CEMP Guidelines for monitoring and assessment of marine litter ingested by sea turtles

(OSPAR Agreement 2020-03)

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1 Introduction

The quantity of litter ingested by sentinel organisms reflects both the spatial and temporal trends in litter in the environment and the harm caused on wildlife and natural habitats. The large distribution of sea turtles (Witt et al., 2007), their use of various marine compartments (Casale et al., 2008) and their propensity to ingest debris (Schuyler et al., 2013; Darmon et al., 2018) make them such a relevant indicator. "Litter ingested by sea turtles" was proposed by France in 2015 and 2016 at EIHA meetings, then retained as a candidate indicator for measuring impact on biota for OSPAR in 2016 (OSPAR- EIHA 16-5-13). Since then, progress has been realized in terms of networking and collection of standardized data and now enables to propose it as a common indicator.

A procedure of harmonization of monitoring approaches has been initiated in EU and Mediterranean between the Marine Strategy Framework Directive (MSFD Criteria D10C3) and the Barcelona Regional Sea Convention (RSC) (common indicator El 18). In OSPAR area, according to the distribution of sea turtles, the monitoring has been proposed to be applicable to Zones III, IV and V, as well as in Macaronesia.

The indicator "Litter ingestion by sea turtles" was proposed based on the loggerhead turtle *Caretta caretta.* It should be applicable to the leatherback turtle *Dermochelys coriacea*, more frequently observed in OSPAR zone III than the loggerhead (OSPAR, 2009, 2017) and also listed in OSPAR List of Threatened and/or Declining Species and Habitats (OSPAR 2009a, 2009b, 2010), highly prone to ingest litter (Schuyler et al., 2013). The monitoring of litter ingested by sea turtles targets all size items, including micro-litter between 1 and 5 mm, meso-litter from 5 to 25 mm and macro-litter larger than 25 mm. The size of litter items being differentiated, a separation of micro and meso/macro-items would be possible subsequently.

On the whole area (OSPAR-Macaronesia and Barcelona RSCs as well as MSFD), more than 72 stakeholders, mostly rescue centres, stranding networks, research or veterinarian laboratories, are using a harmonized procedure for collecting sea turtle individuals in the field and extracting ingested litter in the laboratory (INDICIT final report, 2019). The quality and quantity of standardized data acquired by this network highlight the collective capacity for a global and continuous monitoring of litter impact on sea turtles through ingestion (INDICIT final report, 2019). Integrating newly trained stakeholders should increase the dataset and provide more accurate assessments necessary to precisely define an EcoQO/GES. Meanwhile, collecting data for the monitoring of litter ingestion in sea turtles, will also provide an important knowledge for reinforcing measures related to the conservation of sea turtles (OSPAR, 2013a, 2013b).

The present document aims at providing guidelines for implementing the monitoring of litter ingested by sea turtles: deployment of networks for collection of individuals, sampling and collection of standardized data, data banking and analysis, expected targets at the regional and Contracting Parties' levels. This document also intends to highlight the needs for further developments of the candidate indicator.

2 Monitoring

2.1 Purpose

The purpose of the monitoring is to assess the impact of litter ingestion on sea turtles' health as i) an indicator of litter impact on marine wildlife and environment, ii) a source of key knowledge on threats for threatened species listed by OSPAR.

Sea turtles are considered as relevant indicator for evaluating the success of specific environmental measures targeting marine litter, due to their large distribution, their propensity to ingest litter and the monitoring effort undertaken since decades for rescuing and protecting them. The loggerhead species is known as being an opportunistic feeder (Casale et al., 2008; Frick et al., 2009), regularly ingesting litter (Schuyler et al., 2013; Camedda et al., 2014; Pham et al., 2017), probably unintentionally, litter items being presumably mistaken for natural preys or confounded in the food bowl in areas where litter accumulates (Schuyler et al., 2014). Litter is usually found in the digestive tract of necropsied individuals or observed in the faeces of living individuals hospitalized in rescue centres (Casale et al., 2016), generally within two weeks to one month on average (Valente et al., 2008; Darmon et al., 2016). Since the species frequently occupies the upper layers of the water column, individuals are more likely to ingest floating items rather than heavier litter (Matiddi et al., 2017; Pham et al., 2017; Darmon et al., 2017). Indeed, the litter found ingested is essentially plastics (>80%), often pieces of plastic bags or fragments of hard plastic items (Darmon et al., 2017; INDICIT Final report, 2019).

