

MONITORING MARINE LITTER IMPACTS ON SEA TURTLES



PROTOCOL FOR THE COLLECTION OF DATA ON INGESTION AND ENTANGLEMENT IN THE LOGGERHEAD TURTLE

(*Caretta caretta* Linnaeus, 1758)



Recommended quotation

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Monitoring marine litter impacts on sea turtles. Protocol for the collection of data on ingestion and entanglement in the loggerhead turtle (*Caretta caretta* Linnaeus, 1758).

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For any questions related to the protocol, please contact :

coordination@indicit-europa.eu



INDEX

INTRODUCTION	4
A. GENERAL INFORMATION ON LIVE AND DEAD SPECIMEN	5
A.1. FIRST NOTES ON THE DISCOVERY SITE	5
1. Contact	5
2. On the individual	5
3. On the site	5
A.2. DESCRIPTION OF THE ANIMAL'S BODY CONDITION	6
1. Conservation status or decomposition level	6
2. Discovery circumstances	6
3. Probable cause of death/stranding	7
4. Health status	7
5. By-catch engine cause	7
6. Main injuries	8
7. Affected body part	8
8. Entanglement type	8
9. Litter causing entanglement	9
10. Other descriptive parameters	9
A.3. BIOMETRIC MEASUREMENTS	10
B. SAMPLING MARINE LITTER FROM CARCASSES – PROTOCOL FOR DEAD ANIMALS	11
B.1. TURTLE NECROPSY	11
B.2. EXTRACTION OF THE GUT CONTENT	12
B.3. EXTRACTION OF THE MARINE DEBRIS	12
C. SAMPLING MARINE LITTER FROM FAECES - PROTOCOL FOR LIVE ANIMALS	13
C.1. COLLECTION OF FAECES	13
C.2. COLLECTION OF DEBRIS FROM FAECES	13
D. DEBRIS ANALYSIS AND CLASSIFICATION	14
D.1. LITTER CLASSIFICATION	14
D.2. COLLECTION OF DATA	15
REFERENCES	16
APPENDIX 1 – OBSERVATION SHEET	17
APPENDIX 2 – LIST OF MATERIAL	19
For the recovery of the animal and the collection of samples at the discovery site	19
For the collection of samples on dead individuals in laboratory and the extraction of the ingested litter from the digestive tract	19
For the collection of samples on live individuals in rescue centres and the extraction of ingested litter in the faeces ...	19
PROTOCOL FOR THE EXTRACTION OF MICRO-PLASTICS (OPTIONAL)	20
1. Preparatory actions to avoid contamination	20
1. For dead animals	20
2. For live animals	20
2. Extraction of the ingested micro-debris	20

¹ The protocol for dead individuals can be applicable to other sea turtle species.



INTRODUCTION

The following protocol is intended to respond to the MSFD requirements for the indicator 10.2.1 “Trends in the amount and composition of litter ingested by marine animals”. The INDICIT program proposed marine turtles as an indicator species to study marine litter ingestion on biota through the development and the implementation of one major indicator “Litter ingested by sea turtles”. Standardized methodologies for extracting litter ingested from dead and live individuals are exposed in this document. Some modifications have been conducted from the original methodology drafted and tested in Italy since 2012 (Matiddi et al., 2011; MSFD TG Litter, 2013), following the first applications (Camedda et al., 2014; Matiddi et al., 2017) and within the European Project INDICIT (GA n°11.0661/2016/748064/SUB/ENV.C2) as well as thanks to the feedbacks of rescue centres and stranding networks.

Both “basic” and “optional” parameters are proposed to be collected. The **basic parameters** (thereafter noted in bold) correspond to the minimum parameters fundamental to determine the indicator criteria. The *optional parameters* (thereafter noted in bold italic grey) allow acquiring more knowledge on loggerheads’ behaviour and probability to ingest debris and better specify the indicator criteria in development. The optional parameters can also help to better assess the relevance of two new indicators on litter impacts for which pilot studies are in process (“Entanglement with marine debris by biota” and “Micro-plastic debris ingested by sea turtle and fish”). Following the MSFD Technical sub-group on Marine litter (Galgani et al., 2013) and the new Commission Decision (Decision 2017/848 of the 17th March 2017), the minimum size of litter items considered for the indicator “Litter ingested by sea turtles” is 1 mm, thus including both micro (1-5 mm) and macro-plastics (> 5 mm).

