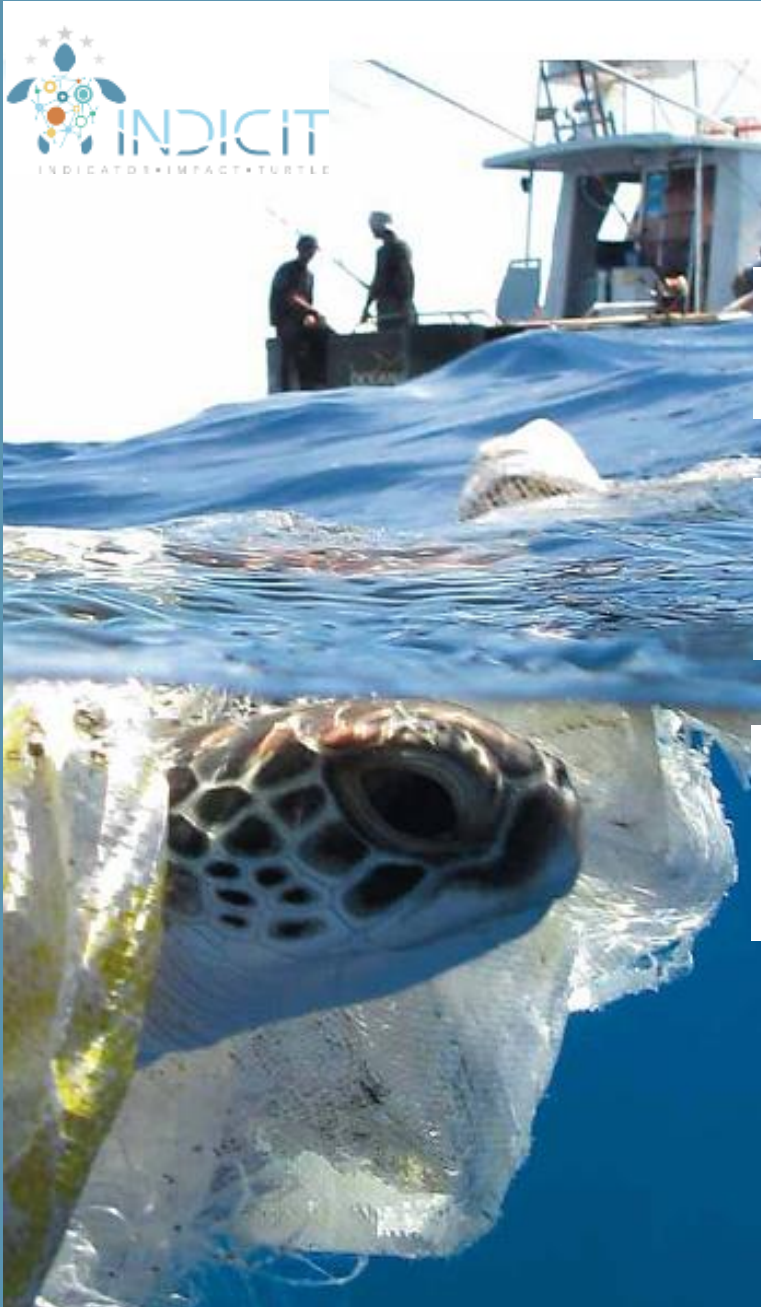




INDICIT tools

Tools Versions September 2018





1

Video-tutorial

2

Protocol for collecting data
on litter ingestion

3

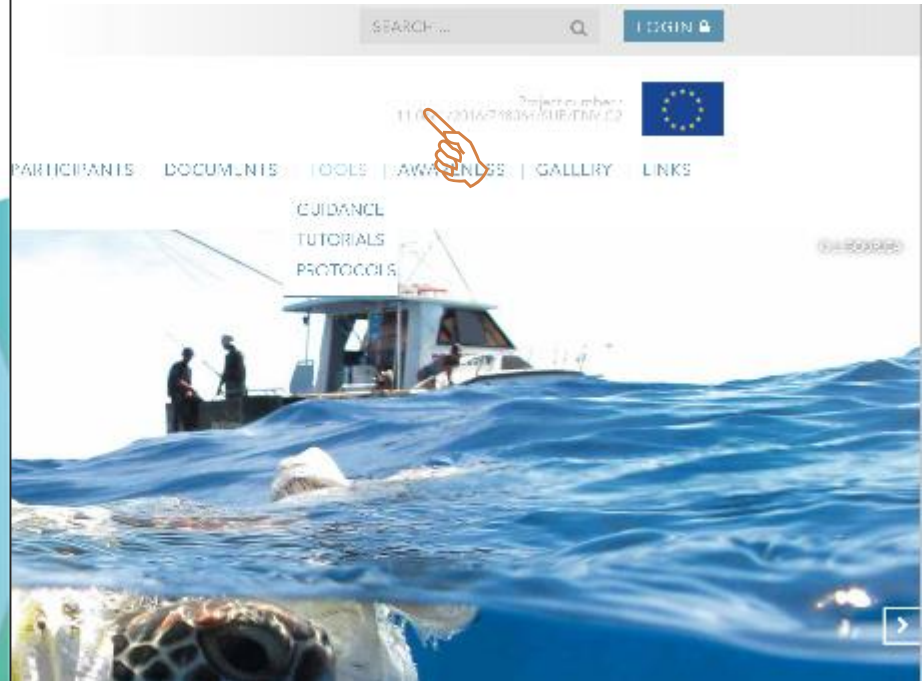
Special focus on
entanglement



 MONITORING MARINE LITTER IMPACTS ON
SEA TURTLES

[Video](#)

PROTOCOL FOR COLLECTING DATA ON LITTER INGESTION



<https://indicit-europa.eu/protocols/>

In brief

➤ Origin:

- Protocol from 2013 MSFD guidance on monitoring marine litter in European seas (Fulmar, Turtles)
- Improvements from experience, results, collaborators' comments

➤ Objectives:

- Collecting, classifying and quantifying the debris ingested by sea turtles
- Assessing body condition
- Evaluating the possible causes of death and impacts caused by debris
- Particular focus on Entanglement

➤ Focus

- Example on *Caretta caretta*, applicable to all species (except *Dermochelys coriacea* for some manipulations → biometric measures)
- Detail Plastic categories (+ other types of debris)

**Comments to make the protocol
evolve are welcome!!**



Special remarks

➤ Sea turtles are protected species

- Only **authorized people** can handle live and dead animals or parts of them.
→ Contact responsible Authorities.
- **CITES permit** asked for sending/receiving specimen or samples

➤ Sanitary precautions

- Be careful to minimize risks for manipulator as for alive animal (zoonosis)
- Mark-off place of intervention to keep away and protect bystanders
- Wear protective suit with glasses, gloves (a cut-resistant pair of gloves below 2 pairs of gloves) and rubber boots