Monitoring and rescuing sea turtles have been widely developed over the sea turtles' distribution range, and more recently, increasing effort has been dedicated to the ingestion of marine litter and impact on these species. Various networks have collected data on litter ingested by sea turtles in the OSPAR-Macaronesia and Barcelona RSCs areas for several years: more than 70 of them were involved in data collection in partnership with the INDICIT project (INDICIT Final report, 2019; Fig. 1). Although no long-term standardized data sets exist, first assessments have been carried out in each of these RSCs and further data will allow finer comparisons and adjustments (INDICIT Final report, 2019). The monitoring generally concerns litter size as defined by MSFD TG-ML definition (MSFD-TGML, 2011), over 1 mm. The differentiation of the micro-items proportion (1-5 mm, Commission Decision (EU) 2017/848 of 17 May 2017) among the ingested litter, as proposed in the SPA/RAC-INDICIT standard protocol (INDICIT, 2018; SPAR-RAC-INDICIT, in press), may provide useful data for further assessment of the specific impact caused by the ingestion of litter in this size range (MSFD Criteria D10C3).

Since the first guidelines proposed by MSFD for initiating a standard procedure in monitoring debris and their impact on biota (MSFD TG Marine Litter, 2013), standardized protocols were further developed (INDICIT, 2018; RAC/SPA-INDICIT, in press). The MSFD TG ML's reference protocol should be improved by considering these protocols which should facilitate the harmonization of approaches between the Atlantic and the Mediterranean. These protocols not only measure the quantity and type of litter ingested by dead and live individuals, they also aim to describe litter impacts on sea turtles' health. This cross-sectional information may be useful for other marine environment status descriptors like MSFD Descriptors 1 (Biodiversity) or 4 (food webs) and for OSPAR assessments of threatened species status and efficiency of measures to protect endangered marine life of the North-East Atlantic.

2.2 Quantitative Objectives

The lack of long term data sets on litter ingestion in sea turtles in the OSPAR area, prevents from proposing temporal trends. However, a regular sampling of sea turtle individuals should allow an-annual reporting and an assessment of the distance to target over a 6-year period, as based on MSFD cycles. A sampling of 30 individuals is generally considered as a minimum to get powerful statistical results and should be expected to be reached per contracting party. More data from OSPAR area will allow specifying this value in the future.

While individuals are generally collected from bycatch and stranding events, all samples should be considered for analysis, since contrasted results among authors (e.g., Casale et al., 2016; Hoarau et al., 2018), underline that no clear influence of the circumstances of finding (e.g., accidental capture or stranding) on the propensity to ingest debris has been detected until now. Sampling must thus be opportunistic with an effort maintained all year-round in order to reach a sufficient sample size to detect potential changes. Thereby, more powerful analyses will be realized later with greater hindsight. Acquiring more standard data will allow considering more qualitative results and evaluating the sample size at which changes in specific litter types could be detected.

The monitoring is carried out in the OSPAR III, IV and V zones as well as in Macaronesia (Fig. 1). However, networking should be developed in Portugal and Atlantic Spain mainland, where not all the stakeholders have agreed to share existing data at the present time. The list of conditions requested for their involvement, elaborated by INDICIT project (INDICIT Final communication report, 2019), may be used for developing the monitoring network at a larger spatial scale.

Fig. 1. Stakeholders involved in 2018 in the monitoring of litter ingested by sea turtles in the OSPAR-Macaronesia area. Pictograms show rescue centres (turtle, blue), stranding network (eye, orange) and research lab (flask, rose) (from INDICIT Final report, 2019).