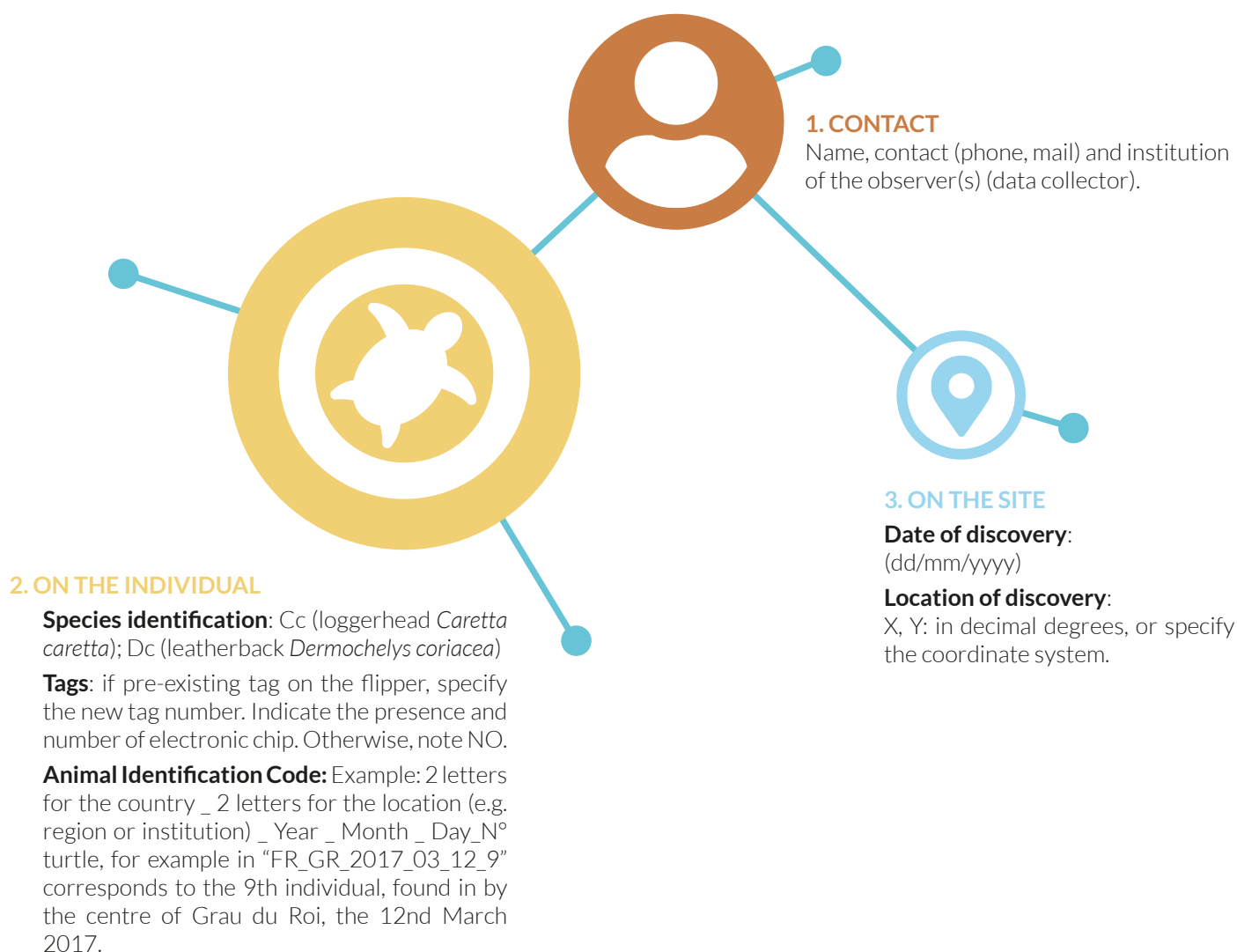
An **observation sheet** is provided in Appendix. In order to facilitate banking and statistical analysis, data must be filled in the INDICIT corresponding **standardized table**, by respecting the units and proposed menu choices, and specifying remarks or other proposals in the last column “Note”. **All boxes must be filled**, either by the information (data) or by 0 or “NA” (information not available or not evaluated).

NOTE: The loggerhead sea turtle (*Caretta caretta*) is a protected species, therefore only authorized people can handle live and dead animals or parts of them. Upon finding the animal, its management and recovery should be reported and coordinated with the responsible Authorities. Note that a CITES permit is asked if a specimen or sample has to be sent/received.

NOTE: Sanitary precautions must be paid for the handling of dead or live wild animal to minimize risks of infectious diseases such as zoonosis. The intervention zone must be marked-off from the bystanders and handling necessitates to wear a protective suit with glasses, gloves and rubber boots, then carefully separated and disinfected or thrown. Ideally, a cut-resistant pair of gloves can be worn below two pairs of gloves, one of them being changed for writing or in case of cutting.

A. GENERAL INFORMATION ON LIVE AND DEAD SPECIMEN

A.1 FIRST NOTES ON THE DISCOVERY SITE



NOTE: Taking pictures of the animal before handling is very important to verify the condition of the recovery. Using a tape measure can show the order of magnitude in the pictures. Please specify if pictures are taken in the column "Photo at finding" of the Excel file.

A.2

DESCRIPTION OF THE ANIMAL'S BODY CONDITIONS

1. Conservation status or decomposition level

Note the **status** according to these 5 levels:



Lvl 1: ALIVE



Lvl 2: FRESH

Dead recently, turtle in good conditions.



Lvl 3: PARTIAL

Internal organs still in good conditions. Autolysis (swollen). Bad smell. Colour changes in skin.



Lvl 4: ADVANCED

Skin scales raised or lost. Stille possible record CCL and presence of ingested plastic (only FO%) & entanglement.



Lvl 5: MUMMIFIED

Part of the skeleton is part of the body are missing. Internal organs exhibited. GI material lost.

- **For Level 1**, litter can be extracted from the analysis of faeces in rescue centre.
- **Levels 2 and 3** are adequate for litter ingestion analysis from necropsies.
- **Level 4** allows to measure biometric data and assess the presence/absence of ingested plastic (for the evaluation of the frequency of occurrence of litter ingestion (or prevalence, FO%)) and entanglement.
- **Level 5**, for which individuals have usually lost the gastro-intestinal material, the analysis of litter ingestion is not possible².

2. Discovery circumstances

Note the **circumstances** among the 4 categories:

- **Stranding:** Animal found stranded on the beach or in the shoreline,
- **By-catch/Fisheries:** Animal captured actively by fishermen (e.g. ingestion of a hook, trapped in a net, brought back by fishermen, etc.),
- **Found at sea:** Animal discovered on sea surface,
- **Dead at the recovery centre:** The animal arrived alive, but died during his recovery.

² Some tissues (muscle, etc.) can be collected and stored in 95° alcohol or freeze at -20°C for further genetic analysis.

3. Probable cause of death/stranding

If possible, deduce the *probable cause of death or of live individual stranding* from external observations and if possible, from the observation of organs during the necropsy of dead individuals. Also inspect the oral cavity for the presence of foreign material. Then choose among the 10 categories:

- **Bycatch/Fisheries related:** Presence of an ingested hook, decompression sickness, individual trapped in gear net (in this case, fill in the column «Entanglement type» and «Litter causing entanglement»), individual drowned in a fishing gear...,
- **Entanglement in debris:** Entanglement in debris items other than related to fishing activity. Please fill the column «Entanglement type» and «Litter causing entanglement»,
- **Ingestion of litter:** digestive obstruction, perforation or other symptoms,
- **Anthropogenic trauma:** Collision with a boat or a propeller, individual beaten with knife, stick or harpoon...,
- **Natural trauma:** e.g., shark attack,
- **Natural disease:** Related to malnutrition, buoyancy trouble, cachexia, dermatitis, conjunctivitis, rhinitis...,
- **Oils:** Ingestion or external impregnation with oils,
- **Healthy:** No remarkable damages, injury or disease,
- **Unidentified:** Impossible to know the cause of death/stranding,
- **Other:** Please specify in the column «Notes».

