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**Agenda item 4: Guidance on monitoring concerning the biodiversity and non-indigenous species common indicators****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 in the Mediterranean**

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## ANALYTICAL SUMMARY

In the context of the Regional Plan on Marine Litter (RPML) adopted by Decision G.21/7 of the Contracting Parties to the Barcelona Convention at their 18<sup>th</sup> Meeting in Istanbul, which came into force in 2014, one of the measures is linked to the implementing of the Integrated Monitoring and Assessing Programme for the Mediterranean Sea and its Coasts (IMAP) partly based on Ecological Objective 10's pilot indicator on quantities of litter ingested by marine organisms or rates of entangling of these organisms. 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. The target species for indicator EI 18 and for monitoring on the scale of the Mediterranean basin is the commonest species of marine turtle in the Mediterranean, *Caretta caretta*, widely distributed throughout the basin and for which a great deal of information is available.

Generally speaking, the rate of ingestion can depend on many factors, and monitoring protocols, particularly when analysing the litter ingested, must be precise and take into account the entire set of information relative to the field of sampling. Although the number of studies differs according to region, the metrics used are common, based on frequency of observation of turtles that have ingested litter. Number and mass of objects ingested are often used, with a recent tendency to assess average values of density or weight of ingested litter (RAC/SPA, 2017).

Marine turtles are not only vulnerable to ingestion but also to being entangled in marine litter. Entanglement in marine litter and the impact of this interaction on individuals has been described for several hundred species. But the monitoring protocols are still rarely described in the Mediterranean (RAC/SPA, 2017), which has so far hampered the development of a monitoring of this type of impact.

Implementing coordinated pilot experiments based on a strategy of improved information collection seems the most appropriate measure before envisaging the development of regional monitoring. This approach implies developing a protocol suited to the monitoring prior to the rationalising of observation procedures.

The present document describes the most suitable protocols for monitoring the ingestion of marine litter by marine turtles in the Mediterranean, dead or alive. It also describes a protocol intended to assess in a harmonized way rates of entanglement of marine turtles in marine litter, as back-up to the pilot monitoring approach.

The three protocols presented integrate the most recent conceptual and scientific elements, bearing in mind the methods advocated as part of the European Directive Framework Strategy for the Marine Environment (DCSMM) adapted to a monitoring context, as defined in the Mediterranean Regional Plan on Marine Litter, and the experience provided by the European rescue centres and networks already involved in marine turtle monitoring. The data collected using the protocols described in this document can also help when applying international biodiversity conservation policies such as the Convention for the Conservation of Migratory

Species (Bonn Convention or CMS) and the Action Plan for the Conservation of Mediterranean Marine Turtles adopted in 1989 (Barcelona Convention/Specially Protected Areas Protocol).

## 1. CONTEXT

In the Mediterranean, marine litter represents a critical problem because of the big amount present and its effects on marine fauna (RAC/SPA, 2017). As part of the Regional Plan on Marine Litter (RPML) adopted by Decision G. 21/7 of the Contracting Parties to the Barcelona Convention at their 18<sup>th</sup> Meeting in Istanbul, that came into force in 2014, one of the measures is linked to implementing the Integrated Monitoring and Assessment Programme for the Mediterranean and its Coasts (IMAP), partly based on Ecological Objective 10's pilot indicator on amounts of litter ingested by marine organisms or these organisms' rates of entanglement. In its study, published in 2017 (RAC/SPA, 2017) the PRDM selected the most representative species for the common indicator IMAP CI 18. 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. Some elements have already been suggested in this perspective (Table 1).

Species and habitat conservation policies recognise the pressure that human-origin waste 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 1.** 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)
  - 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)

Place	Date of sampling	Date of analysis	Species
No. of sample Observer	<b>Observer</b>		Organ*
	Storage conditions (fresh/frozen, duration)		

Item	Category (code)	Size (**)	Weight	Colour
Comments				

- 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 1996 the Parties to the Barcelona Convention listed 5 species of marine turtle on the List of Endangered and Threatened Species appended to the Protocol on Specially Protected Areas and Biodiversity in the Mediterranean (Barcelona, 1995) after the decades-long drop in numbers of marine turtle populations in the Mediterranean. The Regional Action Plan for the Conservation of Marine Turtles in the Mediterranean, adopted in 1989, advocates several measures, particularly the protection and management of habitats A2) and scientific monitoring (B2), including two sections on stranding data and networks. These stranding networks, the creation and strengthening of which were encouraged by this Action Plan, constitute a monitoring platform that increasingly helps monitor the impact of litter on marine turtles. The crafting of national action plans by the Parties, consistent with this regional plan, also has a part in rounding off this arrangement.

As part of the IMAP, the target species for indicator EI 18 for basin-wide monitoring is the commonest species of marine turtle in the Mediterranean, *Caretta caretta*, widely distributed throughout the basin and for which a great deal of information is available.

This species is very vulnerable to the ingestion of marine litter (Lazar & Gračan, 2011; Campani *et al.*, 2013; Camedda *et al.*, 2014; Darmon *et al.*, 2017; Matiddi *et al.*, 2017).

The ingestion of plastic fragments and other human-origin material may be directly responsible for the deaths of marine turtles from the occlusion or perforation of the digestive tracts (UNEP/MAP, 2015). In the long term, the presence of litter that has been ingested by turtles may have consequences for growth, capacity to move around, and reproduction (Teuten *et al.*, in RAC/SPA, 2017).

Generally speaking, the rate of ingestion can depend on many factors such as area of origin, date of stranding, state of health or length of time the animal is kept captive (Casale *et al.*, 2016).

In these conditions, monitoring protocols, particularly the analysis of litter ingested, must be precise and take into account the whole set of information related to the sampling environment. This data will thus facilitate the interpretation of results and trends.

Although the number of studies differs according to region, the metrics used are common and based on the frequency of observations of turtles having ingested litter. This frequency, expressed as a percentage, is also called 'prevalence'. The number and mass of objects ingested are often used, with a recent tendency to assess average values of the density or mass of ingested litter (RAC/SPA, 2017).

Marine turtles are not only vulnerable to ingestion but also to being entangled in marine litter. This type of interaction may have serious direct (drowning, suffocating, immobilisation, wounds) and/or indirect (deformation during growth, amputation, secondary infection) effects. In certain regions, entanglement is sufficiently frequent for it to even threaten the survival of marine turtle populations.

Being tangled entangled in marine litter, and the impact of this interaction on individuals, has been described for several hundred species (Kühn *et al.*, 2015; Werner *et al.*, 2016). But the monitoring protocols are still rarely described in the Mediterranean (RAC/SPA, 2017), which has so far hampered the development of a monitoring of this type of impact. Moreover, since the elements responsible for the entangling often arise from fishing, it is not always easy to decide whether the entanglement results from active fishing or from interaction with abandoned or lost gear, which thus qualifies as litter.

Implementing coordinated pilot experiments based on a strategy of improved information collection seems the most appropriate measure before envisaging the developing of regional monitoring.

This approach implies developing an appropriate monitoring protocol prior to the rationalising of observation procedures.

At methodological level, the choice of a monitoring approach (for ingestion as well as for entanglement) should satisfy several criteria:

- the availability of reference protocols that are tested, inter-calibrated, reproducible and validated by the specialist community
- the feasibility of respecting the procedure of packaging and preserving specimens and samples (freezing, attaching, eliminating the organic elements of samples, etc), and
- the possibility of banking the data.

These conditions once satisfied, the approach then consists of setting up standardised, quality guaranteed operational procedures. It is necessary to possess reference documents, especially of data collection and sample analysis protocols, to be combined with the monitoring.

This document describes the most suitable protocols for monitoring the ingestion of marine litter by marine turtles, dead or alive, in the Mediterranean. Although some studies on marine turtle juveniles have established the presence (Ryan *et al.*, 2016; Pham *et al.*, 2017), and analysed the quantity, of micro-plastics in their digestive tracts, this document only includes a specific protocol for elements that are larger than 1 mm. Indeed, recent work has shown that despite the importance of ingestion of micro-plastics in the animal kingdom, protocols for extracting and characterising smaller elements have not yet been sufficiently well-designed for micro-plastics of smaller size than 1 mm to be monitored for their impact on marine fauna (Fossi *et al.*, 2017).

Our study also proposes a protocol intended to assess in a harmonized way rates of entanglement of marine turtles in marine litter, as back-up to the pilot monitoring approach.

The three protocols presented integrate the most recent conceptual and scientific elements (Galgani *et al.*, 2014; Fossi *et al.*, 2017; Matiddi *et al.*, 2017; Pham *et al.*, 2017; Provencher *et al.*, 2017). They also bear in mind the methods advocated as part of the European Directive Framework Strategy for the Marine Environment (MSFD) (Galgani *et al.*, 2013) adapted to a monitoring context (as defined in the context of the Mediterranean Regional Plan on Marine Litter) as well as the experience provided by the European rescue centres and networks already involved in marine turtle monitoring.

The data collected using the protocols described in this document can also help when applying biodiversity conservation measures and implementing the Action Plan for the Conservation of Mediterranean Marine Turtles.

## **2. PROTOCOL**

### **2.1. Preparing operations**

#### **2.1.1. Regulatory aspects**

The following protocols describe the technical operations to be implemented when noting information and taking samples on living or dead turtles.

The manipulator must first make sure of the conditions for acting on marine turtles in the country where she is acting and conform to the regulations in force. The operations described can require making requests for permission and may come under many kinds of regulations: 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 zoonoses.

