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Agenda Item 3: Proposal for Updating IMAP Ecological Objective 10 (EO 10) on Marine Litter

Protocols for Monitoring Interactions between Marine Litter and Marine Turtles (Ingestion and Entangling) with a view to Harmonizing Methods of Data Collection for Monitoring and Assessment

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Protocols for monitoring interactions between marine litter and marine turtles (ingestion and entangling) with a view to harmonising methods of data collection for monitoring and assessment

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For more information:

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1. Protocols for monitoring interactions between marine litter and marine turtles¹

The protocol presented under the present document intends to provide technical support and guidance with regards to monitoring the impact of marine litter, especially through ingestion and entanglement, on marine biota. The hereunder presented monitoring protocol provides a response to the requirements under the European Commission (EC) Marine Strategy Framework Directive (MSFD) (i.e. Indicator 10.2.1 “*Trends in the amount and composition of litter ingested by marine animals*” (Criteria D10C3), and the Regional Sea Conventions i.e. OSPAR (Indicator EcoQO3) and Barcelona Convention (10th Ecological Objective (EO10) on Marine Litter of the Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP)).

EO10 of IMAP consists of two Common Indicators and a single Candidate Indicator. EO10 Candidate Indicator 24 is referring to the “Trends in the amount of litter ingested by or entangling marine organisms focusing on selected mammals, marine birds, and marine turtles). Marine turtles have been proposed as indicator species to study marine litter ingestion on biota through the development and the implementation of one major indicator “Litter ingested by sea turtles”.² On the basis of the information available, the approach that uses the monitoring of marine turtles’ ingestion of litter seemed consistent and compatible with the whole set of biological, methodological, environmental, logistical and ethical constraints identified (RAC/SPA, 2017). Some elements have already been suggested in this perspective (Table 3).

Standardized methodologies for extracting marine litter ingested from dead and live individuals are presented to the present document. This document originates from the merge and integration between, the INDICIT protocol (INDICIT³, 2018) established from original methodologies tested first ever in Italy (Matiddi et al., 2011), later transposed into the MSFD guideline (MSFD TG ML, 2013), regularly improved in cooperation with various stakeholders (rescue centres, stranding networks, etc.); and the Marine Litter MED⁴ Project protocol (UN Environment/MAP Specially Protected Areas Regional Activity Centre⁵, 2017).

Species and habitat conservation policies recognise the pressure that waste of human-origin exerts on marine turtle populations as a potential threat. In the context of the Convention for the Conservation of Migratory Species (Bonn Convention or CMS), Resolution 10.4 on Marine Litter and Resolution 11.30 on Managing Marine Litter, have recently been repealed and put together in a new Resolution that will reflect how the context has changed since they were published in accordance with developments made in other surroundings. In this Resolution, the CMS invites the Parties (paragraph 24 b) to draft reports on measures implemented and their relative success in marine litter management. It also invites the Secretariat of the CMS family Accords (paragraph 28 b) to submit data on the impacts of marine litter, including micro-plastics, on the migratory species covered by these Accords with a view to their being examined by the Scientific Council.

Table 3: Types of data and categories of litter, the use of which has been advised in the context of the programmes for monitoring the impact of litter on marine turtles/biota, by UNEP/MAP/MEDPOL and MSFD.

¹ The elaboration of the protocols has been prepared by SPA/RAC in the framework of the EU-funded Marine Litter MED Project, with support of regional experts, in full synergy with the Protocols developed under EU-Funded INDICIT Project.

² As part of the Regional Plan on Marine Litter (PRDM) Decision G. 21/7, one of the measures is linked to implementing the (IMAP), partly based on Ecological Objective 10’s pilot indicator on amounts of litter ingested by marine organisms or these organisms’ rates of entanglement. the PRDM selected the most representative species for the common indicator IMAP CI 18.)

³ <https://www.indicit-europa.eu>

⁴ <https://web.unep.org/unepmap/what-we-do/projects>

⁵ <https://www.rac-spa.org>

a. Data capture sheet, according to UNEP/MAP, suggested by MEDPOL (2016)

Place	Date of sampling	Date of analysis	Species	
No. of sample Observer	Observer		Organ*	
	Storage conditions (fresh/frozen, duration)			
Item	Category (code)	Size (**)	Weight	Colour
Comments				

* Oesophagus, and/or stomach, and/or intestine (if parts have not been distinguished)

** (1 = <2.5 cm, 2 = 2.5-5 cm, 3 = 5-10 cm, 4 = 10-20 cm, 5 = > 20 cm)

b. List of recognised litter codes and categories (from UNEP/MAP, 2016). For the purposes of harmonization, the codes are taken from the main list of litter categories as defined by MSFD

Plastic polymers	Codes	Items
	G2	Plastic bags
	G48	Synthetic rope
	G51	Fishing net
	G119	Sheet-like plastic
	G122	Plastic fragments
	G81-G82	Polystyrene
	G78-79	Plastic fragments (>5 mm)
	G112	Industrial pellets
	G107 to G111, G113 to G116	Other micro-plastics (<5 mm)
Rubber	G125	Balloons
Supra-category 'Natural cloth/textile'	G145	
Supra-category 'Paper/cardboard'	G146	
Supra-category 'Wood' (processed)	G170	
Metal	G183	Fish hooks
	G198	Other metal
Supra-category 'Other'		

In the proposed protocol, both “basic” and “*optional*” parameters are proposed to be collected. The **basic parameters** (thereafter noted in bold) correspond to the minimum parameters which are fundamental to determine the indicator criteria. The ***optional parameters*** (thereafter noted in bold italic grey) aim at acquiring further knowledge on loggerheads’ feeding behaviour and the probability to ingest marine litter and micro-litter, as well as to better specify the indicator criteria which are under development. The ***optional parameters*** can also help to better assess the impacts of litter related to entanglement.

An **observation sheet** is provided in Annex II. In order to facilitate data banking and statistical analysis, data must be filled in the 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 (quantitative or qualitative data), by 0 or by “NA” (information not available or not evaluated). A printable summary of the main manipulations is provided in Annex IV to the present document.

