International Workshop

“MONITORING MARINE LITTER IN ENVIRONMENT AND BIODIVERSITY”

COMMON and Plastic Busters CAP projects - Manfredonia (Italy), 13th - 14th July 2022

Protocol for fish and invertebrates species

Toolkit for monitoring marine litter and its impacts on biodiversity in Med.

Dario Giani– University of Siena

Sampling of invertebrates and fish species

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

Mussels

Fish

Coastal waters

Bogue

Red striped mullet

Sea floor

Open waters

European pilchard

European anchovy

Sampling methodology

Scuba divers

Bottom trawl

Artisanal fixed net

ALIVE fish for BIOMARKERS analysis can be collected by researchers on board during the different fishing activities

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
- Weight each individual
- Length and width of each individual
- Record any visible deformations

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 lenght (cm)	Total weight (g)	GI weight (g)	Muscle	Liver weight (g)

Notes and remarks:

Rack (N_2)

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 lenght (cm)	Fork lenght (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 (N_2)

- Record the name of the species
- Weight the whole fish
- Measure the total and fork length of the fish
- Weight the Gastrointestinal tract

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

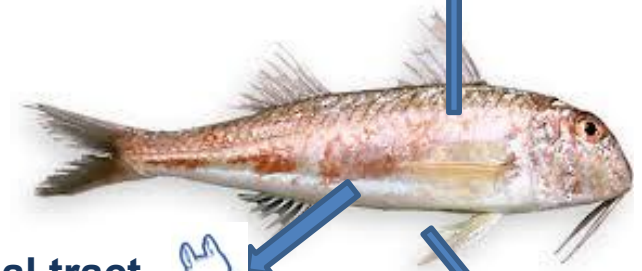
Experimental design: dead species

N= minimum 30 mussel per species

Whole organims

N= minimum 30 fish per species

Muscle



Gastro-intestinal tract

Liver

Experimental design: alive species

N= minimum 30 mussel per species

Alive invertebrates
- whole organism analysis for litter occurrence
- Contaminant analysis
- (OCs, PAH, Trace elements, Plastic additives)
- Biomarkers analysis
- Genetic analysis