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.

### **2.1.2. 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, which, in an emergency, may not be in the minds of the various actors likely to take over from each other when dealing with a dead or living turtle. Without being alarmist, we recommend applying a certain number of basic rules that we shall mention 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 develop which can infect people, especially if they are accidentally hurt while examining and handling. That is why it is vital that the manipulator is equipped with minimal protection, i.e. gloves, ideally two pairs of gloves worn over each other, and protective clothing (overall or coverall, rubber boots or shoes). These gloves will be disposed of in a special rubbish bin, or cleaned and then disinfected if they are to be re-used.

It is important to note that although gloves represent a protection, they can also, once soiled, represent a source of contamination. So the manipulator must be careful to separate clearly those things that must remain clean from the soiled things that must be washed and then disinfected.

If the people providing the information (fishermen, firemen, etc.) have touched the turtle with their bare hands, they must be given advice on hygiene and particularly told to wash their hands carefully after the action, even given a disinfectant soap (chlorhexidine, for example) when they arrive at the place where the turtle will be taken into charge. The same precautions will be taken by manipulators who have not worn gloves.

For the same reasons, live turtles and carcasses must be moved in special tubs (plastic bowls with a waterproof mat for live animals) so that they can be cleaned and disinfected. Samples (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 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 the rules in force in the country 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.

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 centre or a veterinary surgery, where these precautions are already respected.

### 2.1.3. Preparing the premises, equipment and instruments

Before carrying out the operations of dealing with specimens, and storing or taking samples, and analysing 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 Table 2.

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.

**Table 2.** Equipment and material needed to implement the present protocol

OPERATION	OBJECT	MINIMUM EQUIPMENT	OPTIONAL EQUIPMENT
EXTERNAL EXAMINATION	Description of specimens and of the impact of the interaction with litter	Photographic material including a macro lens	Field for placing the specimen pre-photo
MOVING SPECIMENS	Dealing with live turtles	Washable transport tubs  Disposable gloves  Bin bags	Waterproof mat to fit the size of the tub  Ribbons to mark off the action area
	Dealing with dead turtles	Big heavy-duty plastic bags Washable transport tubs	Cool box  Ribbons to mark off the action area
STORING DEAD SPECIMENS	Post-mortem examination postponed	Cold room (+4°C)  Large capacity chest freezer (-20°C)	

		Big freezer chests (-20°C)	
THAWING THE CARCASSES  (To be done 24 to 48 hours before examination according to the size of the carcass)	Post-mortem examination postponed	Cold room (+4°C)	
TAKING FAECES	Collecting excreta in a care pool	Grid/inlet filter to place on the outlet (in the case of dynamic water movement/filtering)  Scoop (less than 1 mm in size)  Coloured plastic beads (diameter <1 cm)	Oven (to dry the faeces)
TAKING THE DIGESTIVE CONTENTS	Extracting and opening up the digestive tract	Scissors, tongs, bistoury and blades, ligatures or clamps  Pools/metallic recipients	
ANALYSING THE DIGESTIVE CONTENTS	Collecting the contents, sorting the litter and the remains of food	Sieves (5 mm, 1 mm), recipients (metal basins or kidney bowls), spatula, tongs  (sorting), absorbent paper  Precision balance (0.01 g precision), magnifying glass/low power stereo microscope	Measuring cylinders  Oven (to dry the faeces)  Labels to help with sorting
PACKAGING SAMPLES	Classifying samples	Plastic zipper bags, variously sized bottles, indelible felt marker	

#### **2.1.4. 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.

#### **2.2. Size of litter considered**

Analysis of litter, especially plastic litter in the environment, has led to litter being classified by size (GESAMP, 2015), type, and different coding according to the group that frames the monitoring methods (Annex I).

Theoretically, classification describes micro-litter as litter whose longest side is less than 5 mm long, and meso-litter as litter whose size is between 5 and 25 mm, and macro-litter as litter bigger than 25 mm. However, in practice, in monitoring programmes (MSFD and MAP) macro-litter often means litter bigger than 5 mm.

In our protocol, we take into account litter whose size is greater than 1 mm and greater than 5 mm.

Two sizes of sieve with various meshes, 1 and 5 mm, will be used to sort the litter by size in order to acquire data on the two size-groups (1 to 5 mm, and >5 mm). The data thus collected will then support choices made to set up monitoring that integrates, or not, small sized litter (1-5 mm).

#### **2.3. Useful definitions**

In order to guarantee optimum harmonization of information collection, certain definitions must be clearly stated. Acceptance of certain terms may differ from one person to the next and thus represent a source of bias.

The glossary (paragraph 6) contains the definition of terms used in the protocols. These concern, *inter alia*, the anatomy of marine turtles, assessment of carcasses, impacts of litter on these species, types of litter and fishing gear encountered, etc.

#### **2.4. Standard codification of observations and samples**

In anticipation of the banking and analysis of data on a national and regional scale, we suggest a standard method of codification that can be used, if need be, alongside the method already used by pre-existing data collection arrangements.

The method is the following, re-appearing in Annex III.

##### **2.4.1. Codifying the species**

We suggest using the traditional methods of codifying a species, giving the initials of its scientific name (initial of genus in capital letters, initials of the species in ordinary type).

Cc= *Caretta caretta*

Dc=*Dermochelys coriacea*

Cm=*Chelonia mydas*

#### **2.4.2. Codifying a specimen and a sample**

For a **specimen** (identifying the individual and the sheet), the code must provide information about the country and site where the animal was found, the species, the date (at least the year), the number of the turtle in a series (year, or month, or day, according to the codification of the date adopted).

Example: For the 1<sup>st</sup> individual of the species *Caretta caretta* (Cc) collected in Tunisia in Sfax by INSTM in 2017, the specimen code will read: Tu-Sf-INSTM-2017-Cc-01.

For a **sample**, added to this last code is information on the type of sample, and the number in the series if several samples of the same tissue were taken during the sampling operations.

Example: For the 1<sup>st</sup> sample of litter in the faeces of the 1<sup>st</sup> individual collected in Tunisia in Sfax by INSTM in 2017, the specimen code will read: Tu-Sf-INSTM-2017-Cc-01-faeces01 (or Oeso01, or Stom01, or Intest01).

#### **2.5. Description of operations**

The general method presented by Claro (2017) is shown below. Data is collected on marine turtles, live or dead, according to complementary external and internal observation protocols which provide information on ingestion and entangling. This information must be put on the data collection sheets that appear in Annex III, according to the following indications and recommendations, which appear in the form of reflex sheets in that same Annex.

##### **2.5.1. External examination of marine turtles**

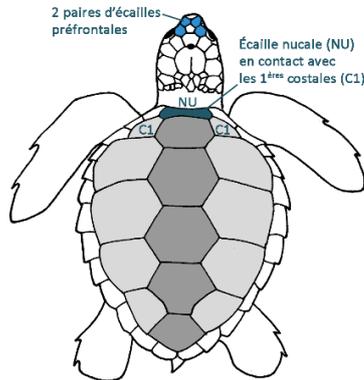
The external examination of marine turtles includes several stages.

###### *2.5.1.1. Identifying the species*

In the Mediterranean, the species most often found are the loggerhead turtle *Caretta caretta*, the green turtle *Chelonia mydas* and, to a lesser extent, the leatherback turtle *Dermochelys coriacea*. The leatherback turtle does not present keratinized scutes but ‘leather’ and ridges that mean it can be identified with confidence.

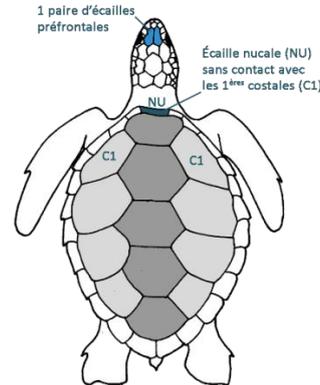
The difference between the green turtle and the loggerhead lies in the number of head scutes (1 pair of pre-frontal scutes in the green turtle, 2 for the loggerhead) and the back, particularly the nuchal (sometimes called cervical) and costal scales (for the loggerhead, the nuchal is in contact with the first costal scute) (Figure 1). On the sheet, tick the box that corresponds to the species you have identified, or the box ‘indeterminate’ if the carcass doesn’t allow you to identify the species with certainty.

**Figure 1.** Criteria for distinguishing the loggerhead turtle *Caretta caretta* (left) and the green turtle *Chelonia mydas* (right). Drawings by V. Hergueta.



2 pairs of pre-frontal scutes

Nuchal scute (NU) in contact with the 1<sup>st</sup> costals (C1)



1 pair of pre-frontal scutes

Nuchal scale (NU) not in contact with the 1<sup>st</sup> costals (C1)

#### 2.5.1.2. Identifying the individual and the gender

The marine turtle examined 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.

In these different cases, note down the figures and other information that enable the animal to be identified on the sheet in the box provided.

Also note the gender which can only be determined by external observation in adult individuals, using Wyneken (2001). On a dead turtle, the internal examination will possibly enable you to identify the gender by examining the genital organs, using Wyneken (2001) description.

### 2.5.1.3. Identifying the state of the specimen and the state of health

#### State of the specimen

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.

