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MEDITERRANEAN ACTION PLAN**

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7th Meeting of the Ecosystem Approach Coordination Group

Athens, Greece, 9 September 2019

Agenda Item 8: Monitoring Protocols for IMAP Common Indicators Related to Pollution and Guidance on monitoring concerning IMAP Common Indicators related to Biodiversity and Non-Indigenous Species

Defining the Most Representative Species for IMAP Candidate Indicator 24 and Related Monitoring Protocols

Note by the Secretariat

The Integrated Monitoring and Assessment Programme for the Mediterranean Sea and Coast and Related Assessment Criteria (IMAP), was adopted in 2016 from the Contracting Parties to the Barcelona Convention (Decision IG.22/7). IMAP provides the requirements for monitoring 23 Common Indicators addressing biodiversity, pollution and marine litter. IMAP also contains one Candidate Indicator 24 on the "*Trends in the amount of litter ingested by or entangling marine organisms focusing on selected mammals, marine birds, and marine turtles.*"

With the aim to improve knowledge of the impact of marine litter on marine fauna and also to facilitate the development of the IMAP Candidate Indicator 24, SPA/RAC in consultation with MED POL elaborated the report on the most representative species for the IMAP Candidate Indicator 24 (UNEP/MED WG.467/Inf.15).

Based on the proposal made to consider marine turtles as the most representative species to assess Candidate Indicator 24 of IMAP, work was undertaken by SPA RAC to elaborate specific protocols for monitoring interactions between marine litter and marine turtles, mainly focusing on ingestion and entanglement; taking also into account the outcome of scientific projects such as the EU-funded INDICIT project (DG ENV 2017-2018).

The Joint Meeting of the Ecosystem Approach Correspondence Group on Marine Litter Monitoring and ENI SEIS II Assessment of Horizon 2020/National Action Plans of Waste Indicators (Podgorica, Montenegro, 4-5 April 2019) reviewed both documents (i.e. the report on the most representative species and the monitoring protocol) and following their revision, both reports were submitted to MED POL Focal Points Meeting (Istanbul, Turkey, 29-31 May 2019) for further review, consideration and approval.

POL and SPA/RAC for the selection of indicator species for monitoring ingestion of marine litter by marine organisms in the Mediterranean, supported by a full report presented under UNEP/MED WG.467/Inf.15. The second part contains the protocols for monitoring interactions between marine litter and marine turtles (i.e. ingestion and entanglement) with a view to harmonize methods of data collection for monitoring and assessment in the Mediterranean.

The Meeting of MED POL Focal Points held in Istanbul, Turkey on 29- 31 May 2019 agreed on the proposed selection of indicator species for monitoring ingestion of marine litter by marine organisms in the Mediterranean, as well as the related Protocol for monitoring interactions between marine litter and marine turtles, and recommended their submission for approval of the 7th Meeting of EcAp Coordination Group.

List of Abbreviations

CITES	The Convention on International Trade in Endangered Species of Wild Fauna and Flora
CMS	The Convention for the Conservation of Migratory Species
EC	The European Commission
EO	Ecological Objective
GES	Good Environmental Status
GESAMP	The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
GI	Gastrointestinal
INDICIT	Implementation of Indicators of Marine Litter on Sea Turtles and Biota In Regional Sea Conventions And Marine Strategy Framework Directive Areas
IMAP	The Integrated Monitoring and Assessment Programme and related Assessment Criteria
MAP	Mediterranean Action Plan
MEDPOL	Mediterranean Pollution Assessment and Control Programme
MSFD	Marine Strategy Framework Directive
OSPAR	Convention for The Protection of The Marine Environment of The North-East Atlantic
RPML	Regional Plan on Marine Litter Management in the Mediterranean Plan for Marine Litter
SPA/RAC	Specially Protected Areas Regional Activity Centre
TG ML	Technical Group on Marine Litter

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1. Introduction

1. In the Mediterranean, marine litter pose a critical problem because of its great quantity and effects on marine fauna. To deal with this problem, UN Environment/Mediterranean Action Plan - Barcelona Convention adopted the first ever legally binding Regional Plan on Marine Litter Management in the Mediterranean (Decision iG.21/71).