Although the current state of knowledge suggests that a same ecological objective can be applicable to the entire OSPAR-Macaronesia area (INDICIT Final report, 2019), more insight is needed to precisely define it. The acquisition of data is required for also evaluating the temporal trend which would reflect the efficiency of restoration measures. Regarding the main categories of litter found ingested in sea turtles, the indicator should be particularly sensitive to measures concerning e.g., plastic bags or litter from fishing activities or agriculture. Further developments are being engaged in order to assess the indicator's temporal and spatial scales more accurately (INDICIT-II project).

The reference level in a natural environment should be zero percent of turtles with litter, and thus zero grams and zero piece of plastics ingested. Since pristine area without litter does not exist, the EcoQO/GES baseline should thus be based from the minimum observed on the current situation. Other options will be considered in future experimental work to refine such a GES. Further work is thus requested for defining it precisely, supported by the collection of standard data across the OSPAR III, IV and V zones and Macaronesia, and considering harmonized approaches with MSFD and Barcelona RSCs.

2.3 Monitoring Strategy:

The parameters to be measured are i) the occurrence of litter ingestion, evaluated as the frequency of necropsied individuals with ingested litter among all individuals, and ii) the quantity of ingested litter expressed in mean dry mass per category (two decimal places) and mean number of pieces per category (two decimal places). Data are collected following a standardized approach as proposed in INDICIT and RAC/SPA protocols (INDICIT, 2018; RAC/SPA-INDICIT, in press). In these protocols, and to support research, other data are proposed to be collected optionally, in order to better characterize litter impacts. It concerns especially the mass of ingested natural food, the mass of ingested litter per size category especially the fraction micro from 1 to 5 mm and upper classes (meso/macro) or per class of colour This optional data collection also aims at better understanding the biological factors which may influence litter ingestion, and better identify the harms caused by litter on individuals' health status (sub-lethal effects and lethal doses) including entanglement (Claro et al., 2018). Such knowledge being useful for taking measures regarding biodiversity and plastics strategies, the collection of those data might be considered by OSPAR Parties.

2.4 Sampling Strategy

A protocol, adapted from the MSFD Guidelines (MSFD-TGML, 2013) has been improved by stakeholders from Atlantic and Mediterranean for supporting MSFD and RSCs monitoring programs harmonization, in the frame of INDICIT European project (INDICIT, 2018; http://indicit-europa.eu; also available in Spanish and French). This protocol has then been harmonized with the RAC/SPA protocol for the Mediterranean (INDICIT-RAC/SPA, in press). In this document, stakeholders will find the precise list of all manipulations to carry out, from the finding of living or dead individuals in the field, to the extraction, quantification and classification of ingested litter that they can record in an Observation sheet (Appendix 1). A list of equipment required for field sampling as well as for laboratory is also provided in this protocol (Appendix 2). The main procedures are explained below.

2.4.1. Costs

The time required to collect samples and extract the marine litter ingested depends on the specimen's autolysis status. An average of 5 hours with two manipulators should be considered for the collection of data from necropsied individuals, including the external and internal exam of the body. The cost of material, which may vary among countries, can be evaluated from the list provided in Appendix 2.

2.4.2. Field sampling

Authorizations. The finding of individuals can only be made by authorized people, after contacting local authorities. The conditions requested to handle a protected species may depend upon the country's rules. CITES permits are also required to move the specimen or samples from and/or to countries having ratified the Washington convention, which lists all sea turtle species in Annex 1. The finding is usually operated by volunteers or staff from NGOs or public institutions, stranding networks, rescue centres, or research or veterinarian laboratories. Depending on countries and regions, a local coordinator could be useful to organize all operations including the collection of data.

Sanitary precautions. Precautionary sanitary measures should absolutely be considered to avoid any risk of zoonosis. Therefore, data collector and manipulator shall have a kit of materials at disposal (Appendix 2), which should be provided to trained people. To be protected against splash of blood or other liquid, manipulators should wear a complete protective suit with glasses, gloves and rubber boots, which must be carefully disinfected or thrown after use. The intervention zone must be marked-off from the bystanders.