- Carefully separate and disinfect or thrown materials.



➤ 2 types of manipulations

- **Necropsy** of dead turtle: Collect debris from the entire digestive tract (B1, p.11)
- **Faeces analysis** on alive turtle: Collect debris from excretions (C1, p.13)
- General information common to the 2 protocols

Common datasheets

APPENDIX 1 – OBSERVATION SHEET 1/2

OBSERVATION SHEET Litter ingestion by sea turtles	
COLLECTOR:	LOCAL CODE:
INSTITUTION:	
CONTACT:	
Discovery circumstances:	
SPECIES	<input type="checkbox"/> Caretta caretta <input type="checkbox"/> Dermochelys coriacea <input type="checkbox"/> Chelonia mydas <input type="checkbox"/> Other
INDIVIDUAL TAG	log number: Electronic chip N°:
INDIVIDUAL CODE:	
DATE OF DISCOVERY (yyyy/mm/dd):	
LOCATION:	
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"/> By-catch/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"/> Old <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"/> Raft/s <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 - Mortified <input type="checkbox"/> NR
HEALTH STATUS (External signs)	<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"/> Sectioning <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
FAT RESERVES	<input type="checkbox"/> Thin <input type="checkbox"/> Fat <input type="checkbox"/> Normal <input type="checkbox"/> NR
Biometric measurements:	
Curved measurements (0.01 cm)	Straight measurements (0.01 cm)
CClst	SCLst
CCLnax	SCLnax
CCMin	SCLMin
CCW	SCW
CPL	SPL
CPW	SPW
WEIGHT (0.01 kg)	
NOTES AND REMARKS (Discovery and Animal conditions):	