2. One of the steps identified in the Regional Plan on Marine Litter is linked to the implementation of the integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coasts and Related Assessment Criteria (IMAP) and its 10th Ecological Objective (EO10) i.e. Marine Litter, partly based on the Candidate indicator 24 "*Trends in the amount of litter ingested by or entangling marine organisms focusing on selected mammals, marine birds, and marine turtles*".

3. During this process it is essential to improve knowledge of the impact of marine litter on marine fauna and also to assess the IMAP Candidate indicator 24. This particularly involves continuing the work of selecting the most representative species to be used for the development and assessment of the IMAP Candidate indicator 24. MED POL and SPA/RAC have worked in developing and preparing the report "*Defining the Most Representative Species for IMAP Candidate Indicator 24*", which comes up with the following findings:

a. Marine litter affects various compartments of the marine environment and monitoring its impacts on marine organisms is of growing importance.
b. Whatever temporal and spatial scale is considered, marine litter (mainly plastics) interact with a vast range of marine species. The different types of impact of marine litter on these organisms can be classified according to the modes of action such as entanglement, ingestion and transportation of species that may be colonized on them.
c. Until now, no monitoring has been implemented to assess the impact of marine litter on marine organisms in the Mediterranean; but we have good scientific and technical basis to start doing so.
d. On the basis of the available information, the approach that uses monitoring of the ingestion of marine litter by marine turtles is consistent and compatible with the whole set of the identified biological, methodological, environmental, logistic and ethical constraints. The target species for the IMAP Candidate indicator 24 and also for monitoring at basin scale are the marine turtles species, which are most commonly found in the Mediterranean, i.e. *Caretta caretta*. *Caretta caretta* has a wide distribution throughout the Mediterranean Sea and a great deal of information is already available. The potential for developing a monitoring network corresponds to the needs expressed by the Contracting Parties to the Barcelona Convention.
e. The use of cetaceans as indicator species can only be considered on an opportunistic basis, and at the initiative of each Contracting Party that has pre-existing stranding monitoring networks.
f. Although protocols for monitoring the ingestion of marine litter by seabirds have been used for a long time in other marine regions, work is still required to identify the most representative species for developing a monitoring programme on the impact of marine litter on seabirds in the Mediterranean. A pilot monitoring programme of marine litter in cormorants' nests is recommended, at the initiative of the Contracting Parties to the Barcelona Convention.
g. Monitoring the ingestion of micro-plastics by fishes or invertebrates presents a strong potential for developing a monitoring programme on the ingestion of marine litter by marine organisms in the Mediterranean. Supplementary work is however necessary to complete a rigorous protocol which eliminates any risk of contamination of the samples examined and thus of false positives due, for example, to the presence of natural fibres. For these pilot studies or for more in-depth research work, priority should be given to common fish species with a wide distribution and easily fished fish species, which are sensitive to micro particles. The selection of nekto-benthic fishes, already identified as being the most affected (i.e. *Boops*

boops), of important commercial interest (i.e. *Mullus sp.*), or of farmed molluscs such as the mussel Mytilus edulis, could facilitate the monitoring approach.

h. Concerning the entrapment/entanglement of marine species, observations have so far been poorly described, which restricts the development of corresponding monitoring networks. Carrying out coordinated pilot experiments based on a strategy of improved data collection, seems to be the most suitable preliminary step before envisaging developing regional monitoring. Work should focus on the prevalence of entrapment/entanglement of Mediterranean species, the identification and mapping of risk areas (presence of active or ghost fishing gear, distribution of susceptible species, probability of encounters between susceptible species and marine litter, etc.), and the rationalization of observation procedures on the basis of existing arrangements (stranding networks, Marine Protected Areas, Observation networks, opportunistic analyses of diving using submersibles or ROVs/Remotely Operated Vehicles).

4. All the recommended approaches should permit:

i. acquiring of better information to support the implementation of reduction measures; and
ii. defining of a Regional Plan-friendly monitoring strategy.