On the observation sheet, 5 stages are proposed:

1. Alive
2. Fresh: the carcass and tissues are intact and have not been swollen by decomposition gases; dissection or necropsy are possible
3. Partly decomposed: smelly, tissues soft and swollen; dissection or autopsy are possible
4. Advanced autolysis: the skin is discoloured or partly missing, the scutes may be coming unstuck, some parts of the body may be missing; in some cases, you can note the size and the presence of ingested litter or litter entangling the carcass
5. Mummified: the remaining tissues are dry, leathery. The digestive tube has disappeared. No observation of the impact of litter is possible.

In the case of a dead turtle at stage 2 or 3, you can look in the coelomic cavity for the quantity of fat present (especially on the mesentery) to assess the trophic state before death and decide whether the death came suddenly.

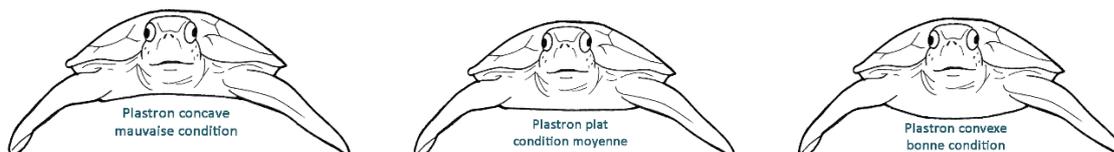
#### State of health of a live turtle

In the case of a live turtle, it is useful, to describe the intensity of the impact of the interaction with litter, to start by a general assessment of its state of health: is it normally active, averagely or strongly debilitated? What is its condition, is it stout?

The data collection sheet presents various options to tick.

The turtle's stoutness is something that allows the animal's trophic state to be assessed. At the level of the neck and the axillary and inguinal hollows, you can see whether the fat is thin (hollow looking), average, or thick (rounded). The concavity of the carapace also enables the turtle's state to be assessed (Figure 2).

**Figure 2.** Assessing the status of a turtle from the concavity of its plastron. Drawings by V. Hergueta, modified after Thomson *et al.* (2009)



Concave plastron, poor condition

Flat plastron, average condition

Convex plastron, good condition

The various criteria for assessment described above appear in Annex III.2.

#### *2.5.1.4. Noting external lesions*

Lesions are important information to be noted for they allow the nature and intensity of the impact of the litter on the marine turtles to be described. The main options are set out in the sheets. You can add additional comments that may be useful when the data is analysed, and the results interpreted.

#### *2.5.1.5. Noting elements that bear witness to an interaction*

The types of litter that interact with turtles are varied. During an external examination, you can observe litter that entangles the body or litter that is visible in the oral or cloacal cavity.

It is important to note these, classifying them in a way that enables analysis of their frequency per type. The data collection sheets offer you several options to be ticked, and if need be you may add comments that seem useful to describe the interaction.

Litter that bears witness to interactions may be usefully kept in zipper bags, bottles or any other container suited to the size or mass of the litter (for example, bundles of entangling rope). Labelling will note the code for the observation or the individuals. It can also be useful to store litter by class by indicating on the zipper bag or bottle the corresponding sort class code (see Annex II).

Similarly, as well as the previously advocated photographs, things that bear witness to the interaction can be photographed in close-up for archiving, after being removed from the turtle.

#### *2.5.1.6. Taking measurements*

There are many ways of measuring the length and width of a marine turtle (Bolten, 1999). In this protocol, we recommend taking *a minima* the most commonly used measurements, that require the least material, i.e. the standard curve width and curve length.

In the context of our protocol, the most important measurement is the length; measuring the width is optional.

Measurements will be taken using a one-metre ribbon and noted on the observation sheet.

#### *2.5.1.7. Taking photographs*

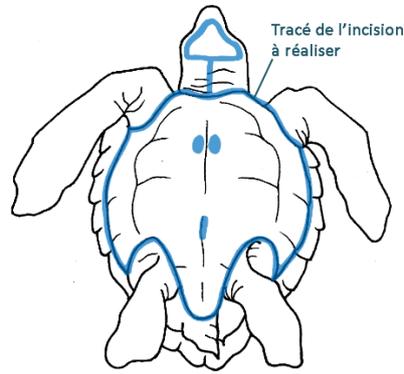
Photographic shots are particularly useful for the *a posteriori* confirming or clarifying of information noted, where there is doubt or difficulty about identifying the species, the lesions, the state of the individuals and the elements responsible for the interaction. Particularly, it can prove difficult in the case of entanglement to identify bits of fishing gear. Photographs will be precious and must be stored, making sure to refer to the code (or sheet) or the animal examined.

### **2.5.2. Internal examination of turtles**

#### *2.5.2.1. Opening up the carcass*

To carry out a dissection or autopsy, place the carcass on its back, trying to wedge it with an object so that it doesn't wobble from side to side. Cut the skin at the joint between the plastron and the marginal scales (Figure 3) and then cut the ligaments of the scapular and pelvic girdles to pull back the plastron and reach the muscles and then the internal organs (Figure 4).

**Figure 3.** Showing the incisions to be made to reach the internal organs. Drawings by V. Hergueta, modified from Wyneken (2001). The three central points show the attachments of the scapular and pelvic girdles.



Incision to be made

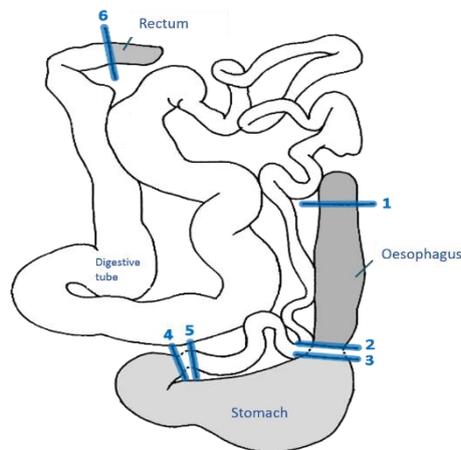
**Figure 4.** Opening up the carcass of a marine turtle during the necropsy in a veterinary laboratory. Photos by D. Gambaiani



#### 2.5.2.2. *Extracting and preparing parts of the digestive tract*

You can access the digestive tract after getting rid of the pectoral muscles and the heart. Tie the digestive tract at four points (Figure 5). Cut the digestive tract above and below the proximal and distal ligatures (ligatures 1 and 6). Helped by an assistant, extract the tract and place it on the lab bench. Ligatures 2 to 5 can also be placed on the bench, once the tract has been extracted.

**Figure 5.** Sketch of the three ligatured parts of the digestive tract to be analysed as part of the protocol on litter ingested by marine turtles. Drawing by V. Hergueta.



If the gender was not identifiable during the external examination, now look at the genital organs to discover the animal's gender.

Separate the three parts (oesophagus, stomach, intestines) by cutting the digestive wall between ligatures 2 and 3, and between ligatures 4 and 5 (Figure 5), to avoid any loss of contents.

#### 2.5.2.3. *Noting internal lesions 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, etc.

#### 2.5.2.4. *Taking photographs*

Photograph every lesion observed, taking care to get an overall view and a close-up (macro-lens). Archive this, referring to the code of the sheet that corresponds to the animal examined, describing the lesion in the description of the subject.

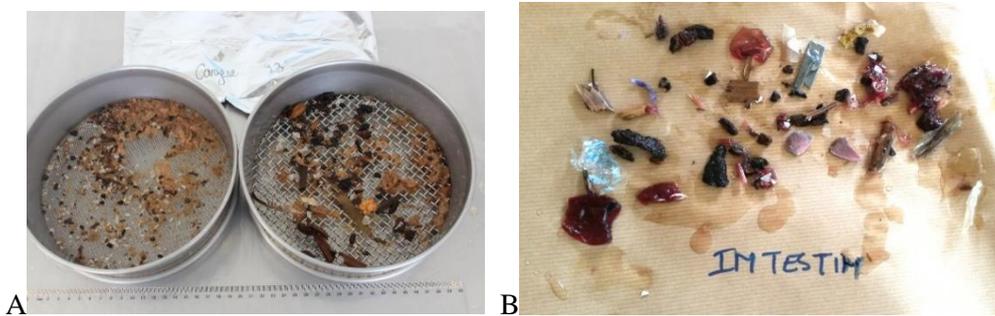
### 2.5.3. Taking samples

#### 2.5.3.1. *Extracting and handling the digestive contents (dead turtle)*

After isolating the portions of the digestive tract, place each of these in a recipient (pan, kidney bowl, etc.). For each portion, after examining possible external lesions (areas of necrosis, perforations), cut the wall. Collect the contents using a spatula and place them in a 5-mm sieve placed above a 1-mm sieve (Figure 6). Rinse with fresh water and possibly alcohol. Drain and dry at ambient temperature or in an oven (30°C). It is also possible to filter first using only the 1-mm sieve and then sieve with the 5-mm mesh after drying, just before analysing the sample.

**Figure 6.** Sifting and drying the digestive contents once recovered

(A): Intestinal contents sieved and rinsed with fresh water in two sieves of 1 and 5 mm mesh respectively. Photos by G. Darmon and F. Claro. (B): Intestinal contents put to dry at ambient temperature. Photo S. Catteau.



#### 2.5.3.2. *Sampling the faeces in the pool (live turtle)*

Faeces excreted by live turtles can contain litter; monitoring the excreta may consequently show the frequency of ingestion and the impact of litter on marine turtles.

If the care pool in which the turtle will be staying is fitted out with a dynamic water circulation and filtering system, care must be taken to place a grid or an inlet filter at the level of the outlet so that the faeces is not evacuated with the water from the pool and thus lost to the monitoring.

