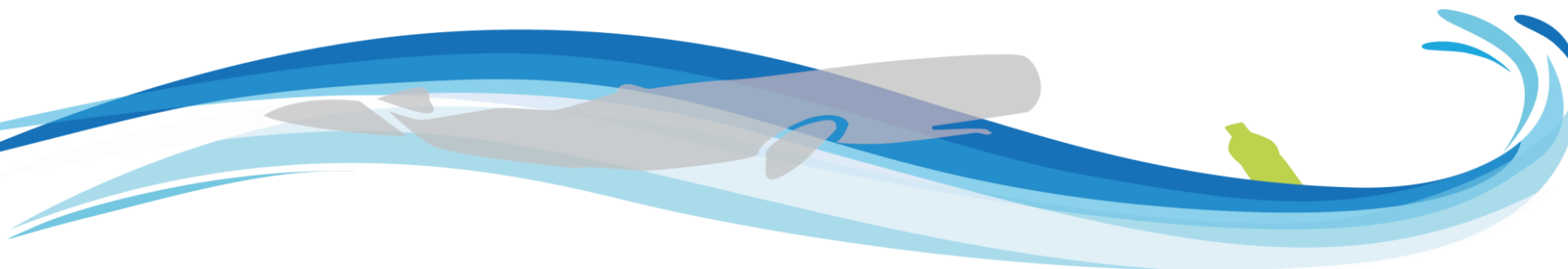
MICROSCOPY

POLYMER ANALYSIS



Use of glass materials washed with micro-filtrated water ($0.45 \mu\text{m}$)

One procedural blanks every two samples

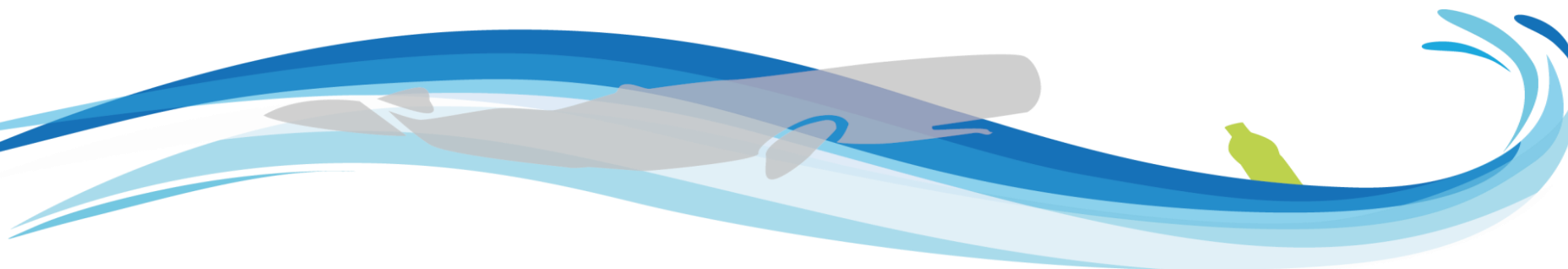


Laboratory analysis: digestion and filtration



In the laboratory, the mussel tissues and fish guts are subjected to digestion of the organic matter by potassium hydroxide (KOH 10%, 50 °C overnight).

When all organic matter has been removed, the samples are filtered on a filtering apparatus (1.6µm glass-fiber filter) under airborne contamination free conditions (e.g. glove box, laminar flow).