4. Health status

Note the *health status* according to the level of body condition (Fig. 2).



Fig. 2. Health status from visual observation of plastron shape (from Thomson et al., 2009)

5. By-catch engine cause

If the animal has been found bycaught, specify among the 6 proposed categories, the *by-catch engine cause*:

- Straight thread
- Trawl
- Drift net
- Fishing rod
- Non identified
- Other: Please specify in the column "Notes".

Please also specify if possible in the column "Notes" the distance from the coast and the duration of the net deployment before the net was brought aboard.

6. Main injuries

In case of injuries, report the **main type of injury** (fracture, amputation, sectioning, abrasion or other) according to Fig. 3. For other type, please specify it in the column "Notes".



FRACTURE

On carapace, head, jaws, plastron or bones, usually caused by boat collisions.



AMPUTATION

Partial (one or more flippers need to be amputated) or total (one or more flippers missing)



SECTIONING

Cuts or shearing produced by different kind of debris usually on flippers or neck.



ABRASION

Lost or wear of scales produced by the friction of material adhered to the animal or causing entanglement.

Fig. 3. Typology of the main injuries observed in sea turtles

7. Affected body part

If the animal presents an injury, report the **affected body part**:

- **RFF**: for the right front flipper
- **LFF**: for the left front flipper;
- **RRF**: for the right rear flipper;
- **LRF**: for the left rear flipper; **neck**; **carapace**; **plastron**; head; **several** (if several parts of the body are impacted) or **other** (please specify in the column "Notes").

8. Entanglement type

If the individual has been found entangled in marine debris, specify the **entanglement type** according to 3 categories:

- **Active**: Related to active fishing gear, e.g., the individual has been released by a fisherman, or a part of the entangling nest has been cut to release the individual or by the individual after entanglement. The presence of a hook is considered as active entanglement,
- **Passive**: The individual entangled in a debris either not related to fishing activity or related to fishing activity but was abandoned at sea for a long time (sign of old age; please specify in the column "Notes"),
- **Undetermined**.

9. Litter causing entanglement

If the individual has been found entangled in marine debris, specify the *litter type in which the sea turtle has been found entangled in* according to the following categories (Fig. 4):

- Pieces of net (N),
- Monofilament line (nylon) (L),
- Rope or pile of ropes (R),
- Plastic bag (Pb),
- Raffia (Rf),
- Other plastics (Ot),
- Multiple materials (Mu),
- Unknown (Unk).

For each category, please specify or describe the entangling debris item in the column "Notes". In particular, specify for items related to fishing activity if the material was colonised e.g. by algae or shells, and appears old (abandoned).



Fig. 4. Classification of litter causing entanglement of marine turtles and biota

10. Other descriptive parameters

Visually inspect the animal's *fat reserves* at the neck. For dead individual, this can be verified when opening the plastron according to the quantity of fat recovering the abdominal muscles (see below, Fig. 7c). Choose among the 3 categories:

- Thin (sunken neck)
- Fat
- Normal.

If possible, specify the *sex* (Male or Female) of the individual (Fig. 5) according to the length of the tail and of the claw in the front flipper, possibly confirming with a visual observation of the genital apparatus during the necropsy for dead individuals. Otherwise, specify by NI (for Not identified) if it is not possible to identify the individual's sex.

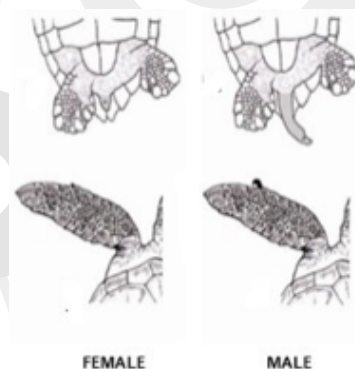


Fig. 5. Determination of the sex (from Wyneken, 2001)

A.3

BIOMETRIC MEASUREMENTS

Following Fig 6., several basic and optional body lengths can be measured (in centimetres, precision 0.01 cm), as well as the **Weight** (in kilograms, precision 0.01g):

- **Maximum Curved Carapace Length (CCLmax)**
 - Minimum curved carapace length (**CCLmin**)
 - Standard curved carapace length (**CCLnt** or **CCL**)
 - Maximum Straight carapace length (**SCLmax**)
 - Minimum Straight carapace length (**SCLmin**)
 - Standard Straight carapace length (**SCLnt**)
 - Curved plastron length
 - Straight plastron length
 - Curved plastron width
 - Straight plastron width

Use a measuring tape to measure curved lengths and a sliding calliper for straight lengths.