Once the animal is hospitalised, monitor its appetite. When the animals starts to feed, put a coloured ball (marble?) (plastic, about 1 cm in diameter) into its food; this will serve to calculate the duration of the digestive transit and identify the date of the last sample.

The faeces will be collected every day in the pool using a scoop (Figure 7) until the coloured ball (marble?) is excreted. The faeces taken will be decanted into a 1-mm sieve and rinsed with fresh water, then into a pan and/or onto absorbent paper. Then what has been sieved will be allowed to dry at ambient temperature.

**Figure 7.** Sampling the faeces in a care pool, packaging and drying a sample. Taking the faeces using a scoop (left), method of packaging a sample after it has been dried at ambient temperature (right). Photos by CESTMed.



#### 2.5.3.3. Taking photographs

Take one-off photographs (litter never having been observed in the list of elements ingested), or systematically, of the litter ingested over a field, making sure to write down the animal's code there. Make sure that a close-up is taken that contains all the litter or the litter sorted by class (macro lens, flash if need be). Archive this, making sure to refer to the code of the animal examined (or its sheet), describing the lesion in the description of the photograph.

#### 2.5.4. Analysing ingested litter

To facilitate the analysis of the litter, you will find in Annex II labels that make it easy to sort by category.

After drying the digestive contents or faeces, place your labels on the bench and separate the alimentary remains from the other elements. The alimentary remains can be kept in zipper bags or correctly labelled bottles for future analysis of the diet.

The remaining part must then be examined by the naked eye or using a magnifying glass, possibly a low-power stereo microscope.

Various categories of litter are defined by Galgani *et al.* (2013), OSPAR and MEDPOL (Annex I) and are used to characterise the state of the marine environment, particularly during drives to count the litter on the beaches.

The various categories have been given codes that differ according to organisation, but a correspondence table has been drawn up to deal with this (Annex I). Cases where the animal's entangling or ingestion are due to litter come under the main categories.

In the present protocol, we distinguish 9 categories of litter. Types and examples of litter coming into each category are described on the labels to help sorting available in Annex II.

Before sorting, and weighing, the litter by category we recommend cutting out the labels and putting them on the bench so that the litter corresponding to each category can be put with the corresponding label (Figure 8).

Once the sorting has been done, all the litter in each category will be put into a container previously placed on the scales to determine the tare weight. The mass, noted with a 0.01 g precision, will then be noted in the corresponding boxes on the data collection sheet.

**Figure 8.** Measuring (A), sorting (B) and weighing (C) litter ingested by a marine turtle. Photos by G. Darmon (A) and S. Catteau (B and C).



Two optional operations can, according to the availability of operators, be carried out to help in the experimenting of the protocols and the work of developing indicators.

The total volume of litter can be noted by a double measuring using a measuring test tube partly filled with water. This can help in assessing from what volume of ingested litter, according to the animal's size, an obstruction or occlusion can be observed.

Additionally, information on colour can help to understand whether marine turtles choose litter of certain colours, the proportions of litter of these same colours being monitored in the environment.

If you decide to carry out this last operation, you will proceed as follows: in each portion of litter belonging to one category, you will count the number of bits of litter of the colours that appear in Table 3, and then add up the number of bits of litter observed for each class of colour.

**Table 3.** Colours to be borne in mind to sort the litter (according to the EMODNET classification)

Class of colour
BLACK/GREY
BLUE/GREEN
BROWN
TRANSPARENT/TRANSLUCID
WHITE/CREAM
YELLOW
ORANGE/PINK/RED

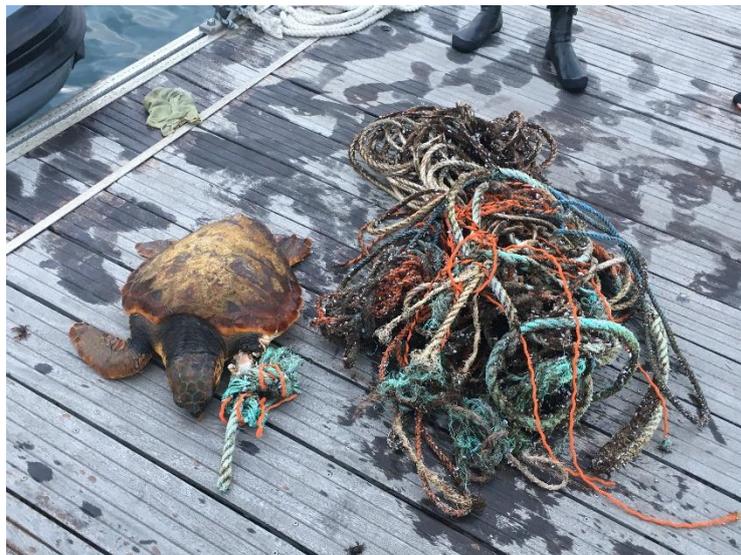
### 2.5.5. Labelling, packaging and banking the samples

Once dried, the samples will be packaged for later analysis or archiving (litter already sorted) in zipper bags or bottles and labelled, making sure that all useful information is noted and, if need be, using the code recommended in paragraph 2.4.

### 2.5.6. Entangling data

Data collection on entangling involves noting the information on the type and nature of the litter in which the turtle is entangled, the areas of the body concerned, the impact of this entanglement on the turtle. The data collection sheet offers a set of boxes to tick, which will enable the operator to be guided in his choice of what to do, helped by the definitions in the glossary. When the turtle is entangled in a material resulting from fishing activities, it is usually impossible to state that the thing responsible for the entangling can be called litter. Indeed, a turtle accidentally caught in fishing gear during a fishing operation can be set free by the fisherman with a bit of the gear still attached, whether this is a line with or without a hook, a net or a trawl or even a fishing pot. That is why the only category that we believe can be surely identified as halieutic-origin litter is the bundle of rope of mixed fishing (various colours and types). Indeed, its mixed make-up shows that the mass has been formed from several floating ropes that got wound up in each other due to gyres. We believe that the other elements from fishing cannot be taken into consideration, even if some of them can indeed constitute litter (gear that has been abandoned, lost, pulled off and discarded). However, it is not possible to check this.

**Figure 9.** Bundle of entangled fishing rope responsible for the amputation of a loggerhead turtle which had got caught up in it. Credit, A. Liria Loza



### 3. PROSPECTS AND RECOMMENDATIONS

#### 3.1. Structuring the data collection and analysis of samples

This report provides protocols that enable data on the ingestion of litter by turtles and on their being entangled in marine litter to be collected and harmonized on a Mediterranean scale.

With a view to the correct application of these protocols, and the setting up of regional monitoring, it will later be necessary to structure the data collection, analysis of samples and network of actors responsible for these activities. Also, an enhancing of capacities can be envisaged.

Table 4 presents recommendations for actions to be implemented to help set up this monitoring. Prior to the choice of the most appropriate monitoring strategy, assessment of existing arrangements (collecting specimens, analysis), and their technical nature, will allow the degree of cover of the Mediterranean region and the additional means to be used to apply the present protocols to be identified.

**Table 4.** Recommendations for actions to be implemented with a view to setting up a programme to monitor the interactions between litter and marine turtles (entangling, ingestion) in the Mediterranean

AIMS AND RECOMMENDED ACTIONS	INFORMATION/TOOLS REQUIRED	SPECIFIC INFORMATION
<b>Structuring the collection (data/samples)</b> <i>Assess existing collection arrangements</i>	Geographical coverage	Collection sectors covered
	Nature of technical skills	Dissection, veterinary diagnosis, sampling, reception of live turtles  Equipment, logistics, staff
	Existing means	Equipment, logistics, staff, training
	Necessary means Regulatory constraints	Protected species, animal experiments, health (zoonoses)
<b>Structuring the analysis of samples</b> <i>Assess analysis structures (laboratories)</i>	Geographical coverage	Collection sectors covered
	Field of competence	Autopsy, veterinary diagnosis, analysis of samples and data  Equipment, staff
	Existing means Necessary means	Equipment, staff, training

	Regulatory constraints	Protected species, animal experiments, health (zoonoses)
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### Enhancing capacities

<i>Organise practical training courses</i>	Identify trainers and trainees	Photographs of types of litter and lesions
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<i>Organise inter-calibration workshops</i>	Identify pertinent teams	
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<i>Produce/circulate technical back-up tools</i>	Photographic atlases	
	Video guides and tutorials	
	On-line resource platform	

### Enhance network functioning

<i>Produce/circulate directories of contacts</i>	Coordinated by country	Data producers and laboratories e.g. Medasset, MedTurtle, Rehab, Turtleingest
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<i>Encourage exchanges</i>	Create and participate in discussion lists	
	Organise regular workshops/meetings	e.g. in the context of the MedTurtle/RAC/SPA conference

In the Mediterranean, most existing data collection arrangements are strandings networks and marine turtle care centres (Casale *et al.*, 2010; Ullmann and Stachowitch, 2015), which often have limited means, and are based on an emergency network that must be permanently kept up. Collection of specimens and data on these specimens (characterisation of individuals, taking of samples) is an integral part of the daily job that these networks and care centres are doing. Their action enables not only the rehabilitating of live marine turtles to guarantee better protection of their species but also the increasing of knowledge about these populations by scientific exploitation of stranded and by-catch individuals. For these networks and care centres, implementing protocols for analysing digestive contents and cases of entangling represents an increase of work of several hours per dead turtle, a time for taking and preparing samples that is proportional to the duration of care for the living turtles. These activities also require specific skills and equipment that according to the case may be available in veterinary or research laboratories near to the place of action and collection arrangements. These operations, without being incompatible with the mission of the care networks and centres, correspond to a different mode of action. The care networks' and centres' actions are more based on emergencies: priority is given to getting as quickly as possible to the place where the stranding or by-catch has been reported.

