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MONITORING APPROACHES FOR ASSESSING THE PRESENCE OF MARINE LITTER IN BIOTA

DARIO GIANI

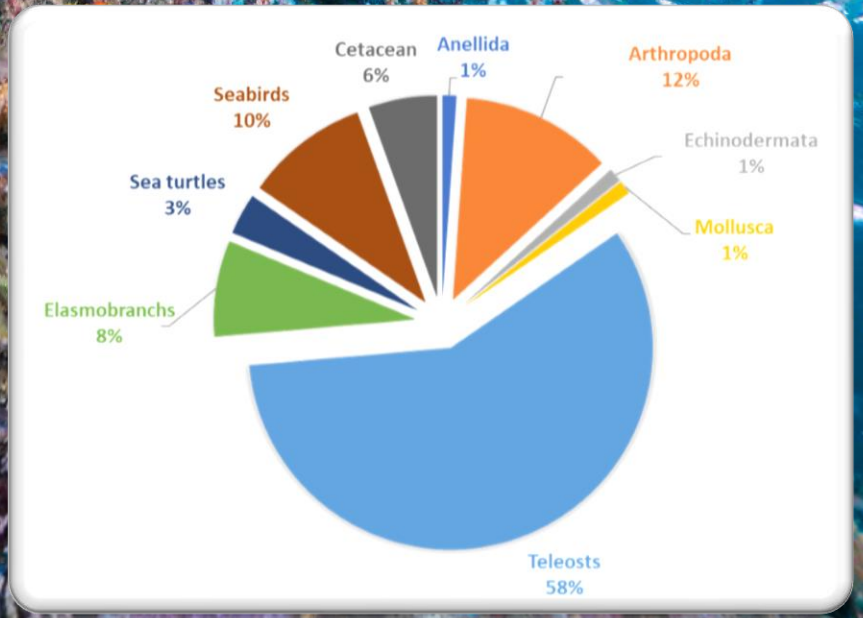
UNIVERSITY OF SIENA

Mediterranean e-course on marine litter monitoring & mitigation

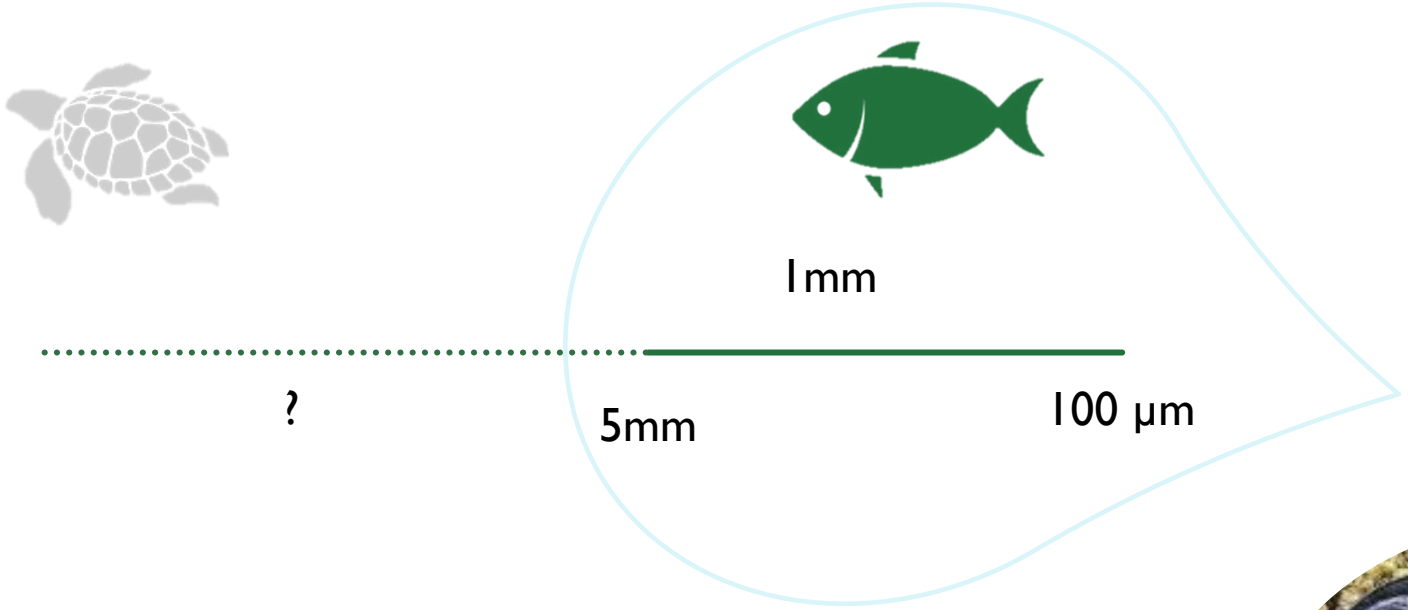
17 & 19 January 2023, 10.00 – 14.00 CET

MARINE LITTER INGESTION IN MEDITERRANEAN SEA SPECIES

- MORE THAN 70 PAPERS REPORTED PLASTIC INGESTION
- 138 SPECIES WITH RECORDS OF PLASTICS INGESTION
- FISH (66%) AND INVERTEBRATES (15%) REPRESENT THE 81% OF TOTAL INVESTIGATED SPECIES



MARINE LITTER INGESTION IN FISH AND INVERTEBRATES



Guideline published for the *Marine Strategy Framework Directive* suggests that the contents of fish stomachs may be analyzed to determine trends of microplastic ingestion