1. Preliminary Information

A. Regulatory aspects

The following protocols describe the technical operations that should be implemented during the recording of information and while taking samples from live or dead marine turtles. The surveyor will have to ensure beforehand the conditions of intervention on the sea turtles in the country where he/she intervenes and to comply with the regulations in force. These operations may require making requests for permission which may lie under different regulations. The requests that may be required are described as follows: i) action on protected species, if the species enjoy national protected status, ii) action on a live wild animal in the context of an animal experiment, even if the activities described here are not intrusive, and iii) the arrangements advocating health precautions to be taken regarding infectious diseases and zoonosis.

If specimens have to be moved for analysis to and/or from a state that is a signatory to the Washington Convention (CITES), it will also be necessary to make a request for a 'CITES permit' since all species of marine turtle appear in Annex 1 to this Convention.

B. Rules of hygiene

Action on specimens of marine turtles, whether these are dead or alive, must respect a certain number of rules of basic hygiene. We recommend applying a certain number of basic rules mentioned below.

Marine turtles may carry agents that are pathogenic to human beings (see Baron, 2014 for references) such as salmonella, mycobacteria, *Leptospira*, *Pseudomonas sp.*, *Aeromonas sp.*, amoeba etc. On the carcass, different anaerobic bacteria are developed and can infect people, especially if they are accidentally hurt while examining and handling.

The intervention zone must be marked-off from the bystanders and handling necessitates to wear a protective suit with glasses, gloves and rubber boots. Note that although gloves represent a protection, they can also, once soiled, represent a source of contamination. Thus, the surveyor must be very careful while separating those items that must remain away from the soiled items. For the soiled items a different process should be followed including washing and disinfection, or to be thrown in separate bins.

If the people providing the information (e.g. fishermen, firemen, etc.) have touched the turtle with their bare hands, they must be given advice and instruction on hygiene and should be particularly told to wash their hands carefully after the action. A disinfectant soap (e.g. chlorhexidine) could be also provided to them when they arrive at the place (e.g. rescue center) where the marine turtle will be delivered. The same precautions will be taken by surveyors who have not worn gloves.

For the same reasons, live turtles and carcasses must be moved in special tubs (e.g. plastic bowls with a waterproof mat for live animals) so that they can be cleaned and disinfected. Samples (e.g. digestive tracts) will be packed into watertight bags and if possible, put in a cool-box for transport to avoid any contamination of the vehicle and also to restrict the process of autolysis*⁶ of the tissues (decomposition). After external examination of a dead turtle, or an autopsy, there are several options for eliminating the carcass or remains according to national rules where the operations are being carried out. If the turtle is examined at the site of the stranding and must be got rid of by municipal workers, for example, or by slaughterhouse workers, it is always preferable to wrap the carcass in a closed, hermetically sealed double bag and inform the agents who are taking over of the precautions to be taken.

⁶ The glossary (Annex I) contains the definition of terms used in the protocols, marked in the text with an asterisk

All soiled elements, gloves, protective clothing, absorbent paper and disposable instruments must be thrown into the bag before it is closed if an incineration is anticipated, or special bins that will be treated in a way that suits this type of organic waste. Finally, it is understood that the ideal conditions for the external and internal examination of a turtle, and for the taking of samples, are those found in a laboratory. For dead turtles, it is recommended that there be a case-by-case study of the possibilities of carrying out the dissections*/necropsies in premises that are well-equipped and with competent technical staff. This means, particularly, veterinary analysis laboratories or scientific research laboratories. As regards live turtles, the examination is usually done in a care center or a veterinary surgery, where these precautions are already respected.

C. Preparing the premises, equipment and instruments

Before carrying out the operations of dealing with specimens, and storing or taking samples, and analyzing them, it is necessary to prepare the premises, equipment and instruments that are to be used. The elements that are useful for this preparation are summarized in Annex III to the present document.

If the examination and dissection cannot be done in laboratory conditions, it is recommended that an action zone be marked off and material prepared somewhere near the carcass, with a toolbox in which soiled instruments will be placed at the end of the operation to be cleaned later, and two big bin bags to receive the carcass to be got rid of as well as disposable sharp things. If the examining and opening up of the carcass is done after moving it to the premises, these must at least have a water tap, an examination table and material that can be washed down (metal), if possible, fitted with a drainage canal, under which a bin will be placed to receive the tissues and non-sharp things to be thrown away at the end of the operation.

D. Preparing the team, distributing roles

For reasons of hygiene (see above), it is recommended that at least two people are involved in the operations: one to operate, protect himself and handle the soiled objects; the other to take photos, note information etc. The second person can assist the surveyor by wearing two pairs of gloves, one of them being changed for writing. For surveyors, cut-resistant pair of gloves must be worn below the two pairs of gloves, one of them being changed for touching materials to keep clean or in case of cutting the first pair.

E. Size of marine litter considered

The new Commission Decision (Decision 2017/848 of the 17th May 2017) provides the different sizes of marine litter for D10C3 “primary” criteria as litter (>5mm) and micro-litter (<5mm). For the D10C3 “secondary” criteria, both marine litter and micro-litter are quantified. The MSFD Technical subgroup on Marine litter (MSFD TG-ML, 2013) recommends, for practical reasons, to consider micro-plastics between 1 and 5 mm when it is impossible to characterize chemically or physically the type of smaller microplastics. Consequently, the micro-litter size range for this criterion is considered at 1-5mm, for practical reasons when visual observations is the only possible method of characterization.

GESAMP (2016) provides the definition of micro-plastic as any plastic particle < 5mm. Moreover, the categories meso-plastic (5-25mm) and macro-plastic (> 25mm) can be used.) For more precise definitions, a glossary is provided under Annex I to the present document.

F. Useful definitions

In order to ensure optimum harmonization during the collection of information, certain definitions must be clearly provided. Acceptance of certain terms may differ from one person to the other and thus may represent a source of bias. The glossary (Annex I) contains the definition of terms used in the

protocols, marked in the text with an asterisk *. These concerns, inter alia, the anatomy of marine turtles, assessment of carcasses, impacts of litter on these species, types of litter and fishing gear* encountered, etc.