APPENDIX 1 – OBSERVATION SHEET 2/2

INDIVIDUAL CODE:							
Extraction of ingested litter							
PROTOCOL	<input type="checkbox"/> Necropsy <input type="checkbox"/> Observation of carcass						
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
CESOPHAGUS							
STOMACH							
INTESTINES							
Marine debris measurements							
CESOPHAGUS		STOMACH		INTESTINES			
DRY MASS		NUMBER		DRY MASS		NUMBER	
Ind. Plastic							
USE SHE							
USE THR							
USE FOA							
USE FRAG							
Other (USE polyl)							
Non plastic							
FOO (wt. Food)							
NFO (wt. no food)							
TOTAL							
TOTAL DEBRIS		NUMBER OF ITEMS		NUMBER OF ITEMS			
dry mass		micro (< 5mm)		very transparent			
number of items		meso (5-25mm)		dark coloured			
vol. LHM		macro (> 25mm)		light coloured			

The screenshot shows the Microsoft Excel interface with the following elements:

- Ribbon:** Fichier, Accueil, Insertion, Mise en page, Formules, Données, Révision, Affichage.
- Formulas Bar:** Contains the formula `=Caretta caretta) / De (=Demochelys coriacea) / Cm (Chelonia mydas) / Other`.
- Worksheet Grid:**
 - Columns:** A, B, C, D, E, F, G, H, I, J, K, L.
 - Row 1 Headers:** AREA, SPECIES, TAG / CHIP, ID CODE, DATE OF DISCOVERY, YEAR OF DISCOVERY, LOCATION AT FINDING, GEOGRAPHICAL COORDINATES, CIRCUMFERENCE.
 - Row 2 Data:**
 - A:** (MED / ATL)
 - B:** Country Stakeholder Partner Institution
 - E:** Co (=Caretta caretta) / De (=Demochelys coriacea) / Cm (Chelonia mydas) / Other
 - F:** No tags / 0000-0000 / 0000000000000000
 - G:** [Conservation, Management, Research, Monitoring, Policy, Development, Conservation Number] CC-BB-YYYY-MM-DD-XXX
 - H:** [DD/MM/YYYY ; NA (information Not available)]
 - I:** [YYYY; NA (Information Not available)]
 - J:** [Locality, place, name of the area, etc.] NA (information Not available)
 - K:** X COORD [Decimal degrees] NA (information Not available)
 - L:** Y COORD [Decimal degrees] NA (information Not available)

- ❖ Associated Standard Database
- ❖ 2 tabs: Necropsy / Faeces
- ❖ 1 line per individual
 - Data banking will be managed by other procedures (ex. Clean Atlantic)
- ❖ Data shared in private area

Other tools

APPENDIX 2 – LIST OF MATERIAL

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

Rope (to mark-off the zone)	Pen	
Integral protective suit	Observation sheet	
Glasses and protective mask or shield	Bottle/toploc bags	
Cub-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 freezer (-20°C) with large storage capacity	Clomex (alcohol 6° and/or fish oil mixing or plastic stable clamor)	
Freezer (not mandatory)	Scissors (available with interchangeable blades)	
Garbage bags	Scissors	
For manipulators		
Integral protective suit	Clips with claws	
Glasses and protective mask or shield	Metal containers	
Cub-resistant gloves	Containers for samples (bottle/toploc bags)	
For the analysis of ingested litter		
Gloves	Sieve with 3 mm mesh	
Boots	Sieve with 5 mm mesh (optional – for the study of fine ingested micro-plastics (0.5 mm))	
For notes and report		
Camera	Measuring cylinders (10 ml, 25 ml, 50 ml)	
Pen	Measuring scale (cm)	
Observation sheet	Precision balance (0.01 g)	
Permanent marker	Binocular (optional)	
For biometric measurements		
Measuring tape		
Sliding caliper		