Part I

2. Proposal for the Selection of Species for the Development of the Candidate Indicator 24

5. Monitoring the impacts of marine litter on marine fauna depends strongly on the availability of indicator species to measure the prevalence and effects of ingestion of marine litter and entanglement/strangling. Monitoring these effects can be designed within a multi-species approach in order to cover the range of impacts linked to both the diverse types of marine litter, of varied size (micro-particles and macro-litter) and nature (plastics, metal, glass, etc.), and also with the varied ways of life (sedentary, benthic, nekto-benthic, pelagic, aerial) and feeding (detritus-eaters, suspension eaters, omnivores, carnivores) of the species that interact with it. The multiplicity of approaches needed to take this variability into account thus requires the use of many target species, and this is only possible if infrastructures crafted using diverse skills are in place. In the present state of our knowledge, monitoring can only be done gradually, stage by stage, depending on the degree of maturity of the indicators. initially it is recommended that a pilot monitoring network be developed based on the use of the *Caretta caretta* marine turtle species, the indicator of ingestion of marine litter by this species being at the most advanced stage of development.

6. It seems reasonable to also envisage starting experimental work to test the potential of new indicator species, mainly to measure the impact of micro-plastics, in particular certain species of fish that have a high rate of ingestion and wide distribution (*Boops boops, Mullus sp.*) and invertebrates, particularly the mussel *Mytilus galloprovincialis*, present throughout a vast area of the Mediterranean Basin. Table 1 lists the species/taxa already used, or that could be used, as bio-indicators, and their potential for use in the context of monitoring.

Table 1: Selection of indicator species for monitoring ingestion of marine litter by marine organisms in the Mediterranean

Taxon	Type of litter	Method	Infrastructure	Indicative Species	Priority	Remarks
Birds	macro-litter	Autopsy	Stranding networks, by-catch	To be researched	+	Work needed in the Mediterranean
Cetaceans	macro-litter	Autopsy	Stranding networks, by-catch	All species	+	Small number of species, low rate of ingestion, opportunistic approach only
Cetaceans	micro-plastics	Autopsy / chemical	Stranding networks, by-catch	All species	+	Sampling and measuring difficult
Marine turtles	macro-litter	Autopsy / excreta monitoring	Stranding networks, by-catch, rescue centers	*Caretta caretta*	+++	Necessity of mastering biological parameters
Nektobenthic fishes	micro-plastics	Stomach contents	Coastal fishing and trawling	*Mullus sp., Boops sp.*	++	Wide distribution of species, easily caught
Demersal fishes	macro-litter	Stomach contents	Scientific and commercial trawling	*Scyliorhinus sp.*	+	Opportunistic collection possible
Pelagic fishes	micro-plastics	Stomach contents	Commercial fishing		+	Opportunistic collection possible
Molluscs	micro-plastics	Stomach contents / chemical	Collection, farming, chemical monitoring networks	*Mytilus sp.*	++	Existing collection networks, concerning public health
Crustacean	micro-plastics	Stomach contents / chemical	Collection		+	Work needed in the Mediterranean
Other invertebrates	micro-plastics	Stomach contents / chemical	Collection	Sea cucumbers	+	Work needed in the Mediterranean

7. Concerning the entanglement / strangling, it is still necessary, under the present conditions, to organize the collection of information and to define the monitoring modes (Table 2). The mobilization of stranding networks must be considered as a priority by the Contracting Parties to the Barcelona Convention on a voluntary basis at first for experimental monitoring of entanglement/strangling of the main most sensitive species (mammals, birds, turtles).

8. The potential of monitoring marine litter in nests must be re-examined by experts in order to propose guidelines; to this effect, an experimental monitoring should be set up, particularly in the Mediterranean protected areas and on the basis of voluntary action by the Contracting Parties.

9. As part of future development, we recommend that the potential of surface and underwater observation campaigns (Table 1) be assessed. The interest of shallow diving, especially in Marine Protected Areas, and using submersibles or ROVs (Remotely Operated Vehicles) for greater depths as tools for collecting observations on entanglement/strangling of the most affected species (invertebrates and fishes) must be assessed. This last approach (submersibles/ROVs) should not be dissociated from operations of inventorying or reducing abandoned fishing gear/nets in areas defined as priority areas within the context of the Un Environment/MAP Regional Plan on Marine Litter Management in the Mediterranean.