MOST INVESTIGATED SPECIES IN MEDITERRANEAN BASIN

N° > 140 individuals from at least 4 studies

32% is the average number of specimens with marine litter in GI tract (occurrence)

Boops boops most studied species and revealed the highest occurrence

Species	Occurrence (%)	N° of specimens	N° references
Boops boops	50.9	930	9
Mullus barbatus	27.9	925	14
Sardina pilchardus	31.1	853	14
Solea solea	17.2	690	5
Engraulis encrasicolus	30.2	541	11
Merluccius merluccius	32	400	11
Trachurus trachurus	27.7	267	7
Pagellus erythrinus	31.3	208	5
Trachurus mediterraneus	50.3	207	5
Scomber scombrus	39.5	172	4
Galeus melastomus	7.7	168	4
Scyliorhinus canicula	37.6	141	6

Low-medium-high

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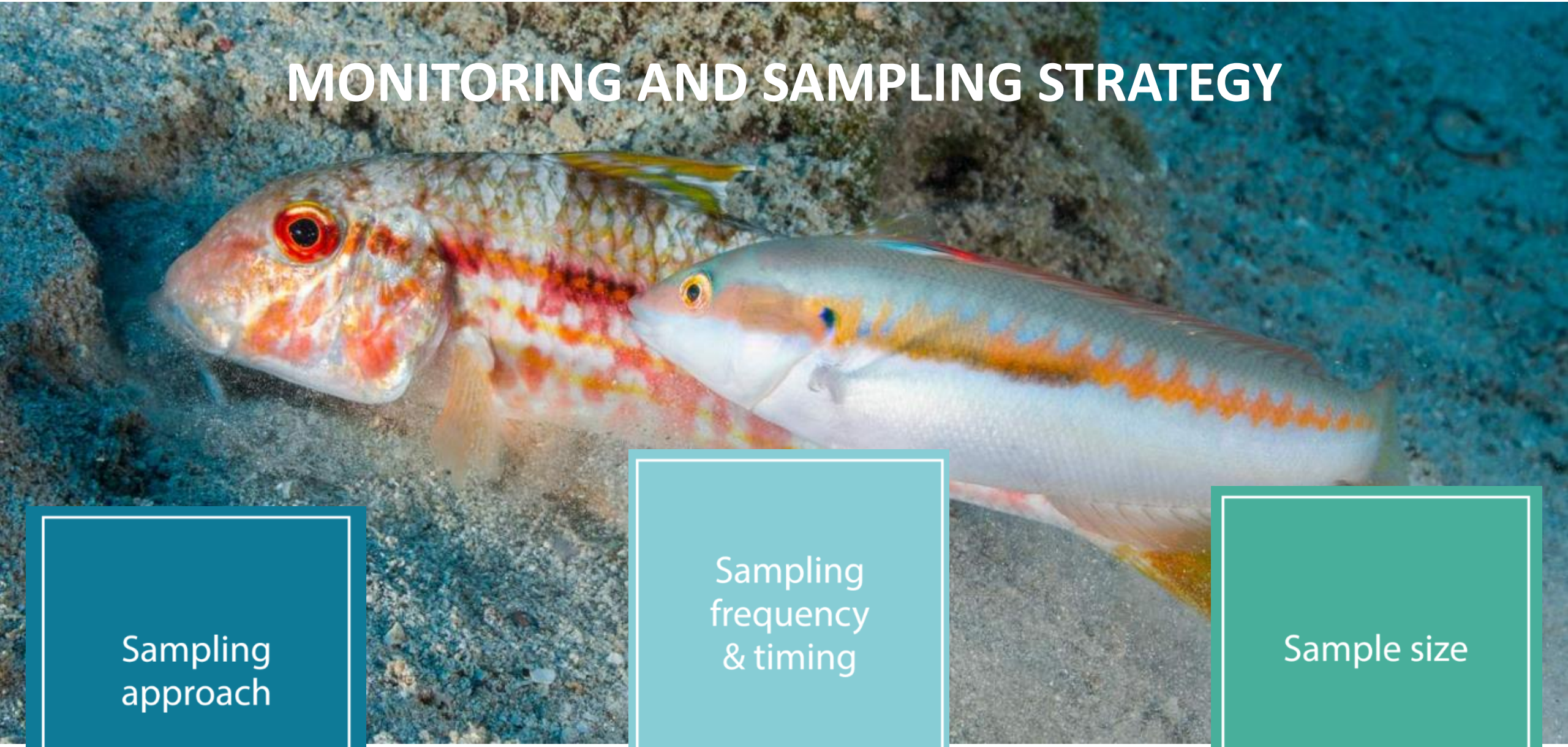
COMMON