Measures. Biometric measures and a first evaluation of the body condition should be recorded on a standard observation sheet as provided in Appendix 1.

If the animal is dead and the autolysis status allows it, the species, the carapace length and a first evaluation of health status will be recorded on the spot or at a later stage in the laboratory. If the animal is alive, the local coordinator and/or the closest rescue centre should be contacted. In any case, it is important to take pictures of the location and of the animal, and of all items considered as important. When taking pictures, a decimetre (or another way) will be used to provide a size reference.

A standard code should be attributed to the individual. INDICIT (2018) recommends a standard identification, with 2 letters for the country, 2 letters for the region/Institution, the year, the month, the day, the individual's arrival number, separated with "_". For example, FR_GR_2017_03_12_9 corresponds to the 9th individual welcomed by the rescue centre of le Grau du Roi in France, the 12nd March 2017.

2.4.3. Spatial and temporal sampling

Sampling should be opportunistic with an effort maintained all year-round thanks to stranding networks' vigilance.

2.4.4. Pre-treatment and storage

Pre-treatment

Examination of specimens. An external and internal examination of both dead and living specimens, is requested for assessing the current health status of the individual, describing injuries, and specifying if ingestion of litter could be the cause of mortality e.g., occlusion, perforation) or morbidity.

For dead animals, a necropsy will be performed, except if the animal is too putrefied (Status 5, INDICIT, 2018; RAC/SPA-INDICIT, in press). The manipulation should ideally be done in a veterinary surgery with full professional equipment.

The procedure for opening the coelomic cavity and extract the litter from the 3 sections of the digestive tract, is detailed in the INDICIT (2018) or the RAC/SPA-INDICIT (in press) protocols. A tutorial for realizing the external and internal exam of a sea turtle and extracting the digestive tract is available in French ("external and internal examination of the turtle"; EPHE, 2017). Another tutorial in English, showing the main manipulations of dead individuals, from the discovery in the field to the necropsy then the extraction of the ingested litter (INDICIT, in press).

Sanitary precautions have also to be taken after necropsy. In laboratory, all soiled disposable equipment shall be thrown into dedicated and closed hermetically bags before incineration by specialized teams. All non-disposable equipment (suit, boot, scissors, sieves, beakers, tubes, necropsy table, etc.) shall be carefully washed with anti-bacterial agent. The material must be cleaned between two necropsies, and the carcass shall be disposed in hermetic double bag before being taken over.

For living animals. The collection of faeces should be made on a daily basis, considering only individuals admitted in rescue centres from at least 1 month and up to 2 months (INDICIT, 2018). The faeces samples are collected during a 1 month-period for evaluating the quantity of ingested litter. The number of current available data from living turtle was not yet sufficient to evaluate EcoQO/GES scenarios from such protocol. Nevertheless, collecting basic and optional parameters can enable acquiring additional knowledge on litter impacts on sea turtles and later define baselines from this approach. The procedure to collect the faeces and extract the litter is detailed in the INDICIT protocol (2018).

Storage

For dead specimens, if the post mortem examination cannot be processed immediately, the entire body or the digestive tract extracted should be kept in a freezer at -20°C. All samples (e.g., faeces, digestive sections) can be placed in plastic bags and frozen at -20°C if further analyses have to be performed later. All samples should be identified using a permanent marker into a double bag. A standard identification is highly recommended, with 2 letters for the country, 2 letters for the region/Institution, the year, the month, the day, the individual's arrival number, as well as the section (Eso/Stom/Intest/Faeces), separated with "_". For example, FR_GR_2017_03_12_9_Faeces corresponds to the faeces of the 9th individual welcomed by the rescue centre of le Grau du Roi in France, the 12nd March 2017. FR_GR_2017_03_12_9_Eso corresponds to the oesophagus of the 9th individual found by the rescue centre of le Grau du Roi in France, the 12nd March 2017.