Table 2: Monitoring arrangements and indicator species to be tested for monitoring entanglement/strangling in the Mediterranean

SPECIES	TYPES OF LITTER	METHOD	EXISTING NETWORKS	SPECIES	PRIORITY	REMARKS
Birds	Fishing gear, macro-litter	Observations, diagnosis	Stranding networks	All species	+	The monitoring must be organized per system with the following priorities:
Cetaceans	Lost nets, ghost nets	Observations, diagnosis	Stranding & observation networks at sea	All species	+	1) Pilot study concerning opportunistic monitoring by stranding networks
Turtles	Lost nets, ghost nets	Video monitoring (diving and ROVs)	Stranding & observation networks at sea	All species	+	2) Evaluation and tests of video/diving monitoring systems in protected areas
Nektobenthic fishes	Fishing gear	Video monitoring (diving and ROVs)	Video monitoring (diving and ROVs)	All species	+++	3) Surface observation test
Pelagic fishes	Lost nets, surface ghost nets	Observations, fishing	networks of sea observation	Big pelagic sharks	++	
Invertebrates	Lost nets, macro-litter	Video monitoring (diving and ROVs)	Protected area monitoring, scientific campaign	All species	+	
Birds	Meso-/ macro-litter	Observation, litter in nests	Nesting monitoring networks	European Shag	+	Indicator of effect partially concerning strangling. To be tested on a pilot scale

Part II

3. Protocols for monitoring interactions between marine litter and marine turtles[1]

10.   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)).

11.   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).

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[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.)

12.   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 (INDICIT[3], 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 MED[4] Project protocol (UN Environment/MAP Specially Protected Areas Regional Activity Centre[5], 2017).

13.   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.

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'		

14.   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.

15.   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.

3.1.   Preliminary Information

3.1.1   Regulatory aspects

16.   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.

17.   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.

3.1.2   Rules of hygiene

18.   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.

19.   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.

20.   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.

21.   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.

22.   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.

23.   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.

3.1.3   Preparing the premises, equipment and instruments

24.   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.

25.   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 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.

3.1.4   Preparing the team, distributing roles

26.   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.

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

3.1.5   Size of marine litter considered

27.   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 sub-group 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.

28.   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.

3.1.6   Useful definitions

29.   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.

3.2.   General Information on Live and Dead Specimens

3.2.1.   First Notes on the Discovery Site

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

31.   **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.

32.   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.

33.   **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.

34.   **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.

35.   **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.

3.2.2.   Description of the animal's body condition

3.2.2.1 Conservation status or decomposition level

36.   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 possible[7].

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[7] Some tissues (muscle, etc.) can be collected and frozen at -20°C for further genetic analysis.
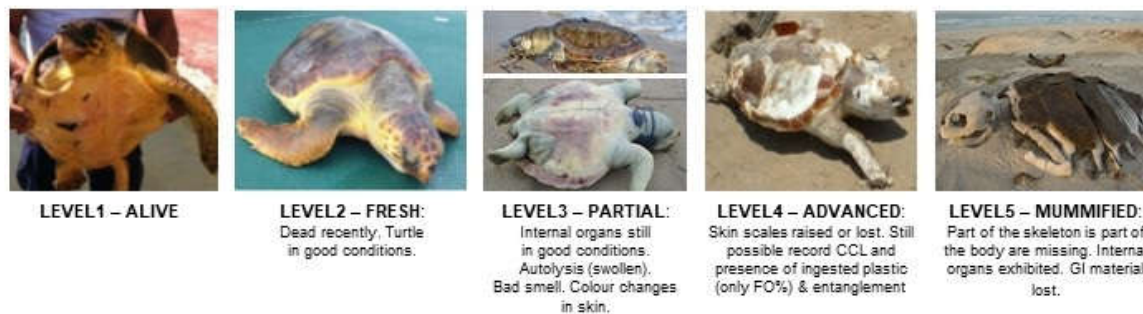
Figure 1: Conservation level or decomposition status

3.2.2.2 Discovery circumstances

37. 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.

3.2.2.3 Possible cause of morbidity and mortality, type of impact

38. 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".

3.2.2.4 By-catch gear

39. 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".

40.   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.

3.2.2.5 Health status

41.   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)

3.2.2.6 Main injuries

42.   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

3.2.2.7 Affected body part

43.   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").