SELECTION CRITERIA OF SENTINEL SPECIES

Selection criteria of sentinel species	
General categories	**Attributes**
1) background information	• Clear taxonomic identification • Scientific knowledge on ecology and biology characteristics
2) habitat information	• Habitat • Home range
3) trophic information and feeding behavior	• Feeding behavior (e.g. feed on schooling, opportunism, feed on pleuston, bentivorous feeders) • Feeding mechanism (e.g. filter feeding) • Trophic level (e.g. large pelagic predators, bioaccumulation) • Keystone species
4) spatial distribution	• Spatial coverage
5) commercial importance and conservation status	• Commercial importance (human health) • Easy availability • Protected, threatened or managed species
6) Documented ingestion of ML	• Data availability on ML ingestion from State of Art

MONITORING AND SAMPLING STRATEGY



Sampling approach

Sampling frequency & timing

Sample size

Sampling approach

MONITORING STRATEGY: INVERTEBRATES

Marine **invertebrate species** such as filter-feeding invertebrates (e.g., mussels), and other invertebrate species (e.g., sea urchins) should be collected following any of the modalities below:

- Collected from the study area.
- Purchased by local fishers and scuba active in the study area
- Collected in adjacent areas with similar conditions with the study area and are re-located in the study area with the use of metal cages. After a period of 3-4 weeks, they can be sampled.

MONITORING STRATEGY: FISH

Sampling approach

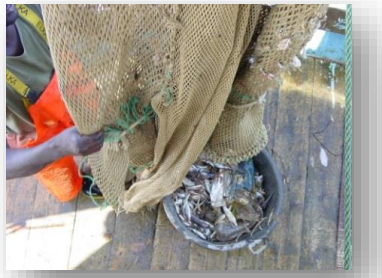
Fish species should be sampled following one of these approaches depending on the type of analysis to be performed:

- For the analysis of **litter ingestion** and associated contaminants, fish species (dead) can be purchased by local fishers active in the study area.

- For the analysis of litter, associated contaminants and **biomarkers**, fish species (still alive!) should be collected in the study area via a dedicated sampling campaign.

Bottom trawl

Artisanal fixed net



MONITORING STRATEGY

Sampling frequency & timing

- The frequency of sampling must be at least once per year, taking into account seasonality.
- A minimum of 30 individuals per invertebrates species should be sampled

Sample size

- A minimum of 30 individuals per fish species should be sampled at each site, preferably for each environmental compartment (i.e., benthic, demersal, pelagic).
- Specimens of endangered species (e.g. Manta ray) occasionally found stranded can also be analyzed in very small numbers