2. General Information on Live and Dead Specimens

A. First Notes on the Discovery Site

Contact: Note the name, contact (phone, mail) and institution of the observer(s) (data collector).

On the individual: Identify the species of the observed marine turtle:

- Cc (loggerhead *Caretta caretta*): 2 pairs of pre-frontals scutes, nuchal scale in contact with the 1st costal;
- Cm (green *Chelonia mydas*): 1 pair of pre-frontals scutes, nuchal scale not in contact with the 1st costal;
- Dc (leatherback *Dermochelys coriacea*): Absence of keratinized scutes, presence of 'leather' and ridges.

In case of doubt about the species identification, refer to identification guide (e.g www.cites.org). If the species cannot be identified, note NI (Non-identified) on the observation sheet.

Tags: If the examined marine turtle has been identified during egg-laying or a prior release, it may have one or two rings attached to one (two) flippers or an electronic chip that has been slid under the skin or into a muscle. To read the chip you need to have a transponder reader. In some relatively rare cases the turtle carries a telemetric monitoring device (tag) that can also help identify it, by contacting the provider or structure whose names appear on the tag. If pre-existing tag on the flipper, specify the tag number. Indicate the presence and code number of electronic chip. Otherwise, note NO.

Animal Identification Code: It is recommended to use a standard identification code. We propose noting: ^{2 letters for the country,} ^{2 letters for the location} (e.g. region or institution): the species, ^{the year, the month}, the day ^{and the number of turtle} per order of collection during the year, separated with “-”. Example: “FR_{GR}CC_20¹⁷_03_12_9” corresponds to the 9th loggerhead individual, found in by the center of Grau du Roi in France, the 12nd March 2017. Thereafter, it will be asked to specific the type of sample.

On the site: Note the **date of discovery** (dd/mm/yyyy), **the location of discovery** and **the coordinates** if available (**X, Y: in decimal degrees, or specify the coordinate system**).

NOTE: Taking pictures of the animal before handling is very important to verify the circumstances of the finding and to *a posteriori* confirm or clarify information noted, in case there is doubt or difficulty in identifying the species, the lesions*, the state of the individuals and the elements responsible for the interaction*. Using a tape measure can show the order of magnitude in the pictures and it is important to refer to the identification code of the animal examined when storing the pictures. Please specify if pictures are taken in the column “Photo at finding” of the Excel file.

B. Description of the animal’s body condition

Conservation status or decomposition level

Two cases are present: the turtle is alive, or it is dead. But it can also seem dead (very slow breathing) and just be in a coma, so it is useful to check by looking for reflexes (oculo-palpebral*, withdrawal reflex when the tail is pinched) before reanimation, if need be. Note the **status** according to these 5 levels presented under Figure 1, hereunder:

- Level 1: litter can be extracted from the analysis of faeces in rescue center.
- 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 possible⁷.

⁷ Some tissues (muscle, etc.) can be collected and frozen at -20°C for further genetic analysis.

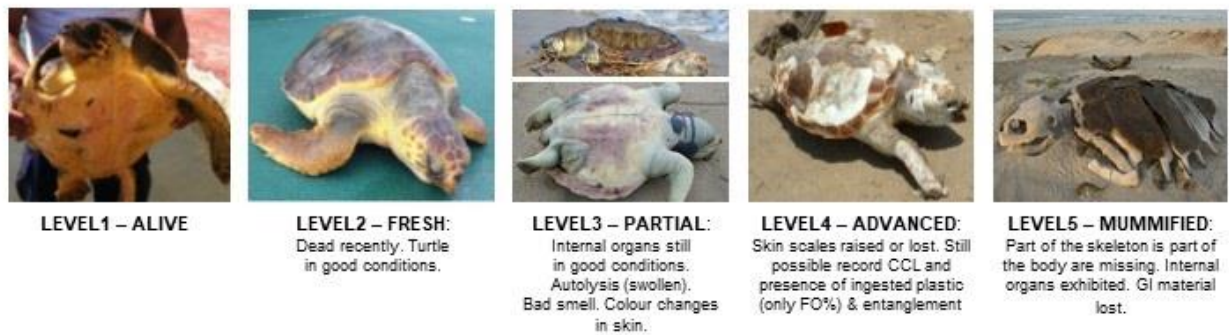


Figure 1: Conservation level or decomposition status

Discovery circumstances

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

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

Possible cause of morbidity and mortality, type of impact

If possible, the *type of interaction with human activities and impact observed or suspected on dead or live stranded individuals* should be deduced from external observations or organs observation during the necropsy* of dead individuals and complement with veterinarian examinations. Also, an inspection of the oral cavity should be conducted for the presence of foreign material. Then a choice among the 10 different categories should be made and the notes and remarks box should be completed with the help of the pathologist (if this is requested):

- Bycatch/Fisheries related: ingested hook, decompression sickness (diagnosable through X rays), individual trapped in a fishing gear, individual drowned in a fishing gear...;
- Entanglement in litter: entanglement in litter other than related to fishing activity. Please fill the column "Entanglement type" and "Litter causing entanglement";
- Ingestion of litter: digestive obstruction or occlusion, perforation or other impacts;
- Anthropogenic trauma: Collision with a boat or a propeller, individual beaten with knife, stick or harpoon, poaching...;
- Natural trauma: e.g., shark attack;
- "Natural disease" (=other symptoms): buoyancy trouble, cachexia, dermatitis, conjunctivitis, rhinitis...;
- Oils: Ingestion or external impregnation with oils;
- Unidentified: Impossible to know the cause of death/stranding, no remarkable damages, injury or disease;
- Other: Please specify in the column "Notes".

✓ *By-catch gear*

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

- Longline;
- Trawler;

- Nets;
- 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 deployment before the gear was brought aboard.

✓ *Health status*

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



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

✓ *Main injuries*

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



Figure 3: Typology* of the most frequent injuries observed in sea turtles

✓ *Affected body part*

If the animal presents an injury, the *affected body part* should be reported:

- 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”).