2.4.5. Analytical procedure

For each digestive section of necropsied individuals or for the whole faeces samples of live individuals, and for each of the 1-5 mm and >5 mm fractions, all anthropogenic materials shall be separated from natural elements, then classified according to categories specified in Table 1. This classification, adapted from MSFD guideline (MSFD TG Marine Litter, 2013) by INDICIT project (INDICIT, 2018), proposes to describe the ingested litter among categories, especially detailed for plastic, which is the main type of litter found ingested in sea turtles (Darmon et al., 2019). It is recommended to further characterize the items found ingested, especially for the fragment category (USE FRAG).

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 threadlike	USE THR	Threadlike materials, e.g. pieces of nylon wire, net-fragments, woven clothing…
		Use foam	USE FOA	All foamed plastics e.g. polystyrene foam, foamed soft rubber (as in mattress filling)…
		Use fragment	USE FRAG	Fragments, broken pieces of thicker type plastics, can be a bit flexible, but not like sheet like materials.
		Other Use plastics	USE POTH	Any other plastic type of plastics, including elastics, dense rubber, and cigarette filters, balloon pieces, soft air gun bullets… Specify in the column "Notes".
	Litter other than plastic		OTHER	All non-plastic rubbish and pollutant
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.)

Table 1: Classification of litter and natural elements (From INDICIT, 2018; RAC/SPA-INDICIT, in press, modified after MSFD TG Marine Litter, 2013)

For each live or dead individual, the presence of litter is noted and the dry mass is evaluated for each natural and litter category. Ideally, further optional parameters should be collected to better evaluate the impacts of plastics, such as the number of pieces (all observed pieces) per category, the total number of pieces per colour (white/transparent, dark or light coloured) and the total volume of ingested plastics (RAC/SPA-INDICIT, in press). The mass is reported in grams with a precision of 2 decimals, as the volume in millilitres. Quantities inferior to 2 decimals are noted 0.001 g or 0.001 mL to differentiate from 0.

2.5 Quality assurance/ Quality Control

The standardized procedures to extract the material from necropsies or faeces, have been tested and validated by INDICIT partners and numerous stakeholders. The data are validated by the person in charge of the data gathering or during their record in the dedicated platform in which drop menus are proposed.

2.6 Data reporting, handling and management

A data entry software coupled with administrative architecture is under development in the framework of Interreg project CLEANATLANTIC to store data collected within monitoring programs on the impact of Marine Litter on sea turtles. This data management process (Figure 2) aims at having a harmonized and structured data with common referential, QA/QC procedures in a sustainable database for all partners from the North-East Atlantic region that are involved in monitoring.

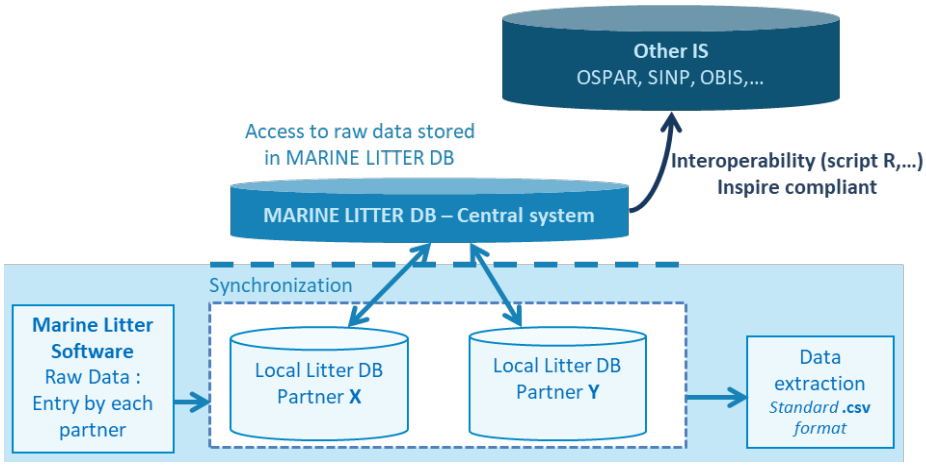

Fig.4. Data management representation

In this collection system, data is managed at local level and is synchronized with a central system. Moreover, data from each partner can be hidden to other partners and a moratorium can be applied on dataset.