Laboratory analysis: characterization of plastics

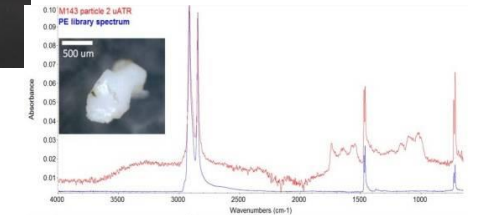
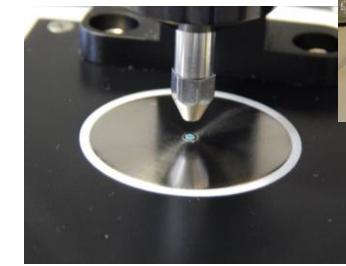
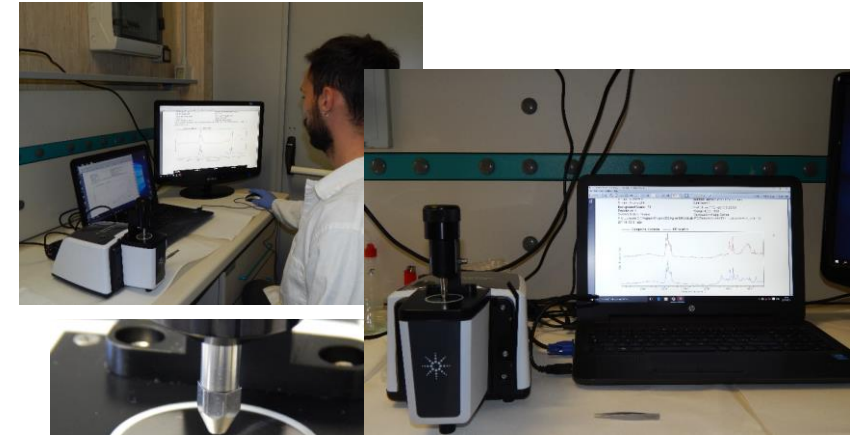


MICROPLASTIC PARTICLES isolated and characterized according to the the **Joint Litter Categories** proposed by **MSFD TG 10 Guidelines**

by:

- Dimension classes
- Shape
- color

A stereomicroscope (Olympus SXE) paired with a camera (Infinity) is used to detect items resembling plastic on the filters.



Fourier-Transform Infrared Spectroscopy (FTIR) is used to confirm the polymer composition of the items detected

POLYMER ANALYSIS is performed at least 10% of the total number of microplastics isolated

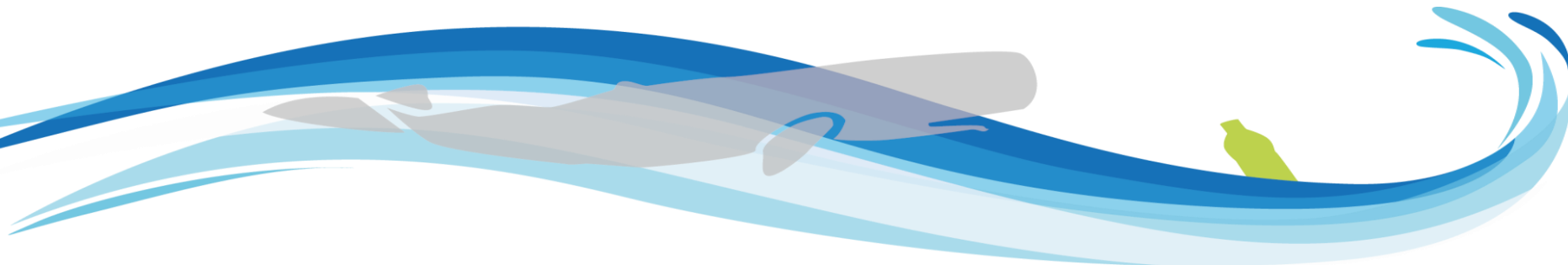
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Laboratory analysis: Report units

For each organism an assessment is made of the:

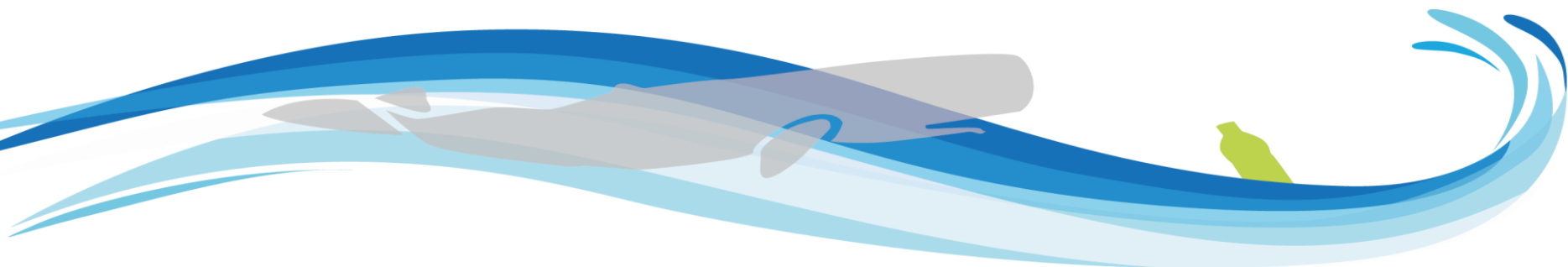
1. Frequency of occurrence (%) of ingested macro- and micro-plastics for each organism is calculated as the percentage of the individuals examined with ingested microplastics.
2. Abundance (N) of macro- and micro-plastics ingested per individual (average number of items/individual) for each species is calculated as a total and per category. Since currently there are inconsistencies in the literature in reporting abundance of ingested litter, it is recommended to report average number of items per individual both considering all individuals examined and only individuals found with ingested macro- and micro-litter.
3. The percentage of the individuals affected in relation with the individuals of the whole sample examined (all species).