✓ *Litter causing entanglement*

If the individual has been found entangled in litter, the *type of material in which the sea turtle has been found entangled in* should be specified, according to the following categories:

- 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).

✓ *Other descriptive parameters*

Visual inspection of the animal’s *fat reserves* at the neck is recommended. For dead individual, this can be verified when opening the plastron* according to the quantity of fat recovering the abdominal muscles (see below, Fig. 6c). Choose among the 3 categories:

- Thin (sunken neck);
- Fat;
- Normal.

If possible, the *sex* (Male or Female) should be noted, which can be determined by gonads analysis or, in adult individuals from the observation of secondary sexual characters (Fig. 4), according to the length of the tail and of the claw in the front flipper. This may be confirmed through a visual observation of the genital apparatus during the necropsy for dead individuals. Otherwise, specify by NI (for Not identified).

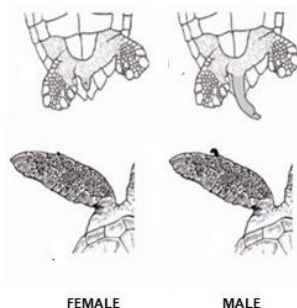


Figure 4: Example of determination of the sex of loggerhead turtle (from Wyneken, 2001)

✓ *Biometric Measurements*

Following Fig. 5, several basic and *optional* body lengths can be measured (in centimeters, precision 0.01 cm), as well as the *Weight* (in kilograms, precision 0.01g). A measuring tape should be used to measure curved lengths and a sliding caliper for straight lengths:

- **Standard curved carapace length (CCLn-t or CCL)**
- *Maximum Curved Carapace Length (CCLmax)*
- *Minimum curved carapace length (CCLmin)*
- *Curved carapace width (CCW)*
- *Standard Straight carapace length (SCLnt)*
- *Maximum Straight carapace length (SCLmax)*
- *Minimum Straight carapace length (SCLmin)*
- *Straight carapace width (SCW)*
- *Curved plastron length (CPL)*
- *Straight plastron length (SPL)*
- *Curved plastron width (CPW)*
- *Straight plastron width (SPW)*

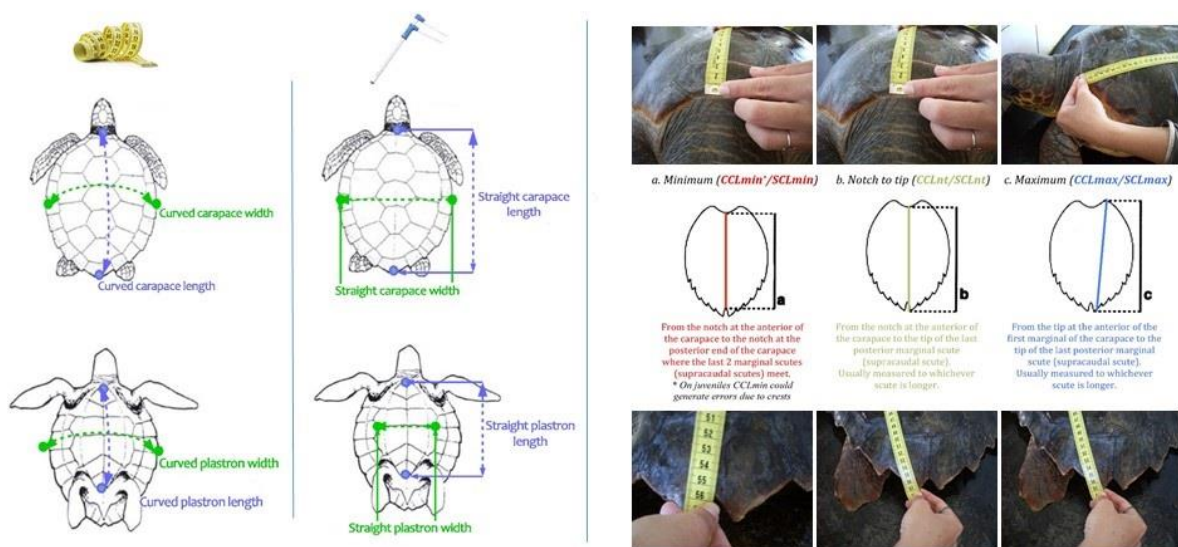


Figure 5: Biometric parameters (carapace and plastron lengths).

3. Sampling Marine Litter from Carcasses – Protocol for Dead Animals

In case of decomposed animal (status of Levels 3 and 4), the integrity of the digestive tract should be checked 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.

Turtle Necropsy

a. Opening of the carcass

The carcass should be placed on its back, trying to wedge it with an object so that it doesn't wobble from side to side. The plastron should be removed and separated from the carapace through an incision on the outside edge (yellow line) (Fig. 6a). The incision should be made with special attention, with the use of a short blade or by cutting with a horizontal tilt in order not to affect the integrity of the interior organs (Fig. 6b).

Once the inside of the plastron is accessed, cut the ligament attachment to the pectoral and pelvic girdle to pull back the plastron and reach the muscles and then the internal organs. Report the *Fat reserves* of the animal (Fig.6c) according to:

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



Figure 6: Sequence of turtle necropsy: a) Ventral view of a dead turtle. 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).

b. Extracting and preparing sections of the digestive tract

Extraction of the Gastrointestinal System: Expose the gastrointestinal system (GI) by removing the pectoral muscles and the heart of the animal (Fig.7a and 7b). 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 carcasse. Isolate the different portions of GI (oesophagus, stomach, intestines) by strangling and cutting between 2 clamps (see the blue solid lines in Fig. 7c) the gastro-oesophageal sphincter and the pyloric sphincter.