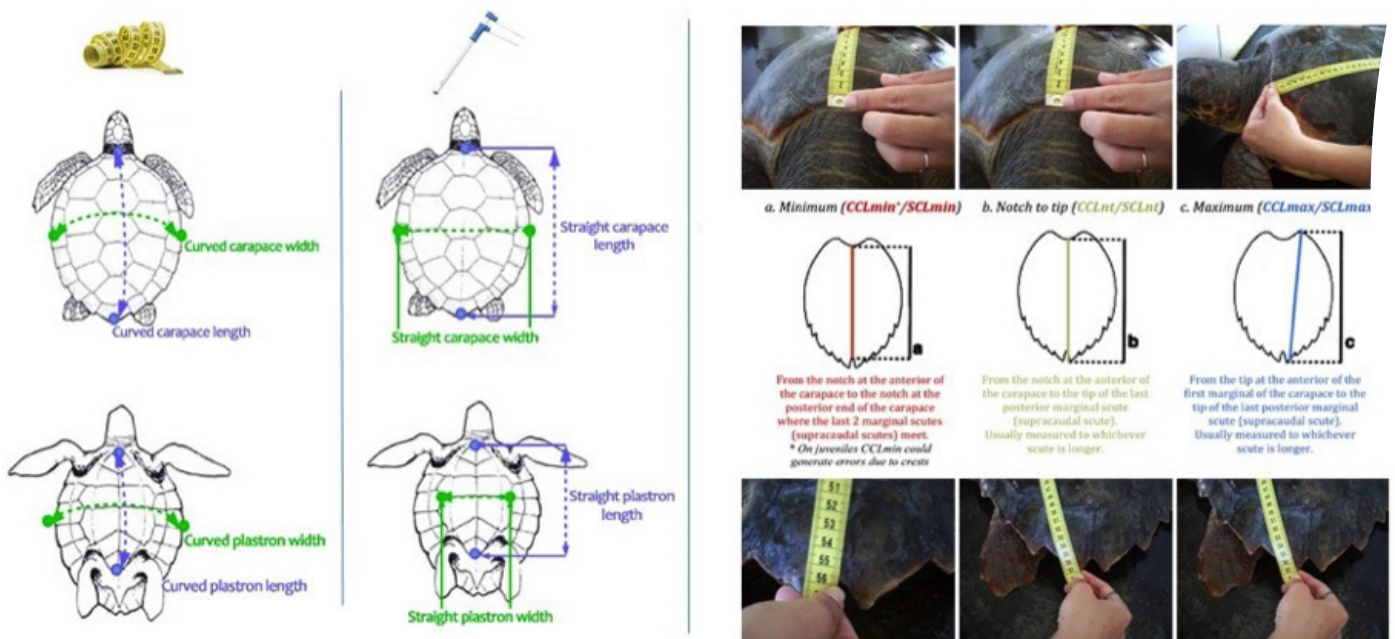


Fig. 6. Biometric parameters (carapace and plastron lengths).

B. SAMPLING MARINE LITTER FROM CARCASSES - PROTOCOL FOR DEAD ANIMALS

In case of decomposed animal (status of Levels 3 and 4), check the integrity of the digestive tract before carrying the turtle in laboratory. In any case (except status of Level 5), if the necropsy cannot be done immediately after the recovery, freeze the carcass at -20°C.

B.1 TURTLE NECROPSY

Follow the steps described in Fig 7:

Remove and separate the plastron from the carapace through an incision on the outside edge (yellow line) (Fig. 7a). Pay attention using a short blade or cutting with a horizontal tilt in order to not affect the integrity of the interior organs (Fig. 7b)

Once the inside of the plastron is accessed, cut the ligament attachment to the pectoral and pelvic girdle. Report the **Fat reserves** of the animal (Fig.7c) according to:

- Atrophy of pectoral muscles (none, moderate, severe),
- Fat thickness in articular cavities and in coelomic membrane (abundant, normal, low or none),
- Then complete the fat reserves informing the trophic status of the animal (thin, normal or fat).

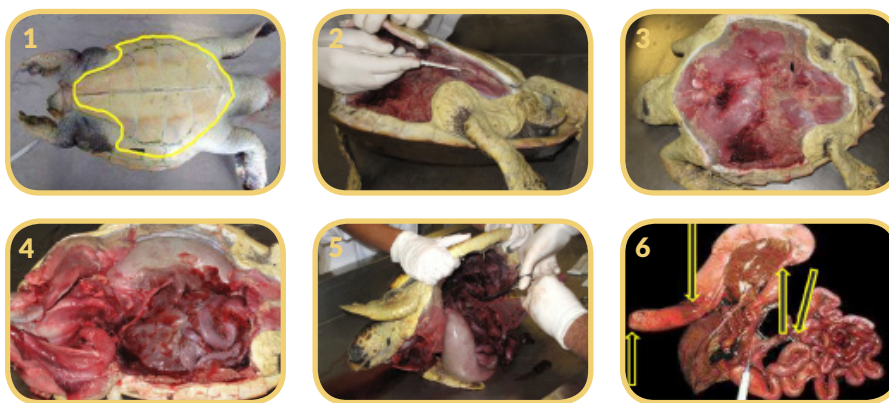


Fig. 7. Sequence of turtle necropsy: **a)** Ventral view of a dead turtle. The yellow line indicates the way to separate the plastron from the rest of the turtle; **b)** Horizontal cuts to prevent affecting the interior organs; **c)** ventral view of the opened turtle (fat reserves (brown) can be observed on the muscles); **d)** Remove the pectoral muscle and the heart; **e)** extraction of the GI; **f)** view of the entire GI. Yellow narrow marks indicate where clamps must be attached in order to separate the 3 different GI sections.

- Expose the gastrointestinal system (GI) by removing the pectoral muscles and the heart of the animal (Fig.7d and 7e). The blood can be emptied from the abdominal cavity by carefully rolling the turtle onto a side.
- Clamp the oesophagus proximal to the mouth and clamp the cloaca, the closest to the anal orifice. Remove the entire GI and place it on the examination surface. This operation is easier if done by at least 2 operators: one person keeps the animal lying on one side, while the other separates the ligaments of the different organs and membranes of the carapace, extracting the GI from the animal.
- Isolate the different portions of GI (oesophagus, stomach, intestines) by strangling and cutting between 2 clamps (yellow narrow marks, Fig. 7f) the gastro-oesophageal sphincter and the pyloric sphincter.

NOTE: If possible, record the **sex** of the animal.

B.2

EXTRACTION OF THE GUT CONTENT

Separate the 3 parts of the GI (oesophagus, stomach, intestines) by adding a second strangling at the cut edge to prevent spillage of the contents (Fig.8a)³. Open each GI section lengthway using a scissor and slide the material directly out of the section in a 1 mm mesh sieve⁴.

NOTE: Each sample which could not be analysed directly must be conditioned in an adapted bottle or zipped bag, identified thanks to a permanent marker with the animal identification code as well as the corresponding section. It is proposed to use 2 letters for the country _ 2 letters for the region/Institution _ Year _ Month _ Day _ N° turtle _ Type of sample (Faeces/Oeso/Stom/Intest) (ex: FR_GR_2017_03_12_9_Oeso corresponds to the oesophagus of the 9th individual found by the rescue centre of le Grau du Roi in France, the 12nd March 2017). The sample is then stored at -20°C, pending the laboratory analyses.

To extract the GI section content, clean out the content with current and abundant tap water (b) to remove the liquid portion, the mucus and the digested unidentifiable matter⁵.

Inspect the content for the presence of any tar, oil, or particularly fragile material, that must be removed and treated separately, and report in the column "Notes" of the INDICIT Excel file.

Rinse all the material collected in the 1 mm sieve; Fig.8b, c) and store it in jars with 70% alcohol or in zipped bags, reporting the label the sample code (individual code and respective GI section).