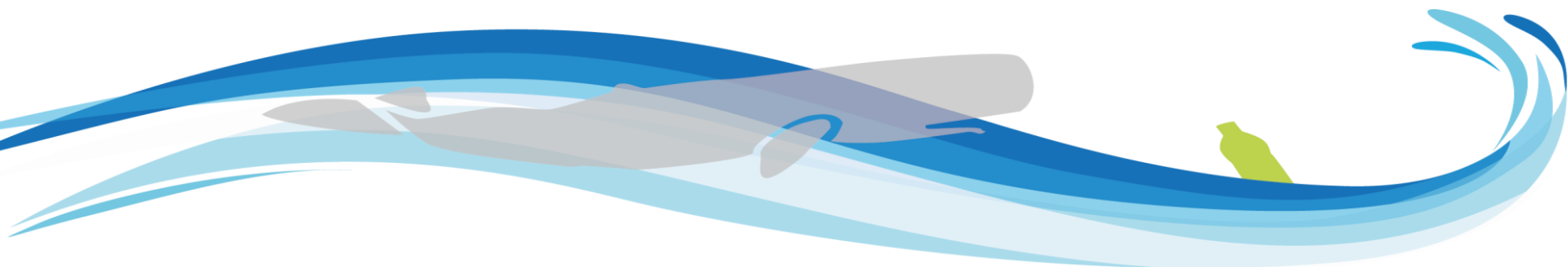
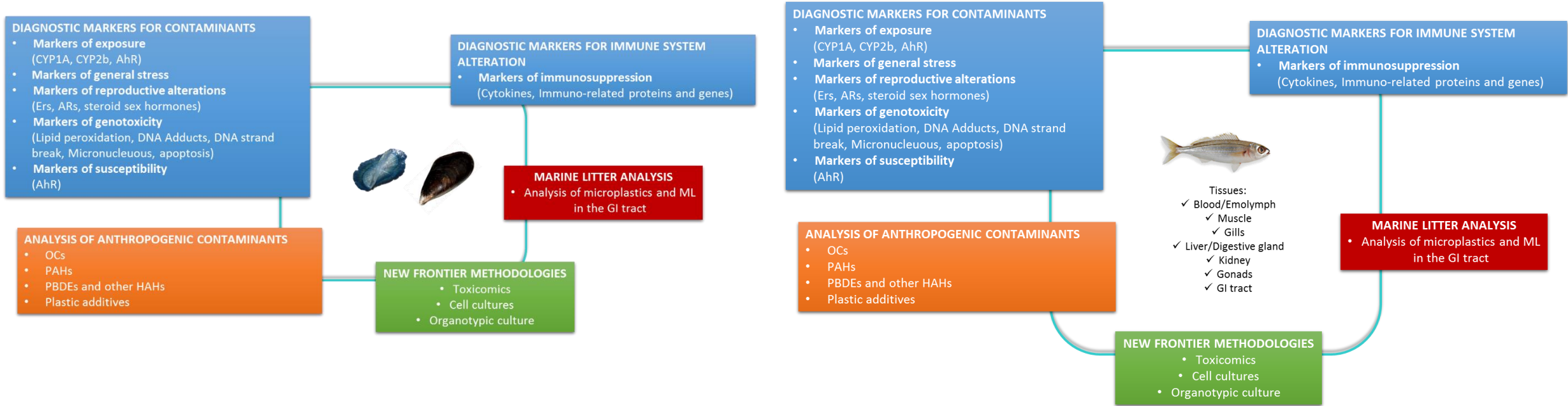
Laboratory analysis: Contaminants analysis



PLASTIC ADDITIVES ANALYSIS will be performed on the 10- 20% of the total number of MPs samples



Laboratory analysis: Biomarkers





Thank you



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