In these conditions, it can be more operational to structure monitoring by choosing local, national or regional reference laboratories that deal with samples (carcasses, frozen digestive tubes, faeces) as an extension of the action of stranding networks and marine turtle care centres. If the logistic and storing means (negative cold) are anticipated in consequence, analysis can be planned or even mutualized to be done in the best conditions, both sanitary and standard. Data banking and analysis can, if need be, be done by these same laboratories and result in a pre-analysis of results in anticipation of national and regional reporting.

### 3.2. Changing protocols

Although the protocols presented here are intended to encourage the standardisation of sample collection and analysis of data on the impact of litter on biota in the Mediterranean, they can and do change. The protocols related to the ingestion of litter were themselves adapted from a first phase of the European protocol's being experimented (Galgani *et al.*, 2013) by certain networks (Italy, France) and by the partners in the INDICIT European project. They must be tested on a new spatial scale. The protocol on the entangling of marine turtles in litter, adapted from an experimental protocol designed as part of the INDICIT European project, is the first protocol to be spread over a regional scale and must also be subjected to a test period. In both cases, the protocols' application periods will help make them as operational as possible to guarantee improved feasibility for monitoring activities.

Organising regular inter-calibration workshops and exchanges is recommended to help perfect the protocols (Table 4). The training courses themselves constitute a chance to test the feasibility of the protocols in new sectors.

Our protocols enable a partial analysis of ingested micro-plastics (>1 mm) and the data resulting from their application can help in work on criteria for classifying and typology being drafted as part of the EMODNET project. This programme is in fact responsible from 2018 on for banking data on micro-plastics at European scale and is open to non-European countries (<http://www.emodnet.eu/chemistry>).

### 3.3. Bringing to attention in the context of environmental policies

Data collected using the protocols suggested in this study will satisfy the information needs of environmental policies to both assess the efficacy of steps taken to keep up, improve or restore the state of the marine environment, and analyse the pressures exerted on marine turtle populations.

Harmonizing the protocols for the collection and analysis of samples between the conventions of the regional seas and the European Community was fundamental and will help guarantee better interpretation of the data. In the field of biodiversity conservation, it is important that this approach be known about and promoted. Information at the CMS technical secretariat could mean that this information appeared on the agenda of a coming CMS technical meeting, for example. Moreover, integration of the approach that monitors the impact of litter on marine turtles could also be borne in mind at the coming revision of the Action Plan for the Protection of Mediterranean Marine Turtles (Barcelona Convention SPA Protocol).

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#### 4.2. Useful links (tutorials)

<https://www.rac-spa.org>

<https://www.indicit-europea.eu>

## 5. 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

**Asphyxia**. A pathological state determined by the slowing or stopping of respiration

**Autolysis**. Destruction of tissues by their enzymes

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

**Dorsal carapace** The dorsal part of a turtle's carapace

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

**Coelomic cavity**. The body's overall cavity that shelters the internal organs and is bordered by a membrane

**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

**Debilitated** (animal). Weakened, lacking in physical force

**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'

**Entangling/entanglement**. Accidentally caught by fishing gear during the fishing operation, or abandoned or lost

**Entangling/entanglement**. Accidentally caught in any kind of wreck either drifting or attached to the bed, whether or not this is fishing material

**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

**Excretion/excreta**. Evacuation from the organism/evacuated secretions or waste (excrement, evacuated faeces)

**Impact**. Effect of something

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

**Intestinal invagination** (or intestinal **intussusception**). The incorporation of one portion of the intestine within the intestinal portion lying below it (like the finger of a withdrawal glove or a telescope). This leads to an intestinal occlusion, a halt in transit. This invagination can happen when the peristalsis of the digestive tube is faced with a mass of ropes, or more or less flexible lines which change in shape and effect during their transit.

**Intestinal occlusion**. Complete halt of the passing of matter and gases in one portion of the intestine. The occlusion can have a mechanical cause (total obstruction by litter) and constitute a veterinary emergency. Without rapid action, the intestine necroses and the animal dies of peritonitis

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

**Macro-litter**. Litter whose longest side is greater than 25 mm long. In this protocol, and the monitoring programmes of the MSFD and MAP, this term also includes meso-litter *sensus stricto*, in a way that extends the term to cover litter whose size is greater than 5 mm

**Meso-litter** (*sensus stricto*). Litter whose longest side is between 5 and 25 mm long

**Micro-litter**. Litter whose longest side is less than 5 mm long

**Mummification**. Drying out of the body (as a result of a dry gangrene and/or of an advanced degradation in the open air)

**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 state**. 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 manipulator to classify observations in the correct category of data

ANNEXES

ANNEX I. List and correspondence of codes the categories of litter taken into account by TSG-ML, OSPAR and UNEP-MAP

(modified from Werner et al., 2016)

Under the heading 'Entanglement', in the red boxes, appears the litter reported as responsible for the entangling of the biota. Under the heading 'Ingestion', the green boxes correspond to the categories of litter ingested by marine turtles (literature and INDICIT project)

Master List of Categories of Litter Items						
TSG_ML General- Code	OSPAR- Code	UNEP- Code	General Name	1st order catégories of aterials	Entanglement	Ingestion
G1	1	PL05	4/6-pack yokes, six-pack rings	Artificial polymer materials		
G2		PL07	Bags	Artificial polymer materials		
G3	2	PL07	Shopping Bags incl. pieces	Artificial polymer materials		
G4	3	PL07	Small plastic bags, e.g. freezer bags incl. pieces	Artificial polymer materials		
G5	112		Plastic bag collective role; what remains from rip-off plastic bags	Artificial polymer materials		
G6	4	PL02	Bottles	Artificial polymer materials		
G7	4	PL02	Drink bottles <=0.5l	Artificial polymer materials		
G8	4	PL02	Drink bottles >0.5l	Artificial polymer materials		
G9	5	PL02	Cleaner bottles & containers	Artificial polymer materials		
G10	6	PL06	Food containers incl. fast food containers	Artificial polymer materials		
G11	7	PL02	Beach use related cosmetic bottles and containers, e.g. Sunblocks	Artificial polymer materials		

Master List of Categories of Litter Items						
TSG_ML General- Code	OSPAR- Code	UNEP- Code	General Name	1st order catégories of aterials	Entanglement	Ingestion
G12	7	PL02	Other cosmetics bottles & containers	Artificial polymer materials		
G13	12	PL02	Other bottles & containers (drums)	Artificial polymer materials		
G14	8		Engine oil bottles & containers <50 cm	Artificial polymer materials		
G15	9	PL03	Engine oil bottles & containers >50 cm	Artificial polymer materials		
G16	10	PL03	Jerry cans (square plastic containers with handle)	Artificial polymer materials		
G17	11		Injection gun containers	Artificial polymer materials		
G18	13	PL13	Crates and containers / baskets	Artificial polymer materials		
G19	14		Car parts	Artificial polymer materials		
G20		PL01	Plastic caps and lids	Artificial polymer materials		
G21	15	PL01	Plastic caps/lids drinks	Artificial polymer materials		
G22	15	PL01	Plastic caps/lids chemicals, detergents (non-food)	Artificial polymer materials		
G23	15	PL01	Plastic caps/lids unidentified	Artificial polymer materials		
G24	15	PL01	Plastic rings from bottle caps/lids	Artificial polymer materials		
G25			Tobacco pouches / plastic cigarette box packaging	Artificial polymer materials		
G26	16	PL10	Cigarette lighters	Artificial polymer materials		
G27	64	PL11	Cigarette butts and filters	Artificial polymer materials		
G28	17		Pens and pen lids	Artificial polymer materials		
G29	18		Combs/hair brushes/sunglasses	Artificial polymer materials		
G30	19		Crisps packets/sweets wrappers	Artificial polymer materials		

Master List of Categories of Litter Items						
TSG_ML General- Code	OSPAR- Code	UNEP- Code	General Name	1st order catégories of aterials	Entanglement	Ingestion
G31	19		Lolly sticks	Artificial polymer materials		
G32	20	PL08	Toys and party poppers	Artificial polymer materials		
G33	21	PL06	Cups and cup lids	Artificial polymer materials		
G34	22	PL04	Cutlery and trays	Artificial polymer materials		
G35	22	PL04	Straws and stirrers	Artificial polymer materials		
G36	23		Fertiliser/animal feed bags	Artificial polymer materials		
G37	24	PL15	Mesh vegetable bags	Artificial polymer materials		
G38			Cover / packaging	Artificial polymer materials		
G39		PL09	Gloves	Artificial polymer materials		
G40	25	PL09	Gloves (washing up)	Artificial polymer materials		
G41	113	RB03	Gloves (industrial/professional rubber gloves)	Artificial polymer materials		
G42	26	PL17	Crab/lobster pots and tops	Artificial polymer materials		
G43	114		Tags (fishing and industry)	Artificial polymer materials		
G44	27	PL17	Octopus pots	Artificial polymer materials		
G45	28	PL15	Mussels nets, Oyster nets	Artificial polymer materials		
G46	29		Oyster trays (round from oyster cultures)	Artificial polymer materials		
G47	30		Plastic sheeting from mussel culture (Tahitians)	Artificial polymer materials		
G48			Synthetic rope	Artificial polymer materials		
G49	31	PL19	Rope (diameter more than 1cm)	Artificial polymer materials		
G50	32	PL19	String and cord (diameter less than 1cm)	Artificial polymer materials		
G51		PL20	Fishing net	Artificial polymer materials		