Fig. 8. Gut content analysis: a) Separated GI sections: Oesophagus (up), stomach (middle) and intestines (down); b) Section opening and gut content lavage; c) Gut content extracted.

B.3

EXTRACTION OF THE MARINE DEBRIS

The collection of the debris items is conducted manually by visual observation directly from the 1 mm sieve (Fig. 8c)⁶. Note the presence of any digestive occlusion or perforation caused by debris in the column "Notes".

³The 3 parts of the GI (esophagus, stomach, intestine) are analysed separately in order to assess possible differences in debris content per section and better assess the digestive transit of marine litter.

⁴To separate micro (1-5 mm) from macro-items (optional parameter), it is possible to superpose a sieve of 5 mm mesh to the sieve of 1 mm mesh (see section F of this document).

⁵Samples can be collected at this stage to determine the diet thanks to eDNA analyses. The protocol can be asked to coordination@indicit-europa.eu.

⁶The biological content can be stored in order to analyse the diet and identify the main ingested preys in relation with marine debris.

C. SAMPLING MARINE LITTER FROM FAECES - PROTOCOL FOR LIVE ANIMALS

C.1 COLLECTION OF FAECES

NOTE: For the homogeneity of approaches allowing the comparability of turtles and regions over time, the collected faeces will be analysed only for the individuals remaining at least 1-month minimum in the rescue centre. The faeces are collected until 2 months after the individual arrival.

Carefully rinse the turtle with water to avoid contamination and place the animal in an individual tank (Fig. 9a).

- Dispose a 1 mm filter in all the discharge tubes of the tank (Fig. 9b).
- Control the water tank daily by filtering through the 1 mm mesh sieve according to the following methods:
 - Collect the faeces manually with a 1 mm mesh dip net (Fig. 9c),
 - Put a 1 mm mesh flexible collector in the drain tube (Fig. 9d),
 - Place a 1 mm mesh rigid sieve under the drain (Fig. 9e).

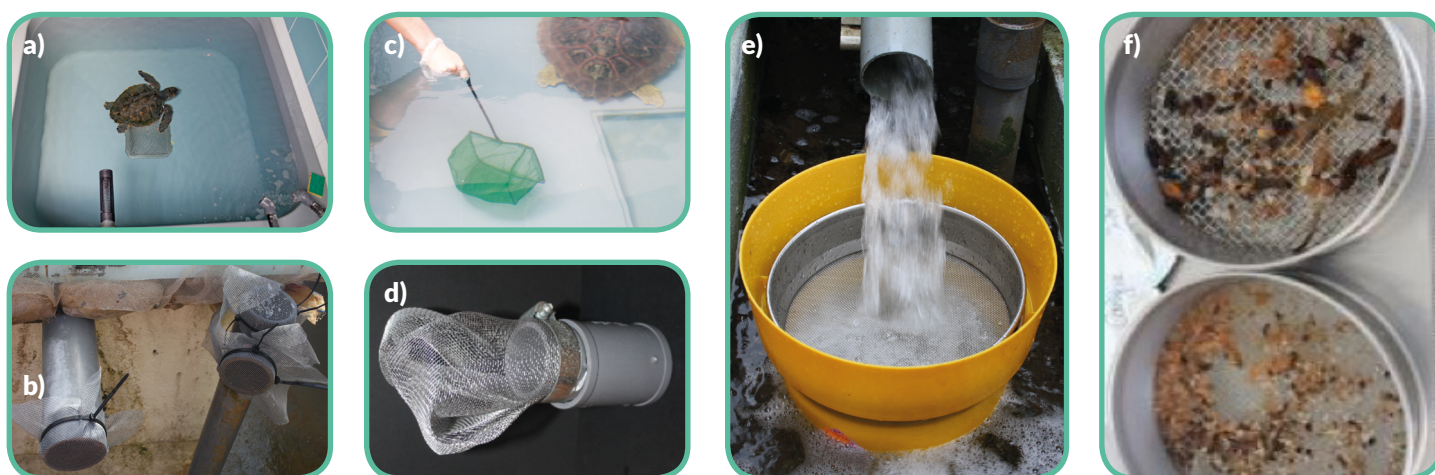


Fig. 9. Sequence of faeces sampling. a) The turtle is disposed in an individual tank; b) A 1 mm mesh sieve is disposed in discharge tubes; c) A 1 mm dip net for handling faeces; d) Collector with 1 mm mesh disposed in discharge tube for filtering water tank; e) A 1 mm mesh rigid sieve down discharge tube for filtering water tank; f) Sample collected in a rigid sieve.

NOTE: Each sample which could not be analysed directly can be conditioned in a tube or a zipped bag, identified with a permanent marker with the animal and the sample identification code, e.g. with 2 letters for the country _ 2 letters for the region/Institution _ Year _ Month _ Day _ N° turtle _ Type of sample (ex: FR_GR_2017_03_12_9_Faeces corresponds to the faeces excreted by the 9th individual found by the rescue centre of le Grau du Roi in France, the 12nd March 2017). The sample is then stored at -20°C or in 70°C alcohol at room temperature, pending the laboratory analyses.

C.2 COLLECTION OF DEBRIS FROM FAECES

Wash the sieves and collectors with abundant water above a rigid sieve (1 mm mesh) (Fig. 9f).
The collection of debris is conducted manually by visual observation directly from the 1 mm sieve.

D. DEBRIS ANALYSIS AND CLASSIFICATION

D.1 LITTER CLASSIFICATION

Specify the **protocol** you use, between “Necropsy” or “Faeces”.

For each GI section of the necropsied individual (Section B of this document) or for faeces (Section C of this document), classify the debris items according to the following categories (Tab 1., Fig. 10)⁷.