With a high level of consistency within each region, data can then be made widely available, also compatible to existing mechanisms (e.g. MSFD, OSPAR/ICES databases and EMODNET). Structure of the data collection system implies the use of:

1) A referential that any user could rely on (e.g. sampling equipment, persons/organisms, parameters…)
2) Common Programs and monitoring strategies
3) Survey / Sampling information describing each sample
4) Quintuplet PMFMU (Parameter Matrix Fraction Method Unit) for each result
5) A storage process under Quality Control
6) User Profiles definition

Two options are proposed for the administrative architecture of the central database, depending on organism IT abilities (i.e. operating team, database server, Oracle License) (Table 2 : Central system architecture proposalsTable).

Table 2 : Central system architecture proposals

Operating team ?	Database server (and administrator) ?	Oracle License ?	Solution #
Yes	**Yes**	**Yes**	1) Replicate existing architecture
Yes	**Yes**	*No*	2) Adapt existing architecture to work with PostgreSQL

The first option is a transfer of the central architecture under use at IFREMER. In that case, each institution will need a trained staff, a database server and an Oracle License. Minor software developments should be required under this option.

The second option offers an adaptation of the existing architecture to use a PostgreSQL central Database. In that case, each institution will need a trained staff, an operating team and a strong IT structure using an open source software. Advanced software development will be necessary under this option.

The following tables summarize both advantages and disadvantages for each of the options (Table) and required developments (Tables 3 and 4).

Table 3: Proposal advantages/disadvantages

	Option	Advantages	Drawbacks
1	Transfer of Central (IFREMER) architecture	• already in place, reduced software development	• Need a strong IT structure • Need a skilled staff • Not only open source (Oracle SGBDr)
2	Adapted architecture using a PostgreSQL central Database	• Open source software • Could be deployed for each different partners	• Need advanced software development • Need a strong IT structure • Need a skilled staff

Table 4 : Development and requirements needed

Needed developments	Option 1	Option 2
Create new Administration Tool	••	••
Adapt existing Data Storage Tool	•	••
Requirements	**Option 1**	**Option 2**
IT structure	••	••
Skilled staff	•	•
Oracle Licence	•	-
Agreement with central system (Ifremer)	••	••

Implementation of these options will require assistance and support.

Figures 3, 4 and 5 show examples of data storage tool under development:

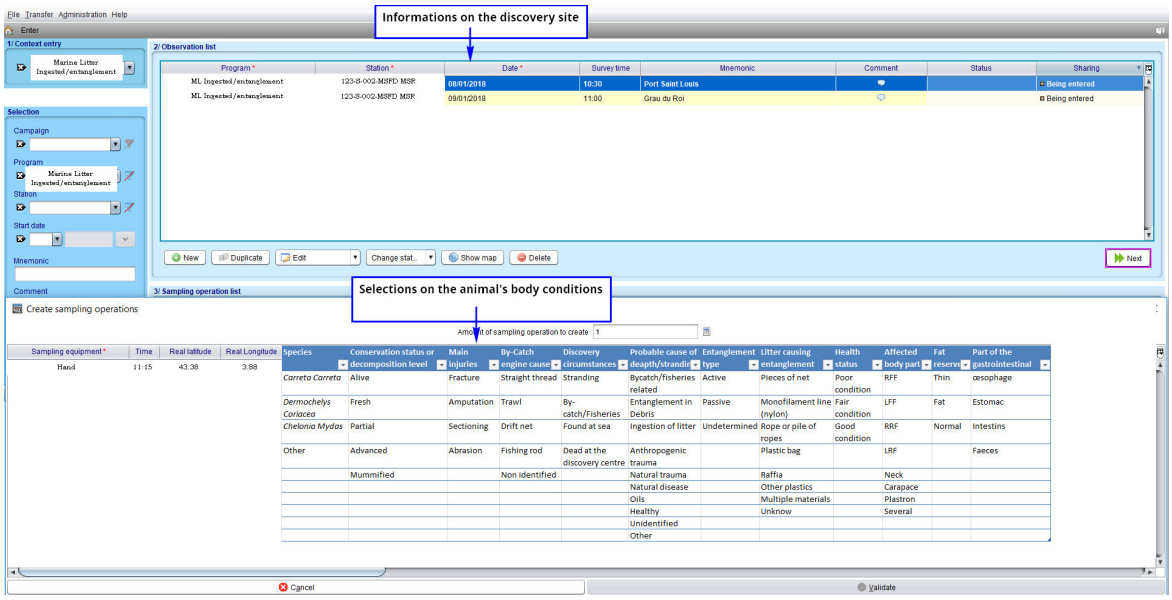

Figure 3: Information on sites discovery on the top & animals body conditions selected thanks to drop menus