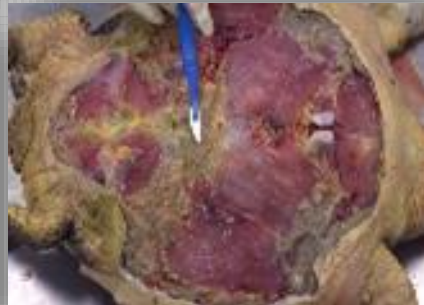
For the collection of samples on live individuals in rescue centres and the extraction of ingested litter in the faeces

In the laboratory room	For the collection of samples and the analysis of the ingested litter	
Freezers (-20°C)	Permanent marker	
Freezer (not mandatory)	Observation sheet	
Garbage bags	Containers for samples (tube/toploc bags)	
For manipulators		
Glasses and protective mask (optional)	Sieve with 1 mm mesh	
Gloves	Sieve with 5 mm mesh (optional – for the study of the ingested micro-plastics (0.5 mm))	
For notes and report	Measuring cylinders (10 ml, 25 ml, 50 ml) (optional)	
Camera	Measuring scale (optional)	
Pen	Precision balance (0.01 g)	
Observation sheet	Binocular (optional)	
Permanent marker	Filter paper with 1 mm mesh (to load of water on filter and discharge)	
For biometric measurements		
Measuring tape	Landing net with 1 mm mesh	
Sliding caliper	Coloured micro-balls diameter < 1 mm (optional)	

~ 5H / individual for protocol necropsy
Preferable to be 2 manipulators



THE PROTOCOL STEP BY STEP



- **“Basic” parameter:** Fundamental for using the indicator and assessing distance to GES
- “Optional” parameters: Acquire knowledge on the factors influencing litter ingestion in sea turtles



*Note the circumstances among the 7 categories:

- Black / Grey

OBSERVATION SHEET - <i>Enter information by sea turtle</i>	
COLLECTOR:	LOCAL CODE:
INSTITUTION:	
CONTACT:	
Discovery circumstances:	
SPECIES	<input type="checkbox"/> <i>Caretta caretta</i> <input type="checkbox"/> <i>Demochelys coriacea</i> <input type="checkbox"/> <i>Chelonia mydas</i> <input type="checkbox"/> Other
INDIVIDUAL TAG	Tag number: Band on flipper(s):
INDIVIDUAL CODE:	CC NB England VN VN DO VN
DATE OF DISCOVERY: <input type="text"/> / <input type="text"/> / <input type="text"/>	
LOCATION:	X COORD: Y COORD:
CIRCUMSTANCES	<input type="checkbox"/> Beach/ Fishing <input type="checkbox"/> Stranding <input type="checkbox"/> Dead at rescue center <input type="checkbox"/> Found at sea <input type="checkbox"/> Other: <input type="text"/> <input type="checkbox"/> NR
BY CATCH/ENGINE CAUSE	<input type="checkbox"/> Longline <input type="checkbox"/> Trawl <input type="checkbox"/> Gillnet <input type="checkbox"/> Harpoon <input type="checkbox"/> Other <input type="checkbox"/> LNR
CAUSE OF DEATH/STRANDING	<input type="checkbox"/> Bycatch/Fishing <input type="checkbox"/> Entanglement in debris <input type="checkbox"/> Ligation of off hook <input type="checkbox"/> Anthropogenic trauma <input type="checkbox"/> Natural trauma <input type="checkbox"/> Natural disease <input type="checkbox"/> Old <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
IF OTHER CAUSING ENTANGLEMENT	<input type="checkbox"/> Net/placement <input type="checkbox"/> Mismanagement - line <input type="checkbox"/> Snagging <input type="checkbox"/> Plastic bags <input type="checkbox"/> Raffle <input type="checkbox"/> Other <input type="checkbox"/> NR