3.2.2.8 Litter causing entanglement

44.   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).

3.2.2.9 Other descriptive parameters

45.   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.

46.   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).
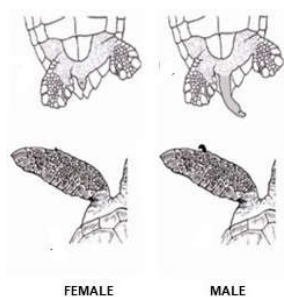

Figure 4: Example of determination of the sex of loggerhead turtle (from Wyneken, 2001)

FEMALE   MALE

3.2.2.10   Biometric Measurements

47.   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)*
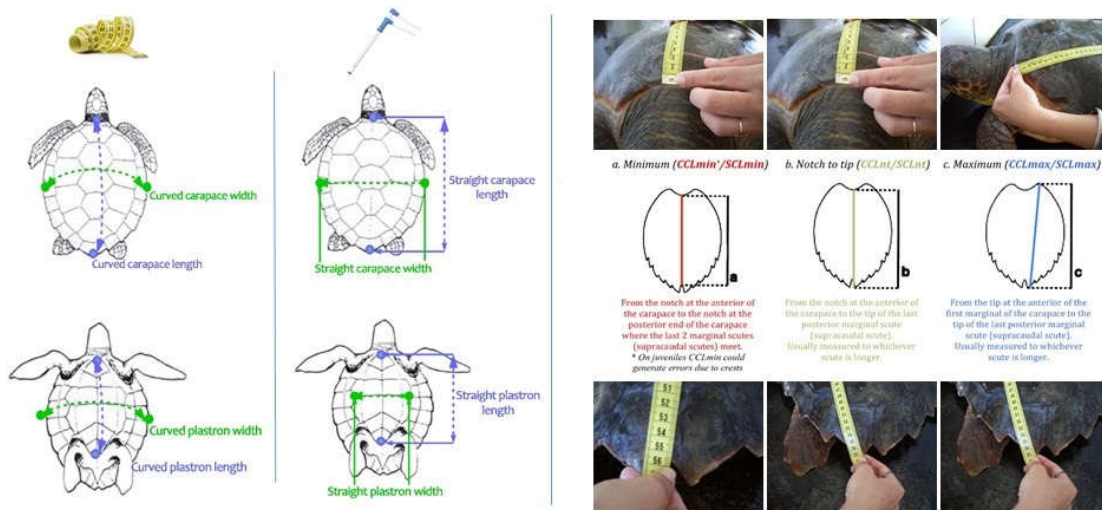
Figure 5: Biometric parameters (carapace and plastron lengths).

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

48.   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.

3.3.1   Turtle Necropsy

3.3.1.1 Opening of the carcass

49.   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).

50.   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).

3.3.1.2 Extracting and preparing sections of the digestive tract

51.   **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.

52.   **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).

53.   **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.

3.3.2   Extraction of Gut Content

54.   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 lenghtway 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 matter[9].

55.   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.

56.   All the material should be rinsed collected in the 1mm sieve (Fig. 8b, c), and should be placesd 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.

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

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

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

58.   **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

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[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.

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 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.

59. **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.
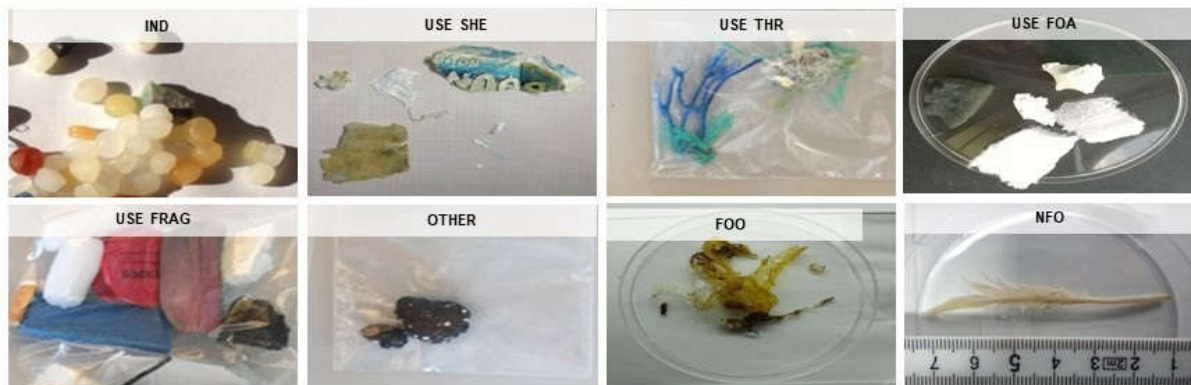
3.5. Marine Litter Analysis and Classification

60. **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.