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

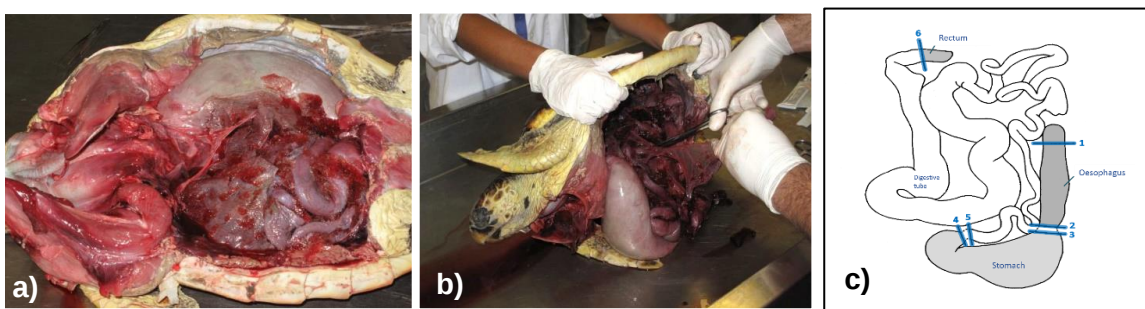


Figure 7: Sequence of extraction and preparation of sections of the digestive a) Remove the pectoral muscle and the heart; b) Extraction of the GI; c) Sketch of the entire GI. Blue lines indicate where clamps must be attached in order to separate the 3 different GI sections. (Drawing by V. Hergueta).

Noting external lesions of the GI that can be attributed to litter: Before opening up the digestive tube, examine the outer wall to observe possible perforations by foreign bodies or areas of necrosis. Also note secondary lesions, particularly a peritonitis following on a perforation of the digestive tube, an invagination of the digestive tube, an occlusion*, etc. Photograph every lesion observed, taking care to get an overall view and a close-up (macro-lens). Pictures must be stored referring to the code corresponding to the animal examined, describing the lesion in the description of the subject.

c. Extraction of Gut Content

The three parts of the gastrointestinal system (i.e. oesophagus, stomach, intestines) should be removed by adding a second strangling at the cut edge to prevent spillage of the contents (Fig. 8a)⁸. Each GI section should be opened lengthway using a scissor and slide the material directly out of the section onto a 1mm mesh sieve. The content should be cleaned with current and abundant tap water (Fig. 8b) to remove the liquid portion, the mucus and the digested unidentifiable matter⁹.

The content for the presence of any tar, oil, or particularly fragile material, should be inspected and should be subsequently removed and treated separately. It should be then reported in the column “Notes” of the INDICIT-UN-MAP Excel file.

All the material should be rinsed collected in the 1mm sieve (Fig. 8b, c), and should be placed in tubes or in zipped bags, reporting the sample code (individual code, respective GI section) and stored at -20°C, pending the laboratory analyses.

NOTE: At this stage, for the optional differentiation of litter and micro-litter, the material should be slid out of the section directly onto a 5mm mesh sieve superposed on a 1mm mesh sieve. Then, proceed with the rinsing and the storing of the material collected as described above, for both 1- and 5- mm sieves, reporting the samples code (individual code, respective GI section and size class (>5mm or 1-5mm)).



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

d. Extraction of Ingested Marine Litter and Other Elements from the Stored Gut Content

The gut contents should be defrosted the stored and both marine litter and other items should be removed manually by visual observation.

4. Sampling Marine Litter from Faeces – Protocol for Live Animals

- a. **Collection of faeces:** For the homogeneity of approaches allowing the comparability of turtles and regions over time, the collected faeces will be analyzed only for the individuals remaining at least **1-month minimum** in the rescue center. The faeces should be collected **only after 2 months** from the arrival of the individual. The turtle should be carefully rinsed with water to avoid contamination and the animal should be placed in an individual tank (Fig. 9a). A filter of 1mm should be disposed in all the

⁸ The 3 parts of the GI (oesophagus, stomach, intestines) are analysed separately in order to assess possible differences in litter content per section and better assess the digestive transit of marine litter.

discharge tubes of the tank (Fig. 9b). The water tank should be controlled daily by filtering through the 1mm mesh sieve according to the following methods:

- Collect the faeces manually with a 1mm mesh dip net (Fig. 9c);
- Put a 1mm mesh flexible collector in the drain tube (Fig. 9d);
- Place a 1mm mesh rigid sieve under the drain (Fig. 9e).



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

NOTE: Each sample which could not be analyzed directly can be conditioned in a tube or a zipped bag and identified with a permanent marker, e.g. with 2 letters for the country _ 2 letters for the region/Institution _ Species _ Year _ Month _ Day _ N° turtle _ Type of sample.

Ex: FR_GR_CC_2017_03_12_9_Faeces corresponds to the faeces, excreted by the 9th loggerhead individual found by the rescue center of le Grau du Roi in France, the 12nd March 2017.

The sample is then stored at -20°C, pending the laboratory analyses.

- b. **Collection of litter and other elements from faeces:** The sieves and collectors should be washed with abundant water above a 1mm mesh sieve (Fig. 9f). The collection of litter and other elements is conducted manually by visual observation directly from the 5mm and 1mm sieve.

NOTE: At this stage, for the optional differentiation of litter and micro-litter, the sieves and collectors should be rinsed above a 5mm mesh sieve superposed on a 1mm mesh sieve. Then, proceed with the collection of litter as described above, for both 1- and 5- mm sieves.

5. Marine Litter Analysis and Classification

- a. **Litter and other elements classification:** The **protocol** that was used should be specified, between “Necropsy” or “Faeces”. For each GI section of the necropsied individual (Section 1 of

this document) or for faeces (Section 2 of this document), classify the litter and other elements according to the following categories (Tab 4., Fig. 10)¹⁰.

Table 4: Classification of ingested litter and other elements for sea turtles content analysis.

CATEGORIES		CODE	DESCRIPTION	
LITTER	PLASTIC LITTER	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 thread	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, balloon pieces, soft air gun bullets... Specify in the column “Notes”.
	Litter other than plastic	OTHER	All non-plastic rubbish and pollutant e.g. cigarette filters...	
OTHER ELEMENTS	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.)	