It is possible to detect the probable cause of death or of live individual stranding from external observations and to establish from the observation of or contact with the carcass of dead individuals. Also important and necessary to the existence of the population, there is a need among the 11 heronries:

- **Bycatch/Fisheries related:** more than a single hook does represent a species, and was mapped in general in this study. In the example, 4 entanglement types and 4 litter causing entanglement, individualized within the fishery.
- **Entanglement in debris:** Entanglement in debris shows that there is no need to fishing out it. Please fill out details of the entanglement types and the cause when available.
- **Immersion of litter:** when litter is not visible, please fill out details.



A. GENERAL INFORMATION

A1. ON SITE:

-> Describe discovery place

Location, coordinates

-> Give contact coordinates

Name, Tel...

-> Identify the individual

Species, Tag, **(standard) Identification code** → all samples contents

-> Take pictures

Use a scale; identify the picture



A2 to A3. DESCRIBE ANIMAL BODY CONDITION

➤ Circumstances:

- **Stranding** → **Stranding cause**
 - Bycatch
 - Entanglement
 - Anthropogenic trauma
 - Natural predation
 - Petrol / oils
 - Natural disease
 - Others
- **Bycatch/fisheries** → **By-catch engine**
 - Longline
 - Trawl
 - Drifting net
 - Other
- **Found at sea**
- **Dead at the Recovery Centre**



A2 to A3. DESCRIBE ANIMAL BODY CONDITION

➤ **Status:** From 1 to 5



- Status 1 → “Alive turtle” Protocol → Faeces (C1), except if dead after the recovery (e.g., at the rescue center). In this case, report to tab “Necropsy”



- Status 2 to 4 → Necropsy



- Status 5 reported but not considered in statistics

A2 to A3. DESCRIBE ANIMAL BODY CONDITION

➤ Impacts of litter

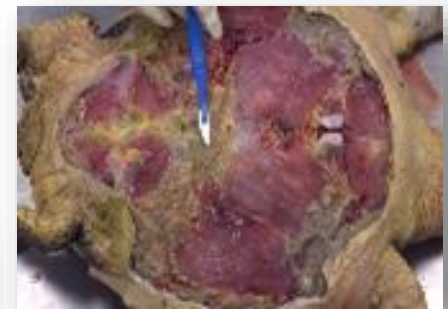
- Probable cause of death or stranding
- Main injuries + Affected body part
- Entanglement characterization (described latter)
- Health status



- **Fat reserves (in Other descriptive parameters)**

Overall evaluation from:

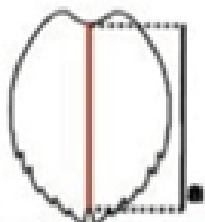
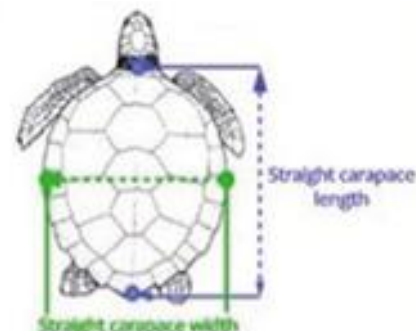
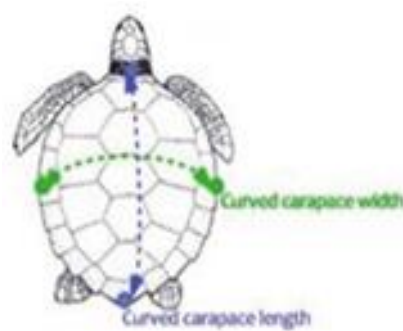
- External examination
- Internal examination



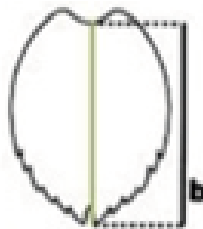
A2 to A3. DESCRIBE ANIMAL BODY CONDITION