61. **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 shorted into the different categories presented under Table 2. In additional 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 35ºC during 12h.

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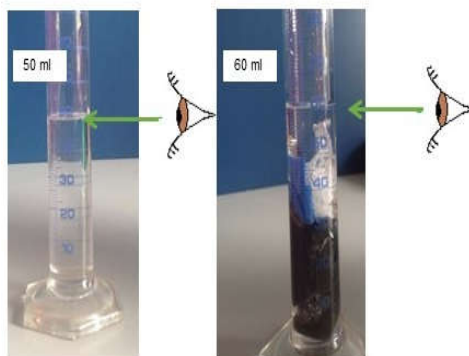
Figure 11: The volume of the plastic litter corresponds to the difference between the volume with (right) and the volume without (left) the plastic litter. The volume is read by considering the bottom of the meniscus formed by the surface water.

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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

UNEP ☺ ☯ **OBSERVATION SHEET - Entangling and litter ingestion** ✦ INDICIT

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 __/__/____ (dd mm eeee) INDIVIDUAL CODE __-__-__-__-__ (CC HH (Region) YY MM DD a')

LOCATION | X CORD | Y CORD

CIRCUMSTANCES	BY-CATH ENGINE CAUSE	CAUSE OF DEATH / STRANDING	ENTANGLEMENT TYPE ☐ Active ☐ Passive ☐ NR
☐ Bycatch/Fishery	☐ Longline	☐ By-catch/Fisheries	
☐ Stranding	☐ Trawl	☐ Entanglement in debris	**LITTER CAUSING ENTANGLEMENT**
☐ Dead at rescue centr	☐ Drift net	☐ Ingestion of litter	☐ Net pieces
☐ Found at sea	☐ Fishing rod	☐ Antrophogenic trauma	☐ Monofilament lines
☐ Other	☐ Other	☐ Natural trauma	☐ Rope/s
☐ NR	☐ NR	☐ Natural disease	☐ Raffia
		☐ Oils	☐ Plastic bags
PICTURES ☐ Yes ☐ No		☐ Healthy	☐ Other
Picture names.		☐ Other	☐ NR
		☐ NR	

Animal body condition:

CONSERVATION STATUS	HEALTH STATUS (Plastron shape)	MAIN INJURIES	AFFECTED PARTS	FAT RESERVES
☐ 1- Alive	☐ Poor (concave	☐ No injuries	☐ Flipper (	☐ Thin
☐ 2- Fresh	☐ Fair (plane)	☐ Fracture	☐ Carapace	☐ Normal
☐ 3- Partial	☐ Good (convex	☐ Amputation	☐ Neck	☐ Fat
☐ 4- Advanced	☐ NR	☐ Sectioning	☐ Head	☐ NR
☐ 5- Momified		☐ Abrasion	☐ Plastron	
☐ NR		☐ Other	☐ Other	

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: INDIVIDUAL CODE:

PROTOCOL: ☐ **NECROPSY** ☐ **OBSERVATION OF FAECES**

Please describe:

VISCERAS STATUS (note the presence of any infection, suspect colour, fluid effusion, perforantion, presence of oil, etc.):

ARRIVAL DATE	/	/
DEPARTURE	/	/
DEAD DATE	/	/

TURTLE BEHAVIOUR AND TREATMENTS:

DIGESTIVE TRACT (note the presence of any infection, suspect colour, fluid effusion, perforantion, presence of oil, etc.):

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

white-transparent

dark coloured

light coloured

NUMBER of plastic items per colour

white-transparent

dark coloured

light coloured

VOLUME of plastic litter

ml

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 devise;
   - 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).