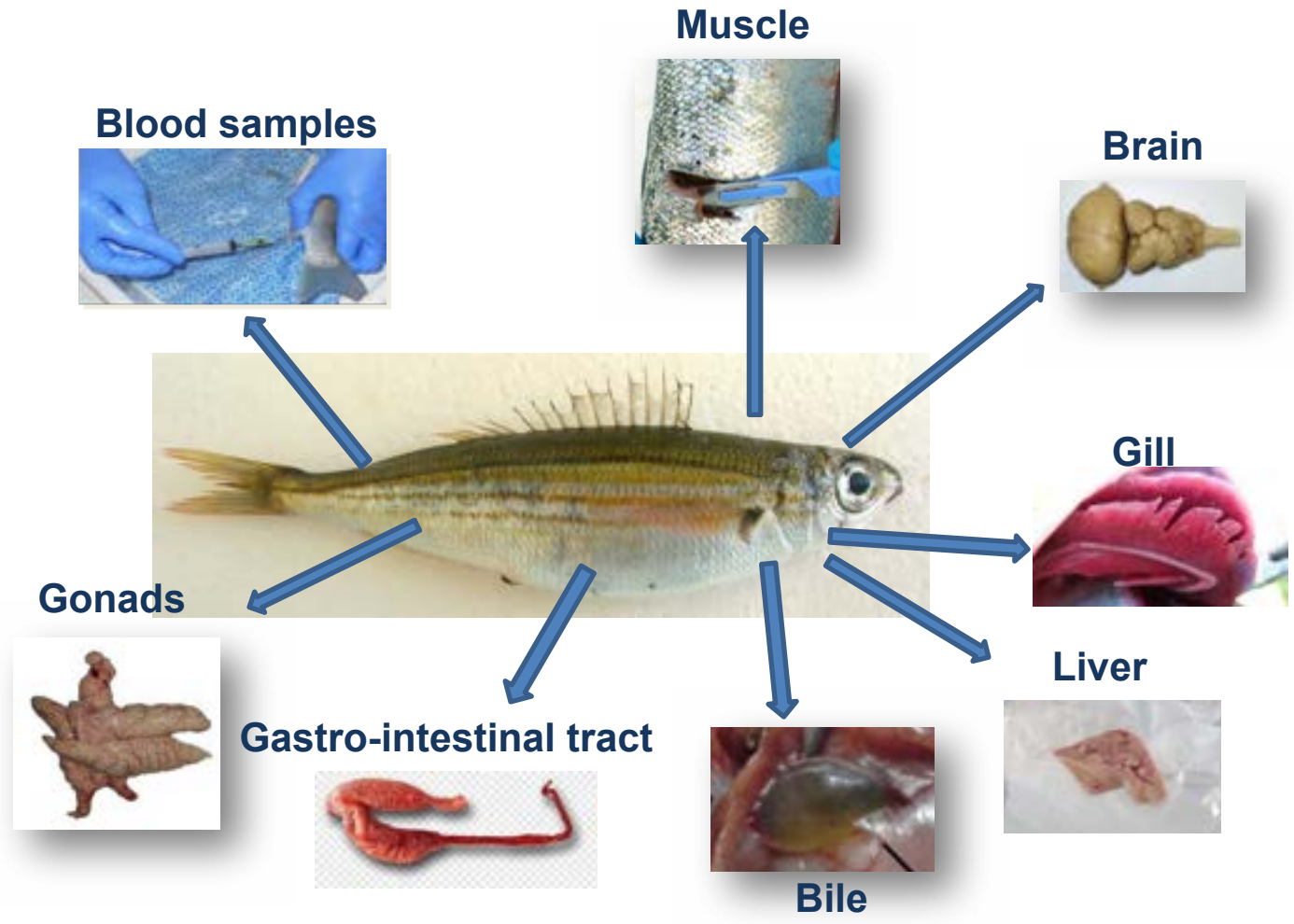
Dead invertebrates
- whole organism analysis for litter occurrence
- Contaminant analysis
- (OCs, PAH, Trace elements, Plastic additives)

Experimental design: *alive species*

N= 20 fish per species

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

Dead fish
- GIT analysis for litter occurrence
- Contaminant analysis
- (OCs, PAH, 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.