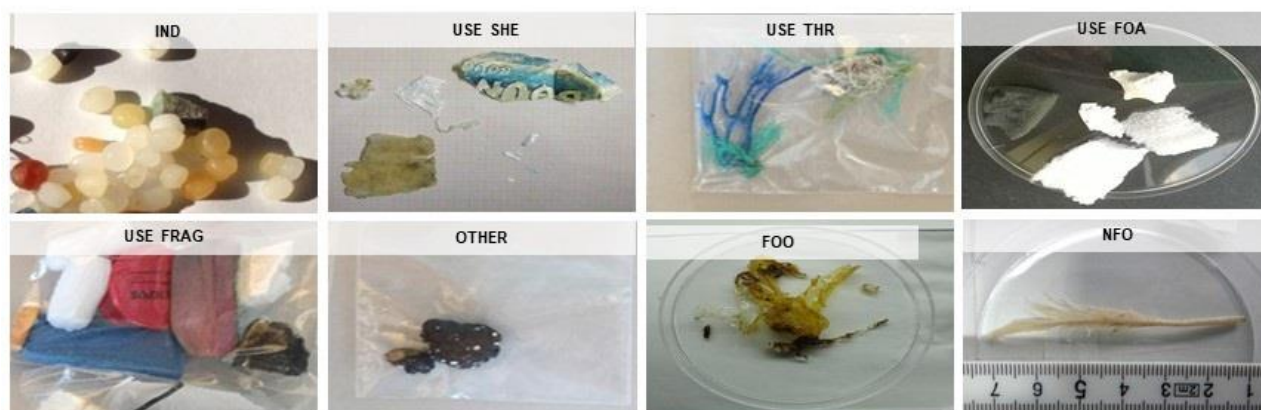


Figure 10: Examples of ingested litter and other elements categories established for marine turtle ingestion.

b. Collection of data: For each GI section of necropsied individuals or for the whole faeces' samples of live individuals, marine litter items and other items should be sorted into the different categories presented under Table 2. In addition the following parameters should be recorded:

- Record for all categories (litter and other elements): The **dry mass** (grams, precision 0.01 g) of each category: dry the sample at room temperature during 24h minimum or in a stove at 35°C during 12h.

¹⁰ The classification of the litter and other elements was adjusted by the INDICIT consortium, based on the MSFD guideline (MSFD TG-ML, 2013) and the INDICIT partners and collaborators (e.g. rescue centers and stranding networks) feedbacks. The different plastic categories can be identified visually and possibly confirmed by stereomicroscopy.

- Record for litter categories only: The **number of fragments** in each category: a fragment is a piece of litter that can be identified. The *number of items* in each category: an item is a set of fragments that seem to originate from the same piece of litter
- Record for the plastic litter categories only: The **total volume of plastic litter** (milliliter, precision 0.01 ml): **measure** the volume of all plastic litter in a graduated beaker and record the water variation (Fig. 11). Push the floating plastic in the water thanks to a rod or a decimeter. The **total number of plastic fragments per colour category**:
 - *Total number of white-transparent plastic fragments;*
 - *Total number of dark coloured plastic fragments (black, blue, dark green...);*
 - *Total number of light coloured plastic fragments (cream, yellow, pink, light green...).*

NOTE 1: In the case where litter and micro-litter were differentiated, proceed with the data collection as described above, but distinguishing both size classes (>5mm and 1-5mm).

NOTE 2: The optional parameters recorded for plastic litter categories can be collected per GI section and per category, for practical and organizational reasons, but it is the total of all the GI – all plastic categories included – that will be noted in the Observation sheet.

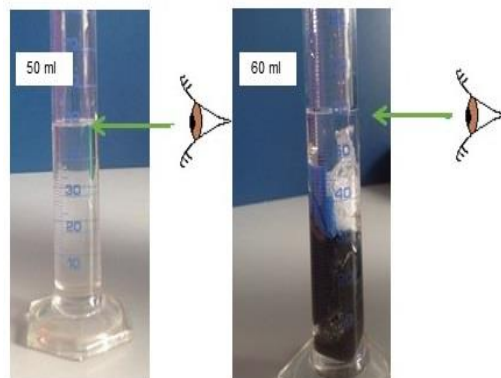


Figure 11: The volume of the plastic litter corresponds to the difference between the volume with (right) and the volume without (left) the plastic litter. The volume is read by considering the bottom of the meniscus formed by the surface water.

ANNEXES

ANNEX I
GLOSSARY

GLOSSARY

Amputation (of a member). For a marine turtle, the loss of a flipper by being cut off, which may result from constriction* or strangling.

Autolysis. Destruction of tissues by their enzymes.

Necropsy. Examination of a carcass to study the causes of death.

By-catch. The accidental catch of a non-target species (of marine turtle, for example).

Cloaca. (Common) orifice of the urinary and genital passages in birds and reptiles.

Constriction. Action of squeezing, pressing around; when this happens at the level of the neck it can suffocate the turtle; when around a member, the blood supply is slowed or even cut off, causing, after a certain time, necrosis and loss of the member.

Dissection (of a carcass). Opening up a carcass according to a defined protocol to study its structure and take samples. When looking for the causes of death, the term used is ‘necropsy’.

Entanglement. Accidentally caught by fishing gear during the fishing operation, or abandoned or lost.

Fishing gear. Material intended for catching marketable aquatic species, e.g. trawls, seine nets, nets, lines and longlines. According to circumstance, the entangling is due to:

- **Abandoned gear** (derelict). The gear is left where the fisherman has intentionally abandoned it;
- **Ghost gear** (e.g. ghost net). Gear left on the seabed and which continues to fish; referred to as ‘ghost fishing’;
- **Lost gear.** Gear unintentionally lost during fishing operations;
- **Wreck.** Object abandoned at sea, drifting or on the seabed;
- **Discarded gear or fishing material.** Old gear or material put aside and often thrown back into the sea; this gear must be collected in containers on land for recycling.

Impact. Effect of something.

Interaction. Reciprocal action that two or more systems exercise on each other.

Occlusion. Complete halt of the passing of matter and gases in one portion of the GI. The occlusion can have a mechanical cause (total obstruction by litter) and constitute a veterinary emergency.

Lesion. Modification of the structure of a living tissue under the influence of a disease, of a reason inducing a pathology.

Macro-litter or litter: artificial polymers (plastic) and “other litter” with a maximum size (or diameter) > 5 mm.