Master List of Categories of Litter Items						
TSG_ML General- Code	OSPAR- Code	UNEP- Code	General Name	1st order catégories of aterials	Entanglement	Ingestion
G52		PL20	Nets and pieces of net	Artificial polymer materials		
G53	115	PL20	Nets and pieces of net < 50 cm	Artificial polymer materials		
G54	116	PL20	Nets and pieces of net > 50 cm	Artificial polymer materials		
G55		PL18	Fishing line (entangled)	Artificial polymer materials		
G56	33	PL20	Tangled nets/cord	Artificial polymer materials		
G57	34	PL17	Fish boxes - plastic	Artificial polymer materials		
G58	34	PL17	Fish boxes - expanded polystyrene	Artificial polymer materials		
G59	35	PL18	Fishing line/monofilament (angling)	Artificial polymer materials		
G60	36	PL17	Light sticks (tubes with fluid) incl. packaging	Artificial polymer materials		
G61			Other fishing related	Artificial polymer materials		
G62	37	PL14	Floats for fishing nets	Artificial polymer materials		
G63	37	PL14	Buoys	Artificial polymer materials		
G64			Fenders	Artificial polymer materials		
G65	38	PL03	Buckets	Artificial polymer materials		
G66	39	PL21	Strapping bands	Artificial polymer materials		
G67	40	PL16	Sheets, industrial packaging, plastic sheeting	Artificial polymer materials		
G68	41	PL22	Fibre glass/fragments	Artificial polymer materials		
G69	42		Hard hats/Helmets	Artificial polymer materials		
G70	43		Shotgun cartridges	Artificial polymer materials		
G71	44	CL01	Shoes/sandals	Artificial polymer materials		
G72			Traffic cones	Artificial polymer materials		
G73	45	FP01	Foam sponge	Artificial polymer materials		

Master List of Categories of Litter Items						
TSG_ML General- Code	OSPAR- Code	UNEP- Code	General Name	1st order catégories of aterials	Entanglement	Ingestion
G74			Foam packaging/insulation/polyurethane	Artificial polymer materials		
G75	117		Plastic/polystyrene pieces 0 - 2.5 cm	Artificial polymer materials		
G76	46		Plastic/polystyrene pieces 2.5 cm > < 50cm	Artificial polymer materials		
G77	47		Plastic/polystyrene pieces > 50 cm	Artificial polymer materials		
G78			Plastic pieces 0 - 2.5 cm	Artificial polymer materials		
G79			Plastic pieces 2.5 cm > < 50cm	Artificial polymer materials		
G80			Plastic pieces > 50 cm	Artificial polymer materials		
G81			Polystyrene pieces 0 - 2.5 cm	Artificial polymer materials		
G82			Polystyrene pieces 2.5 cm > < 50cm	Artificial polymer materials		
G83			Polystyrene pieces > 50 cm	Artificial polymer materials		
G84			CD, CD-box	Artificial polymer materials		
G85			Salt packaging	Artificial polymer materials		
G86			Fin trees (from fins for scuba diving)	Artificial polymer materials		
G87			Masking tape	Artificial polymer materials		
G88			Telephone (incl. parts)	Artificial polymer materials		
G89			Plastic construction waste	Artificial polymer materials		
G90			Plastic flower pots	Artificial polymer materials		
G91			Biomass holder from sewage treatment plants	Artificial polymer materials		
G92			Bait containers/packaging	Artificial polymer materials		
G93			Cable ties	Artificial polymer materials		
G94			Table cloth	Artificial polymer materials		

Master List of Categories of Litter Items						
TSG_ML General- Code	OSPAR- Code	UNEP- Code	General Name	1st order catégories of aterials	Entanglement	Ingestion
G95	98	OT02	Cotton bud sticks	Artificial polymer materials		
G96	99	OT02	Sanitary towels/panty liners/backing strips	Artificial polymer materials		
G97	101	OT02	Toilet fresheners	Artificial polymer materials		
G98		OT02	Diapers/nappies	Artificial polymer materials		
G99	104	PL12	Syringes/needles	Artificial polymer materials		
G100	103		Medical/Pharmaceuticals containers/tubes	Artificial polymer materials		
G101	121		Dog faeces bag	Artificial polymer materials		
G102		RB02	Flip-flops	Artificial polymer materials		
G103			Plastic fragments rounded <5mm	Artificial polymer materials		
G104			Plastic fragments subrounded <5mm	Artificial polymer materials		
G105			Plastic fragments subangular <5mm	Artificial polymer materials		
G106			Plastic fragments angular <5mm	Artificial polymer materials		
G107			cylindrical pellets <5mm	Artificial polymer materials		
G108			disks pellets <5mm	Artificial polymer materials		
G109			flat pellets <5mm	Artificial polymer materials		
G110			ovoid pellets <5mm	Artificial polymer materials		
G111			spheruloids pellets <5mm	Artificial polymer materials		
G112		PL23	Industrial pellets	Artificial polymer materials		
G113			Filament <5mm	Artificial polymer materials		
G114			Films <5mm	Artificial polymer materials		
G115			Foamed plastic <5mm	Artificial polymer materials		
G116			Granules <5mm	Artificial polymer materials		

Master List of Categories of Litter Items						
TSG_ML General- Code	OSPAR- Code	UNEP- Code	General Name	1st order catégories of aterials	Entanglement	Ingestion
G117			Styrofoam <5mm	Artificial polymer materials		
G118			Small industrial spheres (<5mm)	Artificial polymer materials		
G119			Sheet like user plastic (>1mm)	Artificial polymer materials		
G120			Threadlike user plastic (>1mm)	Artificial polymer materials		
G121			Foamed user plastic (>1mm)	Artificial polymer materials		
G122			Plastic fragments (>1mm)	Artificial polymer materials		
G123			Polyurethane granules <5mm	Artificial polymer materials		
G124	48	PL24	Other plastic/polystyrene items (identifiable)	Artificial polymer materials		
G125	49	RB01	Balloons and balloon sticks	Rubber		
G126		RB01	Balls	Rubber		
G127	50		Rubber boots	Rubber		
G128	52	RB04	Tyres and belts	Rubber		
G129		RB05	Inner-tubes and rubber sheet	Rubber		
G130			Wheels	Rubber		
G131		RB06	Rubber bands (small, for kitchen/household/post use)	Rubber		
G132			Bobbins (fishing)	Rubber		
G133	97	RB07	Condoms (incl. packaging)	Rubber		
G134	53	RB08	Other rubber pieces	Rubber		
G135		CL01	Clothing (clothes, shoes)	Cloth/textile		
G136		CL01	Shoes	Cloth/textile		
G137	54	CL01	Clothing / rags (clothing, hats, towels)	Cloth/textile		

Master List of Categories of Litter Items						
TSG_ML General- Code	OSPAR- Code	UNEP- Code	General Name	1st order catégories of aterials	Entanglement	Ingestion
G138	57	CL01	Shoes and sandals ( <i>e.g.</i> Leather, cloth)	Cloth/textile		
G139		CL02	Backpacks & bags	Cloth/textile		
G140	56	CL03	Sacking (hessian)	Cloth/textile		
G141	55	CL05	Carpet & Furnishing	Cloth/textile		
G142		CL04	Rope, string and nets	Cloth/textile		
G143		CL03	Sails, canvas	Cloth/textile		
G144	100	OT02	Tampons and tampon applicators	Cloth/textile		
G145	59	CL06	Other textiles (incl. rags)	Cloth/textile		
G146			Paper/Cardboard	Paper/Cardboard		
G147	60		Paper bags	Paper/Cardboard		
G148	61	PC02	Cardboard (boxes & fragments)	Paper/Cardboard		
G149		PC03	Paper packaging	Paper/Cardboard		
G150	118	PC03	Cartons/Tetrapack Milk	Paper/Cardboard		
G151	62	PC03	Cartons/Tetrapack (others)	Paper/Cardboard		
G152	63	PC03	Cigarette packets	Paper/Cardboard		
G153	65	PC03	Cups, food trays, food wrappers, drink containers	Paper/Cardboard		
G154	66	PC01	Newspapers & magazines	Paper/Cardboard		
G155		PC04	Tubes for fireworks	Paper/Cardboard		
G156			Paper fragments	Paper/Cardboard		
G157			Paper	Paper/Cardboard		
G158	67	PC05	Other paper items	Paper/Cardboard		
G159	68	WD01	Corks	Processed/worked wood		

Master List of Categories of Litter Items						
TSG_ML General- Code	OSPAR- Code	UNEP- Code	General Name	1st order catégories of aterials	Entanglement	Ingestion
G160	69	WD04	Pallets	Processed/worked wood		
G161	69	WD04	Processed timber	Processed/worked wood		
G162	70	WD04	Crates	Processed/worked wood		
G163	71	WD02	Crab/lobster pots	Processed/worked wood		
G164	119		Fish boxes	Processed/worked wood		
G165	72	WD03	Ice-cream sticks, chip forks, chopsticks, toothpicks	Processed/worked wood		
G166	73		Paint brushes	Processed/worked wood		
G167		WD05	Matches & fireworks	Processed/worked wood		
G168			Wood boards	Processed/worked wood		
G169			Beams / Dunnage	Processed/worked wood		
G170			Wood (processed)	Processed/worked wood		
G171	74	WD06	Other wood < 50 cm	Processed/worked wood		
G172	75	WD06	Other wood > 50 cm	Processed/worked wood		
G173		WD06	Other (specify)	Processed/worked wood		
G174	76		Aerosol/Spray cans industry	Metal		
G175	78	ME03	Cans (beverage)	Metal		
G176	82	ME04	Cans (food)	Metal		
G177	81	ME06	Foil wrappers, aluminium foil	Metal		
G178	77	ME02	Bottle caps, lids & pull tabs	Metal		
G179	120		Disposable BBQ's	Metal		
G180	79	ME10	Appliances (refrigerators, washers, etc.)	Metal		
G181		ME01	Tableware (plates, cups & cutlery)	Metal		