TYPE	CODE	DESCRIPTION
Industrial Plastic	IND PLA	Industrial plastic granules, usually cylindrical but also sometimes oval spherical or cubical shapes, or suspected industrial item, used for the tiny spheres (glassy, milky...)
Use sheet	USE SHE	Remains of sheet, e.g. from bag, cling-foil, agricultural sheets, rubbish bags...
Use threadlike	USE THR	Threadlike materials, e.g. pieces of nylon wire, net-fragments, woven clothing...
Use foam	USE FOA	All foamed plastics e.g. polystyrene foam, foamed soft rubber (as in mattress filling)...
Use fragment	USE FRAG	Fragments, broken pieces of thicker type plastics, can be a bit flexible, but not like sheet like materials.
Other use plastics	USE POTH	Any other plastic type of plastics, including elastics, dense rubber, cigarette filters, balloon pieces, soft airgun bullets... Specify in the column “Notes”.
Litter other than plastic	OTHER	All non-plastic rubbish and pollutant
Natural food	FOO	Natural food for sea turtles (e.g., pieces of crabs, jellyfish, algae...)
Natural no food	NFO	Anything natural, but which cannot be considered as normal nutritious food for sea turtle (stone, wood, pumice, etc.)

Tab. 1. Classification of debris items for sea turtles.

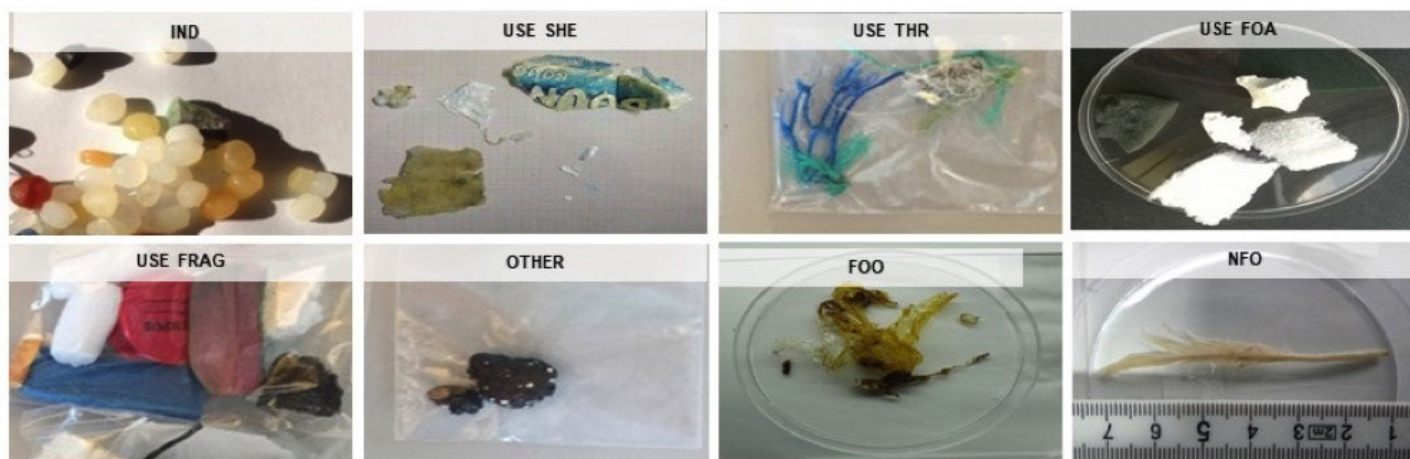


Fig. 10. Examples of litter categories established for marine turtle ingestion.

⁷ The classification of the debris items was adjusted by the INDICIT consortium, based on the MSFD guideline (Galgani et al., 2013) and the INDICIT partners and collaborators (e.g. rescue centres and stranding networks) feedbacks. The categories of debris can be identified visually and possibly confirmed by stereomicroscopy.

D.2

COLLECTION OF DATA

For each GI section of necropsied individuals or for the whole faeces samples of live individuals from 1 to 2 months until its arrival in the rescue centre, record the **dry mass of Food remains (FOO) and of Natural No Food remains (NFO)**, and **for each litter category** record the following parameters:

- **Dry mass** (grams, precision 0.01 g): Dry the sample at room temperature during 24h minimum or in a stove at 35°C during 12h, and record the dry weigh of each litter category in grams,
- **Number of items**: Report all counted items,

For the **individual** (whole samples, i.e. the total GI section of the necropsied individual or the whole faeces collected per live individual from 1 to 2 months until its arrival in the rescue centre), record:

- The **occurrence**: Presence or absence of ingested litter, 1 if at least one debris is found, 0 if no debris is found,
- The **quantity of ingested litter in terms of**:
 - **Total dry mass of litter** (grams, precision 0.01 g): sum of the dry masses reported for non-natural items, after drying the items at room temperature during 24h minimum or in a stove at 35°C during 12h,
 - **Total number of plastic items**: Count all observed plastic items (for the categories, IND PLA, USE SHE, USE THR, USE FOA, USE FRAG, USE POTH), whatever their size,
 - **Total volume of plastic items** (millilitre, precision 0.01 ml): Measure the volume of all plastic categories (IND PLA, USE SHE, USE THR, USE FOA, USE FRAG, USE POTH) in a graduated beaker and record the water variation (Fig. 11). Push the floating debris in the water thanks to a rod or a decimetre,
- The **total number of items per size category**:
 - **Total number of micro-plastics (1-5 mm)**: Count all plastic items whose larger size is between 1 and 5 mm⁷,
 - **Total number of meso-plastics (5 -25 mm)**: Count all plastic items whose larger size is between 6 and 25 mm,
 - **Total number of macro-plastics (> 25 mm)**: Count all plastic items whose larger size is bigger than 25 mm,
- The **total number of items per colour category**:
 - **Total number of white-transparent plastic items**: Count all plastic items being white opaque or transparent,
 - **Total number of dark coloured plastic items**: Count all plastic items of dark colour (black, blue, brown, dark green...),
 - **Total number of light coloured plastic items**: Count all plastic items of light colour (cream, yellow, pink, light green...)⁸,

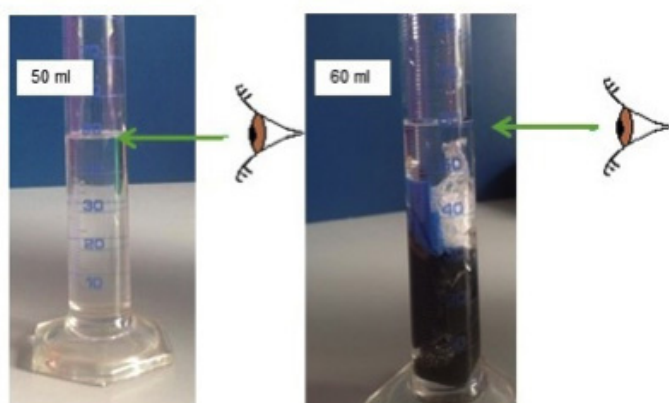


Fig. 11. The volume of the debris items corresponds to the difference between the volume with (right) and the volume without (left) the debris. The volume is read by considering the bottom of the meniscus formed by the surface water.