Meso-litter: artificial polymers (plastic) and ‘other litter’ with size between 5 and 25mm.

Micro-litter: artificial polymers (plastic) and “other litter” with size < 5 mm.

Oculo-palpebral reflex. Reflex in which the eyelids spontaneously shut or blink if the lashes or the internal edge of the orbit are touched with a finger.

Plastron. The ventral part of a turtle’s carapace.

Stranding (of a marine turtle). Said of an animal, dead or alive, that has been washed up on the coast.

Trophic status. Nutritional state in which may be reflected by variable degrees of stoutness, presence of fats in the tissues.

Typology. Approach consisting of defining or studying a set of types; by extension, here it means the listing and describing of types of litter, lesion, etc. that allow the surveyor to classify observations in the correct category of data.

ANNEX II
OBSERVATION SHEET



OBSERVATION SHEET - Entangling and litter ingestion



COLLECTOR

LOCAL CODE

INSTITUTION

Contact:

Discovery circumstances:

SPECIES ☐ *Caretta caretta* ☐ *Dermochelys coriacea* ☐ *Chelonia mydas* ☐ Other

INDIVIDUAL TAG: Tag number:

Electronic chip n°:

DATE OF DISCOVERY

INDIVIDUAL CODE

LOCATION

X CORD

Y CORD

CIRCUMSTANCES

- ☐ Bycatch/Fishery
☐ Stranding
☐ Dead at rescue centre
☐ Found at sea
☐ Other
☐ NR

BY-CATCH ENGINE CAUSE

- ☐ Longline
☐ Trawl
☐ Drift net
☐ Fishing rod
☐ Other
☐ NR

CAUSE OF DEATH / STRANDING

- ☐ By-catch/Fisheries
☐ Entanglement in debris
☐ Ingestion of litter
☐ Anthropogenic trauma
☐ Natural trauma
☐ Natural disease
☐ Oils
☐ Healthy
☐ Other
☐ NR

ENTANGLEMENT TYPE

- ☐ Active ☐ Passive ☐ NR

LITTER CAUSING ENTANGLEMENT

- ☐ Net pieces
☐ Monofilament lines
☐ Rope/s
☐ Raffia
☐ Plastic bags
☐ Other
☐ NR

PICTURES ☐ Yes ☐ No

Picture names:

Animal body condition:

CONSERVATION STATUS

- ☐ 1- Alive
☐ 2- Fresh
☐ 3- Partial
☐ 4- Advanced
☐ 5- Momified
☐ NR

HEALTH STATUS (Plastron shape)

- ☐ Poor (concave)
☐ Fair (plane)
☐ Good (convex)
☐ NR

MAIN INJURIES

- ☐ No injuries
☐ Fracture
☐ Amputation
☐ Sectioning
☐ Abrasion
☐ Other

AFFECTED PARTS

- ☐ Flipper ()
☐ Carapace
☐ Neck
☐ Head
☐ Plastron
☐ Other

FAT RESERVE

- ☐ Thin
☐ Normal
☐ Fat
☐ 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

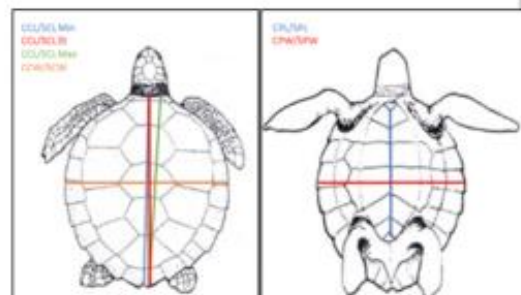
CPW

 cm

SPW

 cm

WEIGHT (0,01kg):



Extraction of ingested debris: PROTOCOL: ☐ NECROPSY						INDIVIDUAL CODE: ☐ OBSERVATION OF FAECES																																
Please describe: VISCERAS STATUS (note the presence of any infection, suspect colour, fluid effusion, perforation, presence of oil, etc.):						ARRIVAL DATE <table border="1" style="display: inline-table; width: 100px; text-align: center;"><tr><td> </td><td>/</td><td> </td></tr><tr><td> </td><td>/</td><td> </td></tr><tr><td> </td><td>/</td><td> </td></tr></table> DEPARTURE <table border="1" style="display: inline-table; width: 100px; text-align: center;"><tr><td> </td><td>/</td><td> </td></tr><tr><td> </td><td>/</td><td> </td></tr><tr><td> </td><td>/</td><td> </td></tr></table> DEAD DATE <table border="1" style="display: inline-table; width: 100px; text-align: center;"><tr><td> </td><td>/</td><td> </td></tr><tr><td> </td><td>/</td><td> </td></tr><tr><td> </td><td>/</td><td> </td></tr></table>							/			/			/			/			/			/			/			/			/	
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DIGESTIVE TRACT (note the presence of any infection, suspect colour, fluid effusion, perforation, presence of oil, etc.):						TURTLE BEHAVIOUR AND TREATMENTS: 																																

Marine litter and other elements measurements:

	OESOPHAGUS			STOMACH			INTESTINES			FAECES		
	Dry mass (0.01g)	Number of fragments	Number of items	Dry mass (0.01g)	Number of fragments	Number of items	Dry mass (0.01g)	Number of fragments	Number of items	Dry mass (0.01g)	Number of fragments	Number of items
IND PLA												
USE SHE												
USE THR												
USE FOA												
USE FRAG												
USE POTH												
OTHER												
FOO												
NFO												

NUMBER of plastic fragments per colour <i>white-transparent</i> <i>dark coloured</i> <i>light coloured</i> 	NUMBER of plastic items per colour <i>white-transparent</i> <i>dark coloured</i> <i>light coloured</i> 	VOLUME of plastic litter ml
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NOTES AND REMARKS (Necropsy, faeces collection and debris measurements):

ANNEX III
LIST OF MATERIAL

LIST OF MATERIAL

For the take-over of the animal and the collection of samples at the discovery site:

Rope (to mark-off the zone)