MONITORING STRATEGY

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

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

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

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

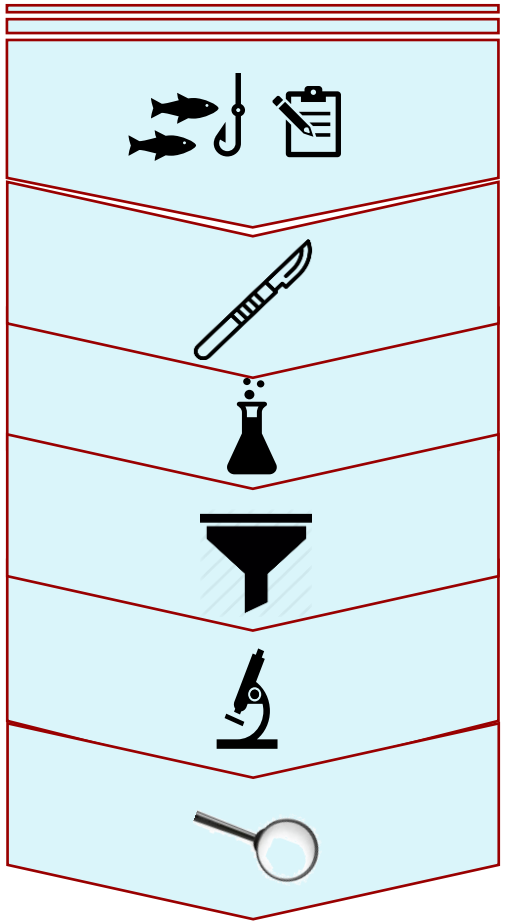
ID code	Species	Sex	Total length (cm)	Total weight (g)	GI weight (g)	Muscle	Liver weight (g)

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

INGESTION OF MICROPLASTICS IN BIOTA: THE ESSENTIAL STEPS



SAMPLING DATA

DISSECTION AND TISSUES COLLECTION

TISSUES DIGESTION

FILTRATION

MICROSCOPY ANALYSIS AND MPs ISOLATION

POLYMER IDENTIFICATION

DISSECTION AND TISSUES COLLECTION

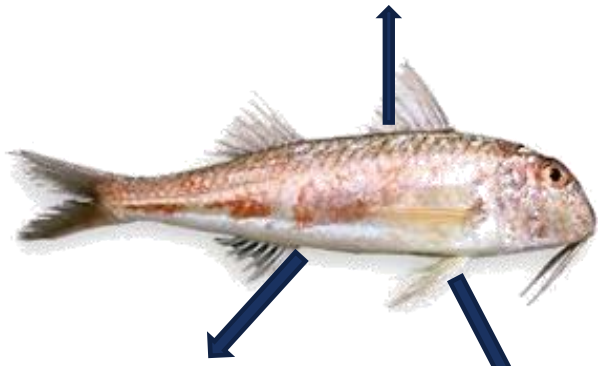
Whole organims

i) Plastic detection

ii) Plastic tracers detection

Muscle

ii) Plastic tracers detection



Gastro-intestinal tract

i) Plastic detection

Liver

TISSUES DIGESTION

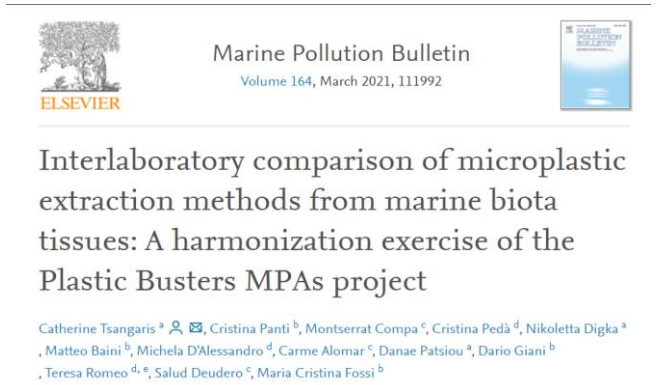
1- Place the GI tract (stomach and intestine) in a glass beaker or tube.

2- Weigh and rinse the gastrointestinal tract with purified water

3- Place a filter paper in a petri dish (blank sample) in the working area during fish dissection to test airborne contamination.

4- The mussel tissues and fish guts are subjected to digestion of the organic matter by **potassium hydroxide**, add 5ml 10% KOH per gram of tissue wet weight (1:5 w/v) at 50 °C overnight.

..for the detailed methodology see *Tsangaris et al., 2021*



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Marine Pollution Bulletin

Volume 164, March 2021, 111992

Interlaboratory comparison of microplastic extraction methods from marine biota tissues: A harmonization exercise of the Plastic Busters MPAs project

Catherine Tsangaris [a], Cristina Panti [b], Montserrat Compa [c], Cristina Pedà [d], Nikoletta Digka [a], Matteo Baini [b], Michela D'Alessandro [d], Carme Alomar [c], Danae Patsiou [a], Dario Giani [b], Teresa Romeo [d, e], Salud Deudero [c], Maria Cristina Fossi [b]

SAMPLE FILTRATION

1- After the digestion of the organic matter, pass the samples through a metal sieve (300 µm) placed above a filtering apparatus and finally filtered under vacuum onto a fiberglass filter (Whatman GF/C, pore size 1.2 or 1.6 µm).