Master List of Categories of Litter Items						
TSG_ML General- Code	OSPAR- Code	UNEP- Code	General Name	1st order categories of aterials	Entanglement	Ingestion
G182	80	ME07	Fishing related (weights, sinkers, lures, hooks)	Metal		
G183		ME07	Fish hook remains	Metal		
G184	87	ME07	Lobster/crab pots	Metal		
G185			Middle size containers	Metal		
G186	83	ME10	Industrial scrap	Metal		
G187	84	ME05	Drums, <i>e.g.</i> oil	Metal		
G188		ME04	Other cans (< 4 L)	Metal		
G189		ME05	Gas bottles, drums & buckets (> 4 L)	Metal		
G190	86	ME05	Paint tins	Metal		
G191	88	ME09	Wire, wire mesh, barbed wire	Metal		
G192		ME05	Barrels	Metal		
G193			Car parts / batteries	Metal		
G194			Cables	Metal		
G195		OT04	Household Batteries	Metal		
G196			Large metallic objects	Metal		
G197			Other (metal)	Metal		
G198	89	ME10	Other metal pieces < 50 cm	Metal		
G199	90	ME10	Other metal pieces > 50 cm	Metal		
G200	91	GC02	Bottles incl. pieces	Glass/ceramics		
G201		GC02	Jars incl. pieces	Glass/ceramics		
G202	92	GC04	Light bulbs	Glass/ceramics		
G203		GC03	Tableware (plates & cups)	Glass/ceramics		

Master List of Categories of Litter Items						
TSG_ML General- Code	OSPAR- Code	UNEP- Code	General Name	1st order catégories of aterials	Entanglement	Ingestion
G204	94	GC01	Construction material (brick, cement, pipes)	Glass/ceramics		
G205	92	GC05	Fluorescent light tubes	Glass/ceramics		
G206		GC06	Glass buoys	Glass/ceramics		
G207	95		Octopus pots	Glass/ceramics		
G208		GC07	Glass or ceramic fragments >2.5cm	Glass/ceramics		
G209			Large glass objects (specify)	Glass/ceramics		
G210	96	GC08	Other glass items	Glass/ceramics		
G211	105	OT05	Other medical items (swabs, bandaging, adhesive plaster etc.)	unidentified		
G212			Slack / Coal			
G213	181, 109, 110	OT01	Paraffin/Wax	Chemicals		
G214			Oil/Tar	Chemicals		
G215			Food waste (galley waste)	Food waste		
G216			various rubbish (worked wood, metal parts)	undefined		
G217			Other (glass, metal, tar) <5mm	unidentified		

**ANNEX II.: Labels for the sorting of litter (to be printed and then cut out)**

<b>USE SHE <i>sheeting</i></b>	<b>USE THR <i>threads</i></b>	<b>USE FOA <i>foam</i></b>
Bits of bags (agricultural, garbage), sheeting/packaging wrapper, paper	Bits of fishing line, nets, rope, cloth	Bits of polystyrene, foam (mattress), polyurethane (building)
<b>USE FRAG <i>thick plastics</i></b>	<b>USE POTH <i>other manufactured items</i></b>	<b>OTHER <i>other non-manufactured items</i></b>
Fragments of thick and/or flexible plastic but not of the 'sheeting' kind (capsules, etc.)	Elastic, cigarette filters, balloons, rubber, hooks, cotton buds, dummies	Except for plastic: tar blobs, etc
<b>FOO <i>turtle food</i></b>	<b>NFO <i>natural elements</i></b>	<b>IND <i>plastic granules</i></b>
Remains of usual food: shells, crustaceans, etc.	Non-food items: pebbles, wood, tree bark, feathers, etc.	Industrial, or probably industrial, plastic granules of variable shapes and colours

### ANNEX III. Summarized technical sheets

#### ANNEX III.1. Coding sheet

For correct classification of specimens, sheets and samples, we recommend the following classification methods.

##### **How to code the species?**

You code the species using the initials of its scientific name (initial of genus in capital letters, initial of the species in small letters).

Cc: *Caretta caretta*

Dc: *Dermochelys coriacea*

Cm : *Chelonia mydas*

##### **How to code a specimen and a sample?**

For a **specimen** (identifying the individual and the sheet), the code must provide information about the country and site where the animal was found, the species, the date (at least the year), the number of the turtle in a series (year, or month, or day, according to the codification of the date adopted).

Example: For the 1<sup>st</sup> individual of the species *Caretta caretta* (Cc) collected in Tunisia in Sfax by INSTM in 2017, the specimen code will read: Tu-Sf-INSTM-2017-Cc-01.

For a **sample**, added to this last code is information on the type of sample, and the number in the series if several samples of the same tissue were taken during the sampling operations.

Example: For the 1<sup>st</sup> sample of litter in the faeces of the 1<sup>st</sup> individual collected in Tunisia in Sfax by INSTM in 2017, the specimen code will read: Tu-Sf-INSTM-2017-Cc-01-faeces01 (or Oeso01, or Stom01, or Intest01).

## ANNEX III.2. Sheet to assess the state of a marine turtle specimen

### States and stages of freshness of a specimen

1. Alive
2. Fresh: the carcass and tissues are intact and have not been swollen by decomposition gases; dissection or necropsy are possible
3. Partly decomposed: smelly, tissues soft and swollen; dissection or autopsy are possible
4. Advanced autolysis: the skin is discoloured or partly missing, the scutes may be coming unstuck, some parts of the body may be missing; in some cases, you can note the size and the presence of ingested litter or litter entangling the carcass
5. Mummified: the remaining tissues are dry, leathery. The digestive tube has disappeared. No observation of the impact of litter is possible.

### Assessing the state of health of a turtle

- Live turtle or fresh carcass:

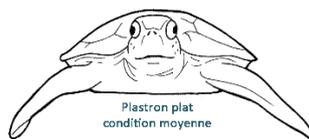
External examination: for the axillary and inguinal hollows, see whether the fat is thin (hollow aspect) (poor condition), thick (rounded aspect) (good condition), or average (average condition).

- Live turtle: Its behaviour and vitality enable a turtle's state of health to be assessed according to three degrees: normally active (good), averagely active (average) or highly debilitated (bad). The concavity of the plastron is also an indicator of its state.



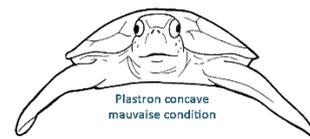
Convex plastron

Good condition



Flat plastron

Average condition



Concave plastron

Bad condition

### ANNEX III.3. Reflex sheet 'Observation of the impact of marine litter'

#### Case of a dead turtle

#### EXTERNAL EXAMINATION

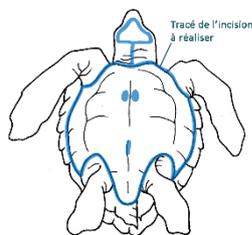
- ✓ Fill in the identification data: species, individual, sheet code, if possible gender



- ✓ Assess the state of the turtle (decomposition)
- ✓ Measure the turtle (curve length)
- ✓ Examine if possible the oral cavity and the cloaca and look for the presence of foreign bodies (litter)
- ✓ Examine the rest of the body and look for lesions due to the litter

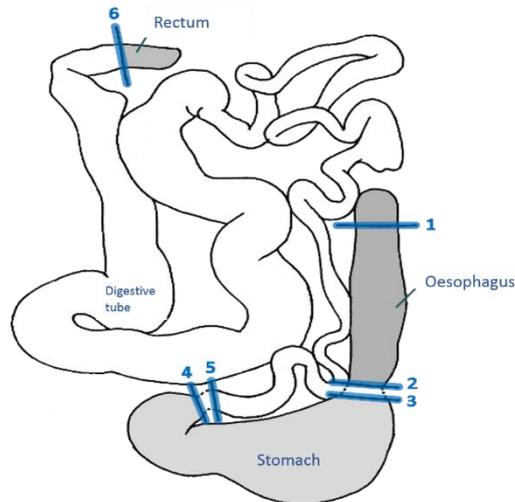
#### INTERNAL EXAMINATION AND EXTRACTION OF DIGESTIVE TRACT

- ✓ Place the turtle on its back
- ✓ Cut and withdraw the plastron and cut the ligaments of the pelvic and pectoral girdlesto pull off the plastron



Incision to be made

- ✓ Assess the trophic state (stoutness) of the animal: atrophy of the pectoral muscle, thickness of the fat in the articular cavities and on the peritoneum
- ✓ Pull out the digestive tract by withdrawing the pectoral muscles and the heart
- ✓ Tie the digestive tract at four points.



- ✓ Cut the ends (ligatures 1 and 6); pull out the digestive tract and put it on the bench
- ✓ Identify the gender of the animal if this is not known
- ✓ Separate the three parts (oesophagus, stomach, intestines) by cutting between ligatures 2 and 3, and 4 and 5
- ✓ Place each part in a bowl.