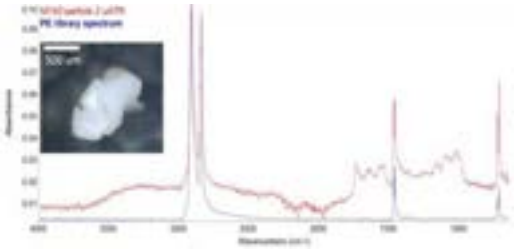
Microplastics analysis:ingestion

DIGESTION

FILTRATION

MICROSCOPY

POLYMER ANALYSIS



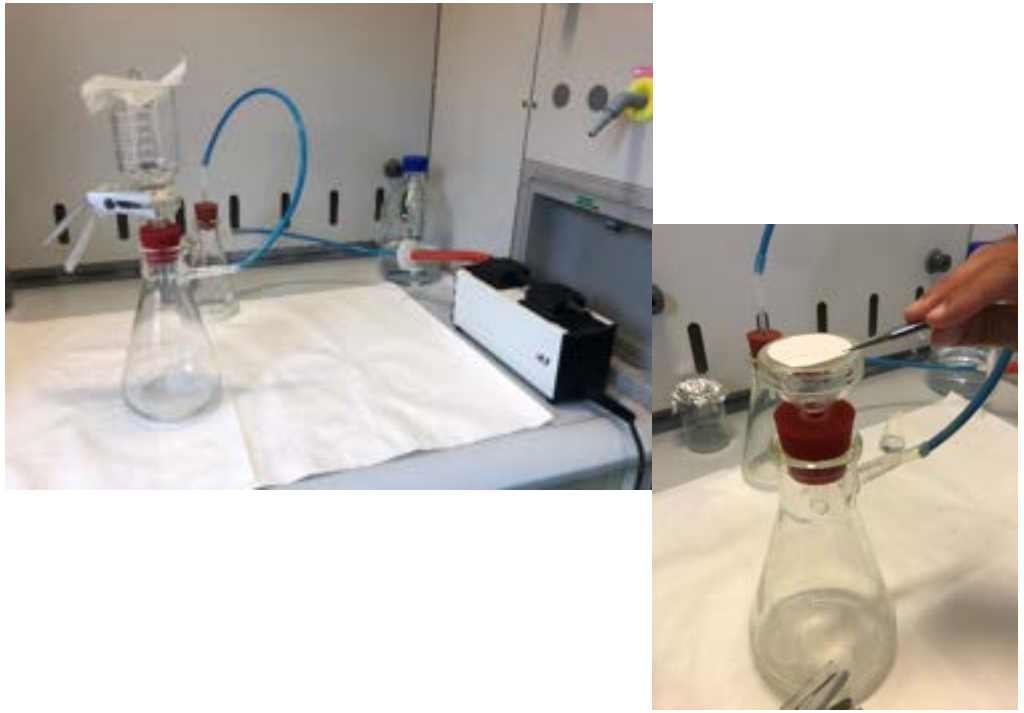
- **TO AVOID CONTAMINATION**

Use of glass materials washed with micro-filtred water (0.45 µm)

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 hidroxide (KOH 10%, 50 °C overnight).

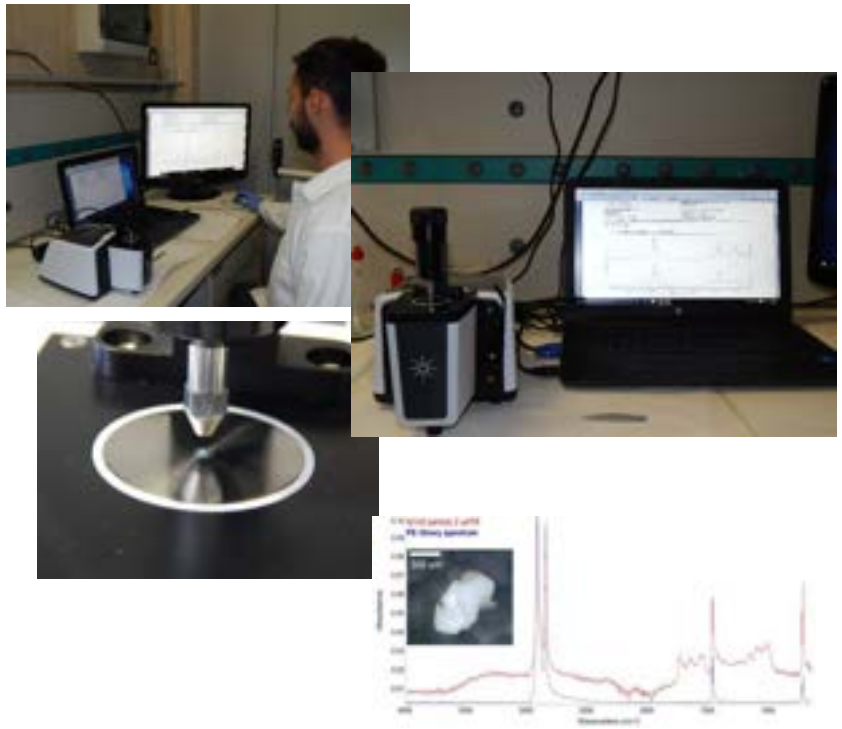


When all organic matter has been removed, the samples are filtered on a filtering apparatus (1.6µm glass-fiber filter)

Characterization of plastics items

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 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

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).

International Workshop

“MONITORING MARINE LITTER IN ENVIRONMENT AND BIODIVERSITY”

COMMON and Plastic Busters CAP projects - Manfredonia (Italy), 13th - 14th July 2022

Thank you!

dario.giani@unisi.it