2- Metal sieves should be covered with aluminum foil and filters must be placed in aluminum foil-covered Petri dishes and dried at room temperature.

3- All filtering procedures took place inside a laminar flow cabinet.

4- Use a procedural blank sample to test the ambient contamination: add similar volume of 10% KOH as that used in the samples in a beaker without sample, and follow the protocol described in the steps above.

SAMPLE ISOLATION AND MICROPLASTICS CHARACTERIZATION

1- After the digestion procedure, check the filter to isolate plastic items with a stereomicroscope.

2- Photograph, count and record the maximum length of plastic particles using image analysis software.

MICROPLASTICS CHARACTERIZATION

Categorize plastic particles according to **shape**, size, and colour.

FRAGMENTS

Irregular shaped hard particles

FILAMENTS

Flat, flexible particle with smooth or angula edges

FILM

Thin and flexible plastics such as plastic bags, food wrappers, or tape

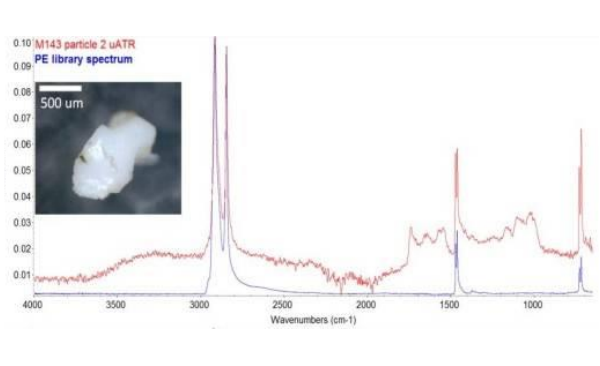
MICROFIBERS

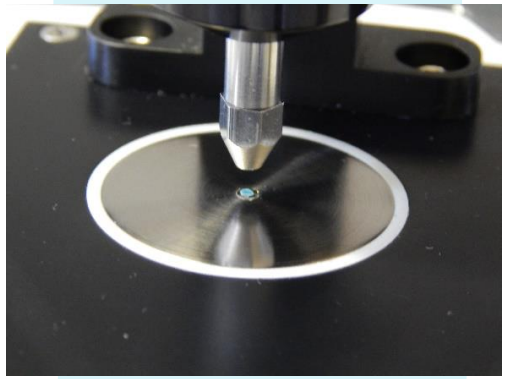
in is a synthetic fiber finer than one denier and having a diameter of less than 10 µm (Jerg and Baumann 1990)

FOAM

Near-spherical or granular particle, with deforms readily under pressure and can be partly elasted

POLYMER CHARACTERIZATION



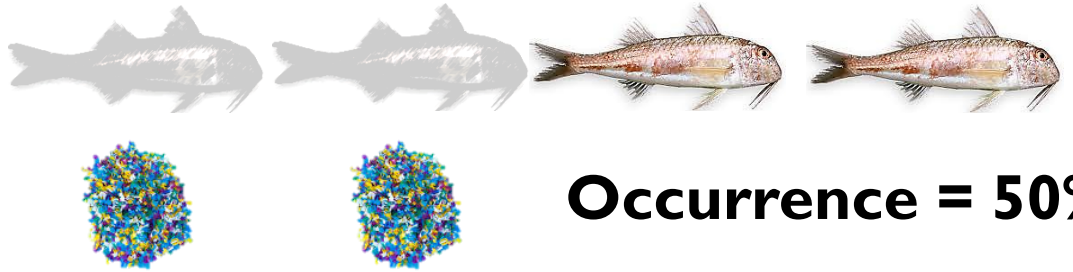


3- Analyse at least 10% of the detected microplastics by FTIR (Fourier Transform Infrared Spectroscopy) to determine the polymer composition and confirm the polymer origin of the detected particles.

COLLECTION OF DATA

For each organism an assessment is made of the:

1. Frequency of occurrence (%) of ingested microplastics for each organism is calculated as the percentage of the individuals examined with ingested microplastics.



Occurrence = 50%

COLLECTION OF DATA

For each organism an assessment is made of the:

2. Abundance (N) of microplastics ingested per individual (average number of items/individual) for each species. 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 particles.

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UNIVERSITÀ DI SIENA 1240

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