⁷ If 2 sieves (of 1 and 5 mm mesh) have been used when washing the digestive or faeces contents, this corresponds to all plastic items found in the 1 mm mesh sieve. You can report to Appendix 3 for a specific protocol to assess micro-debris in sea turtles.

⁸ The color can be detailed in the column "Notes".



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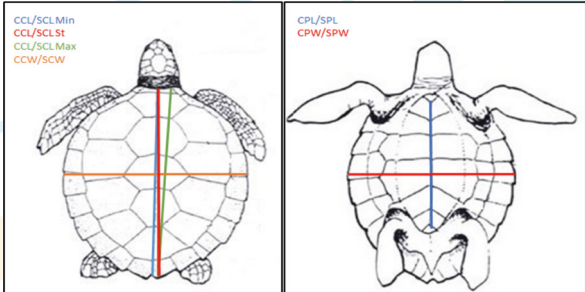
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APPENDIX 1 – OBSERVATION SHEET 1/2

OBSERVATION SHEET - Litter ingestion by sea turtles			
COLLECTOR:		LOCAL CODE:	
INSTITUTION:			
CONTACT:			
Discovery circumstances:			
SPECIES	<input type="checkbox"/> <i>Caretta caretta</i> <input type="checkbox"/> <i>Dermochelys coriacea</i> <input type="checkbox"/> <i>Chelonia mydas</i> <input type="checkbox"/> Other		
INDIVIDUAL TAG	Tag number: _____ Electronic chip N°: _____		
INDIVIDUAL CODE:	CC RR (Region) YY MM DD n°		
DATE OF DISCOVERY (yyyy/mm/dd):			
LOCATION:		X CORD : _____ Y CORD : _____	
CIRCUMSTANCES	<input type="checkbox"/> By-catch/Fishery <input type="checkbox"/> Stranding <input type="checkbox"/> Dead at rescue centre <input type="checkbox"/> Found at sea <input type="checkbox"/> Other <input type="checkbox"/> NR		
BY-CATCH ENGINE CAUSE	<input type="checkbox"/> Longline <input type="checkbox"/> Trawl <input type="checkbox"/> Drift net <input type="checkbox"/> Fishing rod <input type="checkbox"/> Other <input type="checkbox"/> NR		
CAUSE OF DEATH/ STRANDING	<input type="checkbox"/> Bycatch/Fisheries <input type="checkbox"/> Entanglement in debris <input type="checkbox"/> Ingestion of litter <input type="checkbox"/> Anthropogenic trauma <input type="checkbox"/> Natural trauma <input type="checkbox"/> Natural disease <input type="checkbox"/> Oils <input type="checkbox"/> Healthy <input type="checkbox"/> Other <input type="checkbox"/> NR		
ENTANGLEMENT TYPE	<input type="checkbox"/> Active <input type="checkbox"/> Passive <input type="checkbox"/> NR		
LITTER CAUSING ENTANGLEMENT	<input type="checkbox"/> Net pieces <input type="checkbox"/> Monofilament lines <input type="checkbox"/> Rope/s <input type="checkbox"/> Plastic bags <input type="checkbox"/> Raffia <input type="checkbox"/> Other <input type="checkbox"/> NR		
PICTURES <input type="checkbox"/>	Picture names :		
Animal body condition:			
CONSERVATION STATUS	<input type="checkbox"/> 1 - Alive <input type="checkbox"/> 2 - Fresh <input type="checkbox"/> 3 - Partial <input type="checkbox"/> 4 - Advanced <input type="checkbox"/> 5 - Momified <input type="checkbox"/> NR		
HEALTH STATUS (Plastron shape)	<input type="checkbox"/> Poor (concave) <input type="checkbox"/> Fair (plane) <input type="checkbox"/> Good (convex) <input type="checkbox"/> NR		
MAIN INJURIES	<input type="checkbox"/> No injuries <input type="checkbox"/> Fracture <input type="checkbox"/> Amputation <input type="checkbox"/> Sectionning <input type="checkbox"/> Abrasion <input type="checkbox"/> Other		
AFFECTED PARTS	<input type="checkbox"/> Flipper () <input type="checkbox"/> Carapace <input type="checkbox"/> Neck <input type="checkbox"/> Head <input type="checkbox"/> Plastron <input type="checkbox"/> Other		
FAT RESERVES	<input type="checkbox"/> Thin <input type="checkbox"/> Fat <input type="checkbox"/> Normal <input type="checkbox"/> NR		
Biometric measurements:			
Curved measurements (0,01cm)		Straight measurements (0,01cm)	
CCLst	cm	SCLst	cm
CCLmax	cm	SCLmax	cm
CCLmin	cm	SCLmin	cm
CCW	cm	SCW	cm
CPL	cm	SPL	cm
CPW	cm	SPW	cm
WEIGHT (0,01kg)			
NOTES AND REMARKS (Discovery and Animal conditions):			