➤ Biometric measures

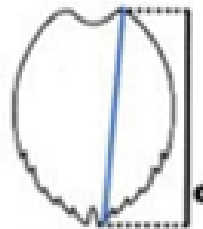
- Curved (CCL) and straight (SCL) carapace lengths (0.1 cm)



Min



Standard



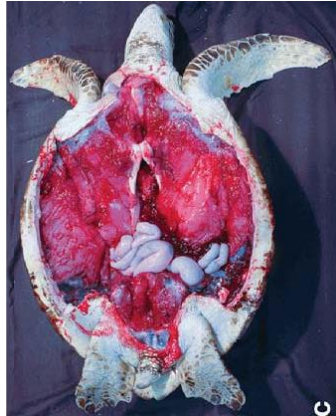
MAX

- Weight (grams)



⇒ With biometric measure, test ≠ in litter ingestion according to stage and the area where turtles may ingest plastics

SAMPLING DEBRIS



**DEAD
TURTLES**

**DIGESTIVE TRAC
(NECROPSIES)**

**ALIVE
TURTLES**

**FAECES
ANALYSIS**



**STRANDING
CAUSE**

**IND2:
*Entanglement on
marine debris***

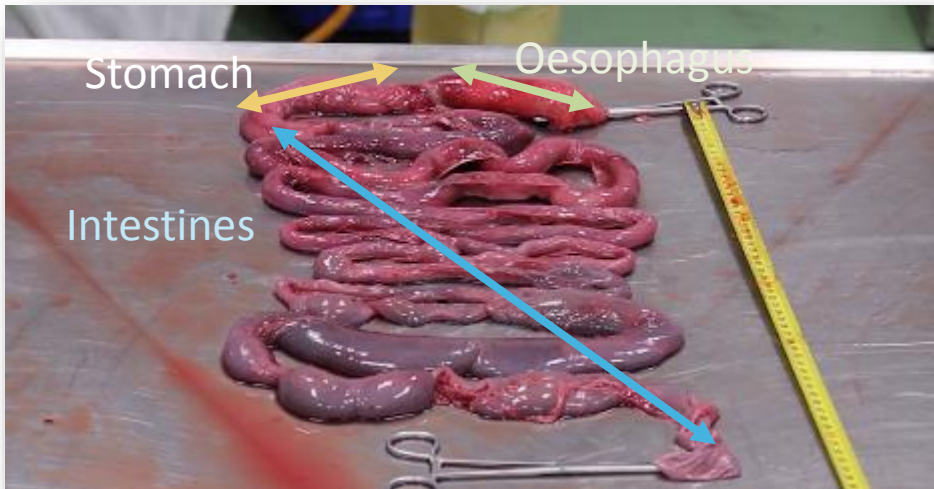
**IND1:
*Debris ingestion (>1mm)***

**IND3:
*micro-debris ingestion (1-5mm)***

B) SAMPLING FROM DEAD INDIVIDUALS: (DIGESTIVE TRACT ANALYSIS)

- Various possible sampling → from the cleaner to the dirty (e.g., cranial cavity first)
- Preferable to be 2 manipulators to turn/maintain the animal body

Clamp and separate each section



B) SAMPLING FROM DEAD INDIVIDUALS: (DIGESTIVE TRACT ANALYSIS)

- Various possible sampling → from the cleaner to the dirty (e.g., cranial cavity first)
- Preferable to be 2 manipulators to turn/maintain the animal body



C) SAMPLING FROM ALIVE INDIVIDUALS: (FAECES ANALYSIS)