Integral protective suit

Glasses and protective mask or shield

Cut-resistant gloves

Gloves

Boots

Camera

Measuring tape

Pen

Observation sheet

Bottle/zipped bags

Cooler

Permanent marker

Transport bins or containers for the turtle

Garbage bag

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

Cold chamber or chest freezers (-20°C) with large storage capacity

Proofer (not mandatory)

Garbage bags

For surveyors

Integral protective suit

Glasses and mobcaps

Protective mask or shield

Cut-resistant gloves

Gloves

Boots

For notes and report

Camera (+ scale decimeter)

Pen

Observation sheet

Permanent marker

For the necropsy and the collection of the GI content

Clamps (at least 6) or roast wire

Scalpel

Scissors

Clips with claws

Metal containers

Metal spoon

Containers for samples (Bottle/zipped bags)

For the collection of samples

Sieve with 1 mm mesh

Sieve with 5 mm mesh

Measuring cylinders (2 L, 1L, 50cL; precision 0.1L)

Measuring decimeter

Precision balance (capacity 4kg, precision 0.01 g)

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

In the laboratory room

Freezers (-20°C)

Proofer (not mandatory)

Garbage bags

For surveyors

Glasses

Protective mask

Gloves

For notes and report

Camera (+ scale decimeter)
Pen
Observation sheet
Permanent marker

For the collection of samples

Containers for samples (tubes/zipped bags)
Metal spoon
Sieve with 1mm mesh
Sieve with 5mm mesh
1mm mesh rigid sieve
1mm mesh flexible collector (drain tube)

For the analysis of the ingested litter:

For surveyors

Glasses
Protective mask
Gloves

For notes and report

Camera (+ scale decimeter)
Pen
Observation sheet
Permanent marker

For the analysis of the ingested litter

Measuring tape

Decimeter

Precision balance (capacity 1kg; precision 0.01)

Measuring cylinders

Metal spoon / clamps

Binocular (*optional*)

ANNEX IV
REFLEX SHEETS

A. FIRST NOTES ON THE DISCOVERY SITE

***Note:** The loggerhead sea turtle (*Caretta caretta*) is a protected species in some countries, 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. A CITES permit is asked if a specimen or sample has to be sent/received.*

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.

On the discovery site, note the following information on the observation sheet:

1. General information:

- Contact information of the observer/collector of the animal;
- Species;
- Presence of pre-existing tags/electronic chips/telemetric monitoring device;
- New numbers of tag and electronic ship, when it applies;
- Animal's identification code;
- Date and location of discovery;
- Coordinates (*optional*);
- Pictures/Videos.

2. Animal's body condition:

- Conservation status or decomposition level;
- Discovery circumstances;
- Probable cause of death/stranding (*optional*);
- By-catch gear (*optional*);
- Health status (*optional*);
- Main injuries (*optional*);
- Affected body parts (*optional*);
- Entanglement type (*optional*);
- Litter causing entanglement (*optional*);
- Other descriptive parameters (*optional*);
 - Fat reserves
 - Sex
- Biometric measurements.

B. EXTRACTION OF MARINE LITTER FOR DEAD ANIMALS: Necropsy protocol

1. Turtle's necropsy:

- Open the carcass by removing the plastron;
- Note fat reserves;
Thin / Normal / Fat;
- Expose the gastrointestinal system (GI);
- Clamp the esophagus and the cloaca;
- Remove the GI from the carcass;
- Note external lesions on the GI and specify when attributed to litter.

2. Extraction of gut content and collection of ingested litter:

- Separate the 3 sections of the GI (oesophagus, stomach, intestines), and for each section:
- Rinse all the material collected over a 1mm mesh sieve (or superposed 5- and 1-mm mesh sieves – optional)
- Inspect the content and separate marine litter from other elements
- Collect marine litter and other content in separated zipped bags or bottles, noting the animal's identification code, the GI section (and optionally the litter class size (1-5, >5))
Example: FR_GR_2017_03_12_9_Oeso
- Freeze at -20°C if analyses cannot be performed successively.

C. EXTRACTION OF MARINE LITTER IN LIVE ANIMALS: Faeces protocol

Note: Collect faeces from individual remaining at least 1 month in the rescue center only and up to 2 months after the individual's arrival

1. Collection of the daily faeces:

- With a 1 mm mesh dip net;
- From a 1 mm mesh flexible collector disposed around the drain tube;
- From a 1 mm mesh rigid sieve disposed under the drain.

2. Collection of marine litter:

- Rinse the sieves and collector with abundant water above a 1mm mesh sieve (or superposed 5 and 1 mm mesh rigid sieves – optional);
- Inspect the content and separate marine litter from other elements;
- Collect marine litter and other content in separated zipped bags or tubes, noting the animal's identification code, the protocol (and optionally the litter size class (1-5, >5));
Example: FR_GR_2017_03_12_9_Faeces
- Freeze at -20°C if analyses cannot be performed successively.

D. MARINE LITTER ANALYSIS

1. Litter and other element classification:

CATEGORIES		CODE	DESCRIPTION	
LITTER	PLASTIC LITTER	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 thread	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, balloon pieces, soft air gun bullets... Specify in the column “Notes”.
	Litter other than plastic	OTHER	All non-plastic rubbish and pollutant, e.g. cigarette filters	
OTHER ELEMENT	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.)	

2. Collection of data for >5mm and 1-5mm

For each GI section of necropsied individuals or for the whole faeces samples of live individuals, sort litter and other elements into the different categories exposed above (Tab. 1) and record the following parameters:

- **For all categories (litter and other elements):**
 - Dry mass (grams, precision 0.01g) of each category.
- **For marine litter only:**
 - Number of fragments (i.e a piece of litter that can be identified in each category);
 - Number of items (i.e. a set of fragments that seem to originate from the same piece of litter) (*optional*).
- **For plastic litter only (*optional*):**
 - Total volume of plastic litter fragments;
 - Total number of plastic fragments and/or items per colour category:
White-transparent / Dark coloured / Light coloured

Note: In the case where litter and micro-litter were differentiated, proceed with the data collection as described above, but distinguishing both size classes (>5mm and 1-5mm).