#### **ANNEX III.4. Reflex sheet ‘Observation of the impact of marine litter’**

##### **Case of a live turtle**

##### **EXTERNAL EXAMINATION AND MONITORING OF THE DIGESTIVE TRANSIT**

- ✓ Take the animal to the authorized rescue centre
- ✓ If the pool is not yet fitted out with this, put an inlet filter over the place where the water is evacuated (if dynamic)
- ✓ Place the animal on the medical examination table
- ✓ Carry out the external examination and fill in the identification data as indicated on sheet III.3
- ✓ Place the animal in the care pool, and when the animal starts feeding, insert into its food a coloured ball (plastic, about 1 cm in diameter) which will help calculate the duration of the digestive transit duration

##### **COLLECTING THE FAECES**

- ✓ Every day, collect the faeces in the pool using a scoop, until the coloured ball is excreted.



- ✓ Every day, decant the faeces sampled into a sieve with 5-mm mesh placed above another with 1-mm mesh, and rinse with fresh water
- ✓ Dry at ambient temperature

### ANNEX III.5. Reflex sheet 'Observation of the impact of marine litter'

#### Extracting the digestive contents (dead turtle)

- ✓ Place the portion of the digestive tract in a kidney bowl or a bowl
- ✓ Open it up and see whether there is an occlusion (blockage) then empty the contents into the kidney bowl or pan using a spatula
- ✓ Note possible lesions on the digestive wall: presence of ulcers or perforations caused by objects made of hard plastic, etc.
- ✓ Inspect and sort the contents: the presence of tar, of particularly fragile material that must be taken out and separately treated, separate the remains of food
- ✓ Above a sink, decant the contents of the kidney bowl or pan into a 5-mm mesh sieve placed over a 1-mm mesh sieve, then rinse with fresh water and filter



- ✓ Rinse all the elements collected by the filter in alcohol at 70% and rinse again in fresh water
- ✓ Dry at ambient temperature (or in an oven)
- ✓ Go through the same operations for each portion
- ✓ Package the samples in a zipper bag or a plastic pot, label and freeze, or analyse immediately (see following stage).

### ANNEX III.6. Reflex sheet 'Observation of the impact of marine litter'

#### Sorting and analysing the litter (dead or live turtle)

- ✓ Separate the remains of food from the other things in the sample
- ✓ Sort the marine litter by type using the sort labels and, if need be, a magnifying glass (low power stereo microscope)



- ✓ Dry the fragments of litter at ambient temperature
- ✓ Classify the litter by category



- ✓ Weigh the dry mass for each category of litter.



**ANNEX III.7. Data collection sheets on interactions between marine litter and marine turtles**

(See the 3 following pages)

Contracting Party	<b>Monitoring the impact of marine litter on marine Turtles</b>		
	<b>DESCRIPTION SHEET OF SPECIMEN</b>		
Name/first name of compiler			
Date (dd/mm/yy)			
Place of examination (commune, post code)			
Institution:	Telephone	Email:	@
Type of data collection arrangement	Strandings Network <input type="checkbox"/> Rescue centre <input type="checkbox"/> laboratory <input type="checkbox"/> Other <input type="checkbox"/>		
<b>LIVE TURTLE</b> 1 <input type="checkbox"/>		<b>DEAD TURTLE</b> <input type="checkbox"/>	
CODE	SHEET:	INDIVIDUAL:	
DATE OF DISCOVERY (dd/mm/yy)			
INFORMER'S NAME/ADDRESS,PHONE NUMBERS ETC...			
PLACE OF DISCOVERY ( <i>decimal coordinates</i> )	City/harbour Y	<i>coordinates*</i> : X	
DATE OF EXAMINATION			
SPECIES	<i>Caretta caretta</i> <input type="checkbox"/> <i>Dermochelys coriacea</i> <input type="checkbox"/> <i>Chelonia mydas</i> <input type="checkbox"/>		
INDIVIDUAL IDENTIFICATION	N° of ring or ship:		
CARAPACE CURVE LENGTH (0,1cm)	Max :	Min :	Standard :
WEIGHT (0,01 kg)			
GENDER *	Male <input type="checkbox"/>	Female <input type="checkbox"/>	Uncertain <input type="checkbox"/>

CIRCUMSTANCES	stranding <input type="checkbox"/> collision with ship <input type="checkbox"/> found at sea <input type="checkbox"/> caught during fishing: dead <input type="checkbox"/> alive <input type="checkbox"/> reanimated <input type="checkbox"/> clarify fishing gear and metiert:
DATE WHEN ENTERED RESCUE CENTRE	
DATE WHEN LEFT RESCUE CENTRE	Released (dd/mm/yy): _____ Dead (dd/mm/yy): _____
STATE AT NECROPSY	fresh <input type="checkbox"/> thawed <input type="checkbox"/>
DEGREE OF DECOMPOSITION	2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5 <input type="checkbox"/>
TROPHIC STATE	<b>Live:</b> neck inguinal & axillary fat thick <input type="checkbox"/> hollow <input type="checkbox"/> <b>plastron</b> concave <input type="checkbox"/> flat <input type="checkbox"/> convex <input type="checkbox"/> <b>Dead:</b> pectoral muscles slight atrophy <input type="checkbox"/> moderate <input type="checkbox"/> severe <input type="checkbox"/> <b>Internal fat :</b> abundant <input type="checkbox"/> normal <input type="checkbox"/> thin <input type="checkbox"/> none <input type="checkbox"/>
STATE OF HEALTH	good <input type="checkbox"/> average <input type="checkbox"/> bad <input type="checkbox"/> precise information:
TYPE OF INTERACTION WITH LITTER	Ingestion <input type="checkbox"/> Entangling <input type="checkbox"/> Additional information below
CAUSE OF MORTALITY/MORBIDITY ( Apprend report of necopsy of veterinarian if need be))	linked to litter <input type="checkbox"/> non-identified <input type="checkbox"/> collision <input type="checkbox"/> fishing <input type="checkbox"/> other <input type="checkbox"/> Precise information:
IMPACT OF INGESTION OF LITTER	occlusion <input type="checkbox"/> obstruction <input type="checkbox"/> perforation <input type="checkbox"/> inflammation <input type="checkbox"/> necrosis <input type="checkbox"/> peritonitis <input type="checkbox"/> other <input type="checkbox"/> Precise information :
<b>If the turtle is being monitored in a rescue centre</b>	
DIGESTIVE PROBLEMS AND OBSERVATIONS WHEN ADMITTED TO THE RESCUE CENTRE	Gas/buoyancy problems (X-ray) <input type="checkbox"/> constipation <input type="checkbox"/> anorexia <input type="checkbox"/> prolapse <input type="checkbox"/> Litter in the beak <input type="checkbox"/> litter coming out of the cloaca <input type="checkbox"/> other: provide precise information:
DATE OF 1st EXCRETION OF LITTER	
DATE OF LAST EXCRETION	
NUMBER (N=), SAMPLE CODES (x to x)	



(USE SHE)																		
(USE THR)																		
(USE FOA))																		
(USE FRAG)																		
(USE POTH)																		
Other litter (except plastic)																		
(FOO)																		
(NFO)																		
SUB-TOTAL																		
VOLUME (ml) (optionnel)																		

BG=Black/grey; BG=Blue/green; BT=Brown/tan; TT=Transparent/translucid; WC=White/cream; Y=Yellow; ORP=Orange-red-pink

<b>LIVE TURTLE (litter excreted in care centre)</b> <i>S=Sample ; M=dry Mass ; N=Number of items</i>																									
<i>Use a second sheet if the turtle excretes litter over a period greater than the number of lines in the table</i>																									
Date (n° S.)	IN		US		US		US		US		OT		FO		NF		TOT	N	N	N	N	N	N	N	
	D	E	SH	TH	FO	FR	PO	H	O	O	N	M	TT	WC	Y	ORP									
	N	M	N	M	N	M	N	M	N	M	N	M	N	M	N	M									

**MEASURING THE LITTER INGESTED (> 5 mm)**

DEAD TURTLE	OESOPHAGUS		STOMACH		INTESTINE		No items	N	N	N	N	N	N	N
							Total	BG	BG	BT	TT	WC	Y	ORP
<b>PRESENCE OF LITTER</b>	Yes <input type="checkbox"/>	No <input type="checkbox"/>	Yes <input type="checkbox"/>	No <input type="checkbox"/>	Yes <input type="checkbox"/>	No <input type="checkbox"/>								
M= dry mass (0.01g) N= number of items	<i>N</i>	<i>M</i>	<i>N</i>	<i>M</i>	<i>N</i>	<i>M</i>								
<b>(IND)</b>														
<b>(USE SHE)</b>														
<b>(USE THR)</b>														
<b>(USE FOA))</b>														
<b>(USE FRAG)</b>														
<b>(USE POTH)</b>														
<b>Other litter (except plastic)</b>														
<b>(FOO)</b>														
<b>(NFO)</b>														
<b>SUB-TOTAL</b>														
<b>VOLUME (ml) (optional)</b>														

BG=Black/grey; BG=Blue/green; BT=Brown/tan; TT=Transparent/translucid; WC=White/cream; Y=Yellow; ORP=Orange-red-pink

**LIVE TURTLE (litter excreted in rescue centre)** *S=Sample; M=dry Mass ; N=Number of items*  
Use a second sheet if the turtle excretes litter over a period greater than the number of lines in the table