APPENDIX 1 – OBSERVATION SHEET 2/2

INDIVIDUAL CODE:								
CC	RR (Region)	YY	MM	DD	n°			
Extraction of ingested litter								
PROTOCOL		<input type="checkbox"/> Necropsy <input type="checkbox"/> Observation of faeces						
ARRIVAL DATE / /		DEPARTURE DATE / /						
DEAD DATE / /								
FAT RESERVES		<input type="checkbox"/> Thin <input type="checkbox"/> Fat <input type="checkbox"/> Normal						
Please describe :								
VISCERAS STATUS (note the presence of any infection, suspect colour, fluid effusion, perforation, presence of oil, etc.):								
DIGESTIVE TRACT (note the presence of any infection, suspect colour, fluid effusion, perforation, presence of oil, etc.):								
TURTLE BEHAVIOUR AND TREATMENTS:								
Capacities of digestive tract section and gut content								
	FULL				EMPTY			
	mass	Vol (V1)	vol (V0)	V1-V0	mass	Vol (V1)	vol (V0)	V1-V0
ŒSOPHAGUS								
STOMACH								
INTESTINES								
Marine debris measurements								
	ŒSOPHAGUS		STOMACH		INTESTINES			
	DRY MASS	NUMBER	DRY MASS	NUMBER	DRY MASS	NUMBER		
Ind. Plastic								
USE SHE								
USE THR								
USE FOA								
USE FRAG								
Other (USE poth)								
Non plastic								
FOO (nat. Food)								
NFO (nat. no food)								
TOTAL								
	TOTAL DEBRIS		NUMBER OF ITEMS		NUMBER OF ITEMS			
	dry mass		micro (1-5mm)		white transparent			
	number of items		meso (5-25mm)		dark coloured			
	volume		macro (>25mm)		light coloured			

APPENDIX 2 – LIST OF MATERIAL

For the recovery of the animal and the collection of samples at the discovery site

Rope (to marke-off the zone)		Pen	
Integral protective suit		Observation sheet	
Glasses and protective mask or shield		Bottle/ziploc bags	
Cut-resistant gloves		Cooler	
Gloves		Permanent marker	
Boots		Transport bins or containers for the turtle	
Camera		Garbage bag	
Measuring tape			

For the collection of samples on dead individuals in laboratory and the extraction of the ingested litter from the digestive tract

In the laboratory room		For the necropsy and the collection of samples	
Cold chamber or chest freezers (-20°C) with large storage capacity		Clamps (at least 6) and/or kistchen string or plastic cable clamps	
Proofer (not mandatory)		Scalpel (possible with interchangeable blade)	
Garbage bags		Scissors	
For manipulators		Clips with claws	
Integral protective suit		Metal containers	
Glasses and protective mask or shield		Containers for samples (Bottle/zipped bags)	
Cut-resistant gloves		For the analysis of ingested litter	
Gloves		Sieve with 1 mm mesh	
Boots		Sieve with 5 mm mesh (optional – for the study of the ingested micro-plastics (1-5 mm))	
For notes and report		Measuring cylinders (10 ml, 25 ml, 50 ml)	
Camera		Measuring decimetre	
Pen		Precision balance (0.01 g)	
Observation sheet		Binocular (optional)	
Permanent marker			
For biometric measurements			
Measuring tape			
Sliding calliper			

For the collection of samples on live individuals in rescue centres and the extraction of ingested litter in the faeces

In the laboratory room		For the collection of samples and the analysis of the ingested litter	
Freezers (-20°C)		Permanent marker	
Proofer (not mandatory)		Observation sheet	
Garbage bags		Containers for samples (tubes/zipped bags)	
For manipulators		Sieve with 1 mm mesh	
Glasses and protective mask (optional)		Sieve with 5 mm mesh (optional – for the study of the ingested micro-plastics (1-5 mm))	
Gloves		Measuring cylinders (10 ml, 25 ml, 50 ml) (optional)	
For notes and report		Decimetre (optional)	
Camera		Precision balance (0.01 g)	
Pen		Binocular (optional)	
Observation sheet		Filtration grids with 1 mm mesh (at the levels of water arrival and discharge)	
Permanent marker		Landing net with 1 mm mesh	
For biometric measurements		Coloured micro-balls diameter < 1 mm (optional)	
Measuring tape			
Sliding calliper			

PROTOCOL FOR THE EXTRACTION OF MICRO-PLASTICS (OPTIONAL)

1. PREPARATORY ACTIONS TO AVOID CONTAMINATION

1. For dead animals

- Make an exhaustive lavage of the digestive track sections before opening by using water above a 1 mm mesh sieve (filtered water).
- Make an exhaustive lavage of all instruments used during the gut content and debris analysis.

2. For live animals

- Make an exhaustive lavage of the turtle before placing the animal into a sampling tank using running water.
- Dispose filter of 1 mm mesh in the water entrance tube of the sampling tank.
- Eviscerate prey food items (fish, squid, crab, etc.) before supplying the turtles in order to avoid contamination caused by possible micro-plastics ingested by the preys.
- Sample an empty tank ("control") located close to the sampling tank in order to control air contamination.

2. EXTRACTION OF THE INGESTED MICRO-DEBRIS

To specifically assess the dry mass of the micro-debris, use two superposed sieves of 1 and 5 mm and collect the items of size between 1 and 5 mm in the 1 mm mesh sieve. **The methodologies for micro-debris collection from gut content and faeces are similar to the one described in the text (section D of the document).**

To better separate the micro-debris, which could be camouflaged in the biological content, it is possible to proceed to a chemical digestion of the organic material by introduce the entire 1 mm mesh filter in a glass jar containing H₂O₂ / KOH, which necessitates between 12 to 24h depending on the sample quantity (Fig.11).

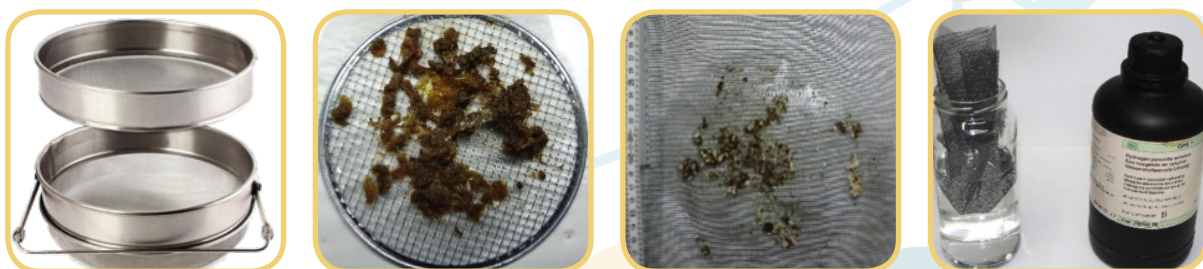


Fig. 11. Micro-debris extraction methodology. a) Two sieves methodology; b) Sample collected in the 5mm rigid sieve; c) Sample collected in the 1mm flexible mesh; d) Digestion of 1mm sample in H₂O₂.