Direct observation



Filtering water tank



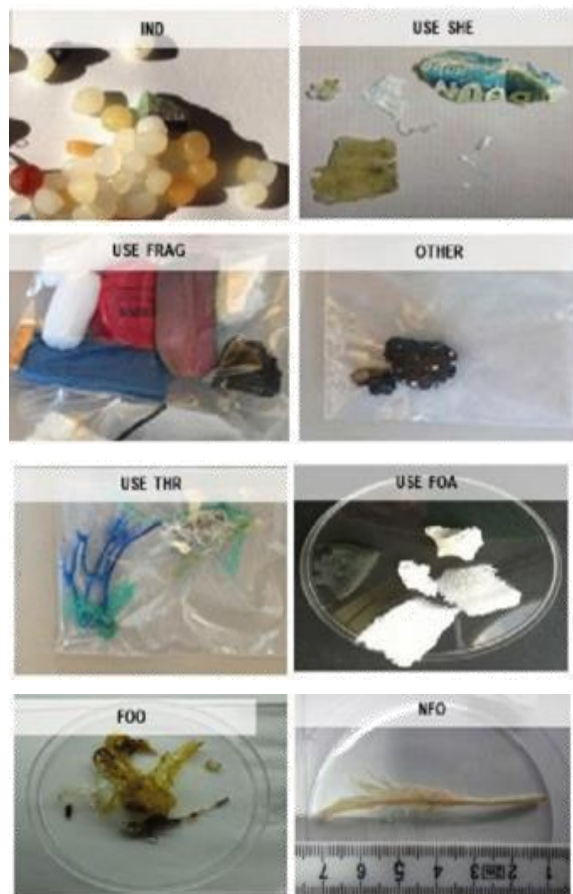
(1mm mesh)



→ Analyse or freeze

D) DEBRIS ANALYSIS AND CLASSIFICATION: LITTER CLASSIFICATION:

→ Adapted from MSFD classification



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.)

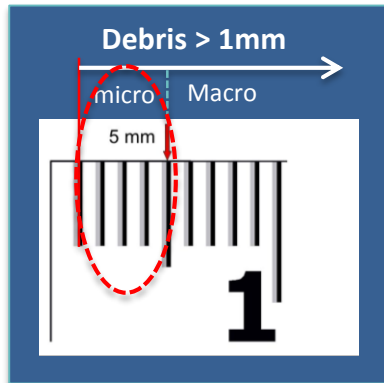
D) DEBRIS ANALYSIS AND CLASSIFICATION: LITTER ANALYSIS:

- **Occurrence (1/0)**
- **By category** in each digestive section / faeces
 - Dry mass (0.01 gram)
 - Number of fragments
 - Number of types of items
- **For plastic only**
 - Total dry mass
 - Total number of fragments
 - Total volume of items
 - Color per category
 - Number per size category

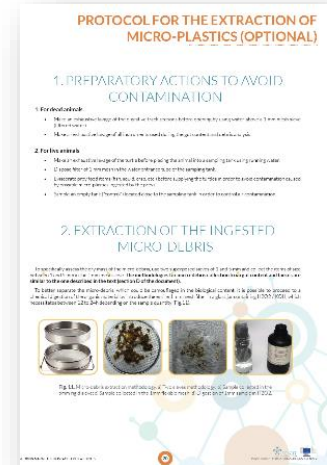


D) DEBRIS ANALYSIS AND CLASSIFICATION:

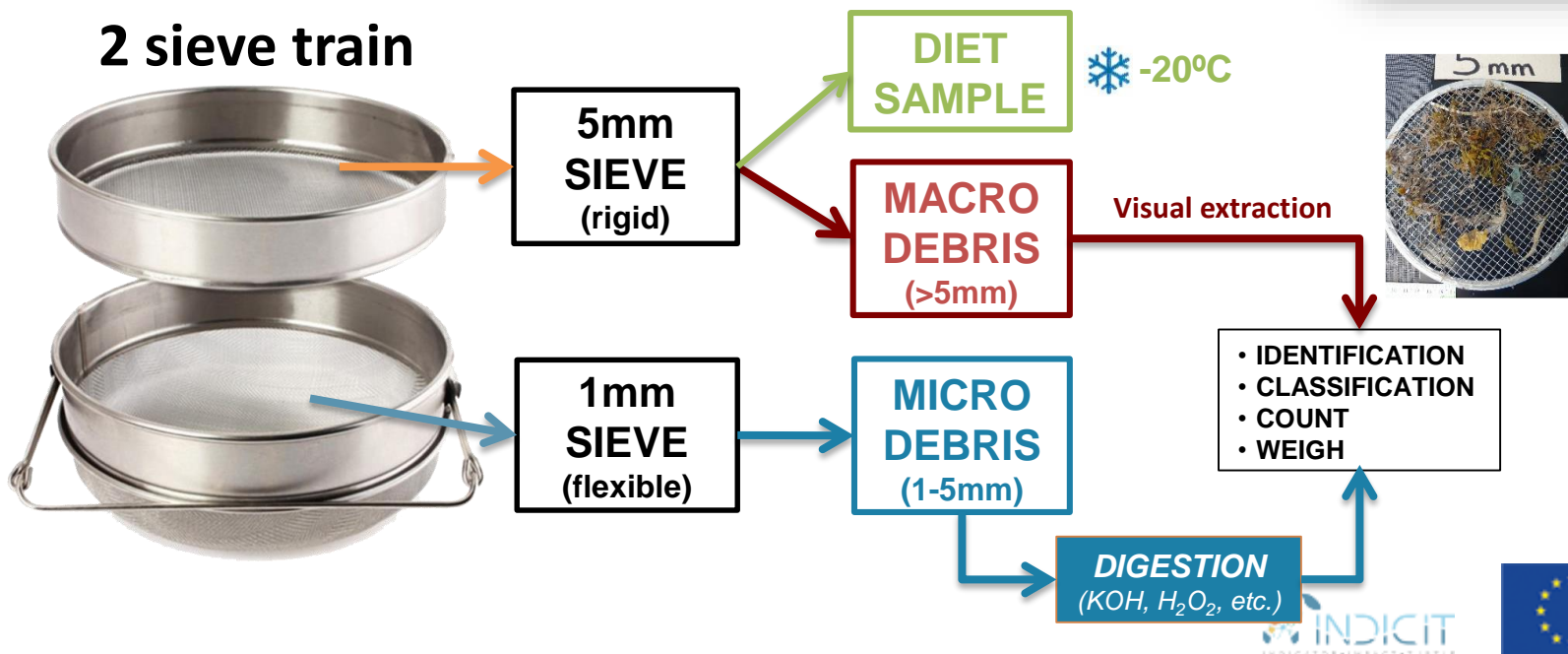
LITTER ANALYSIS:



→ specific analysis for micro (1-5 mm)
(optional protocol)



2 sieve train



Forward the needs and share experience

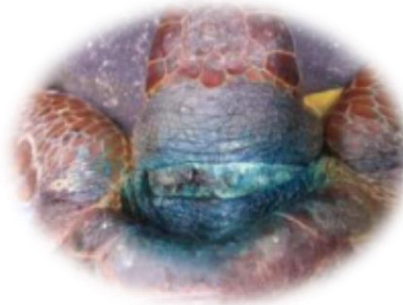
- Training sessions
- Specific material or overall kit
- Share your observations in column “Notes”
- Other ideas
 - Forum (share pictures, ask questions...)





- Probably high impact on marine biota
- Developing a new Litter impact indicator
 - See online [Feasibility study](#)
 - Need standardize typology
 - Passive (litter) ≠ Active (fishing)
 - Requires specific protocol and database
- Sea turtle = a good indicator taxa
 - Available competent network
 - Recurrent observation of entanglement
 - Recognized by experts as fundamental and feasible

- ❖ Probable cause of death/stranding
- ❖ Main injuries & affected body part



❖ Litter causing entanglement

FISHING GEARS



(N) Net pieces



(L) Monofilament line



(R) Ropes

(Mu) Multiple materials



DEBRIS OF USER ORIGIN



(Pb) Plastic bag



(Rf) Raffia sack



(Ot) Other

(UNK) UNKNOWN:



(multiple choice)

- Fishing nets
- Monofilament
- Ropes
- Plastic bags
- Raffia
- Other



Thank you