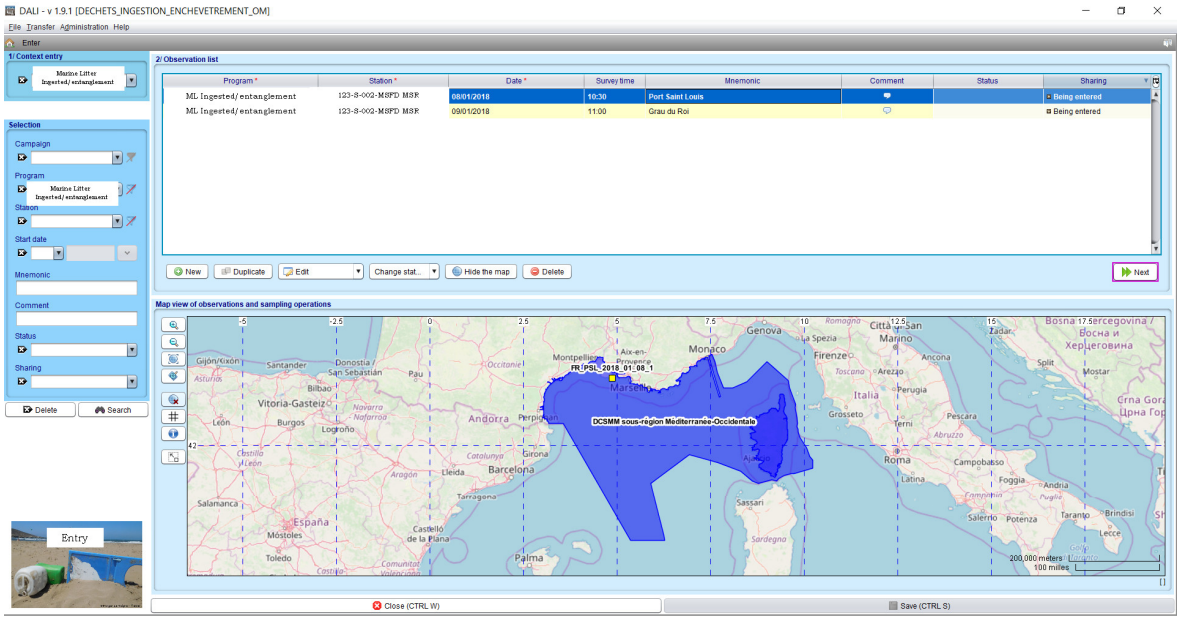

Figure 4: Discovery sites coordinate Control

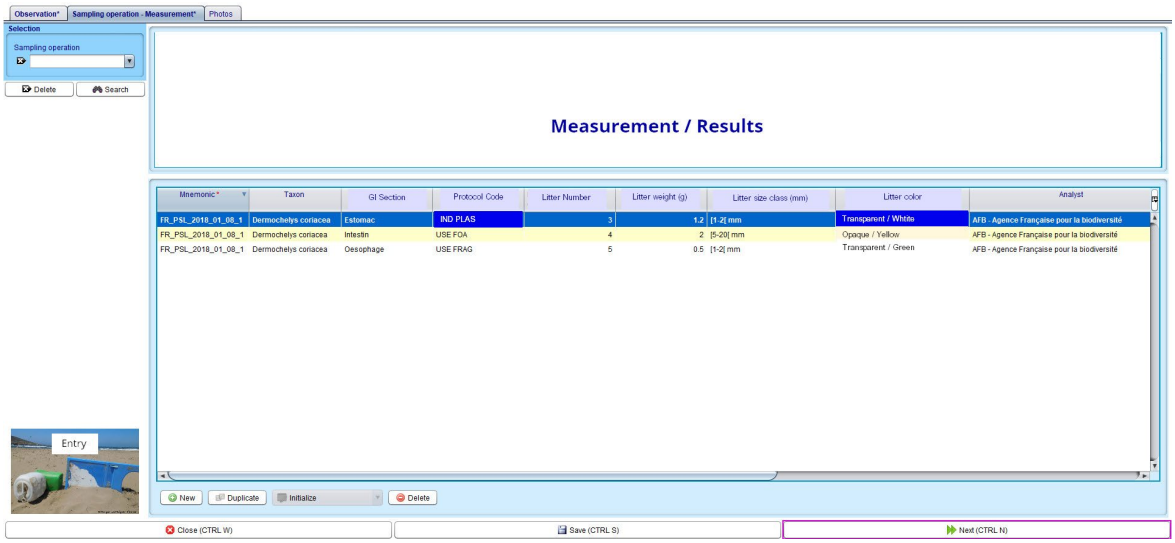

Figure 5: Results - Type (link to code protocol)/Number/Weight/Size/Color of Litter measured in each GI section

3 Assessment

3.1 Data acquisition

Raw data can be analysed to evaluate litter ingestion using the following main parameters:

- The occurrence of litter ingestion, or specifically of plastic ingestion, at the population level for the species concerned. It is calculated from the presence/absence noted per individual, as the percentage of individuals having ingested litter according to the sample size. This can be evaluated per country and averaged for the entire region.
- The mean dry mass of ingested litter in the population, evaluated per country or for the entire region. It corresponds to the arithmetic mean of the sum of masses for all categories of Plastic litter (Table 1) per individual. The standard error is generally provided.
- The mean number of pieces of ingested litter in the population, evaluated per country or for the entire region. It corresponds to the arithmetic mean of the sum of masses for all categories of Plastic litter (Table 1) per individual. The standard error is generally provided.

Other data (INDICIT's optional parameters, INDICIT, 2018, SPA/RAC-INDICIT, in press) can inform on the mean ingested dry mass of litter per type (Table 1)/size/colour category and the mean dry mass of ingested natural food in the population, at the population level and either per country or for the entire region.

Spatial aggregation. The mean digestive transit duration may suppose that the ingestion of plastics represents what the turtle found during the last month. Like fulmars *Fulmarus glacialis,* who can cover large distances in a short time but are proved to be good indicator of litter impact for the OSPAR area (OSPAR, 2019), sea turtles can migrate long distances but they also may use local foraging areas, making them good indicator at the sub-region level. Each Contracting Party can assess its current situation and the distance to the target.

Temporal aggregation. For the harmonization with MSFD, data can be reported once a year and analysed over 6 years.

3.2 Preparation of data

The platform developed by CLEANATLANTIC project (see section 2.6) should help significantly reduce or eliminate the time allocated to data cleaning, thanks to drop menus (a dedicated data manager is needed so far).

3.3 Assessment criteria

As stated in 2.2., the reference level should be based on the cleanest area assessed from the current situation evaluated over the last 6-year period. However, further work is needed on the basis of more data and knowledge for defining precisely the assessment criteria.

3.4 Spatial Analysis and / or trend analysis

Further analysis is requested for defining spatial analysis and/or trend analysis. Data collected from the 1990's in OSPAR area (Darmon et al., 2016; Pham et al., 2017; INDICIT final report, 2019) will be analysed for a better understanding of the factors which may influence litter ingestion in sea turtles, refine the spatial scale and assess temporal tendencies.

4 Change Management

The current availability of datasets is low in certain areas, despite the availability of specimens. Engaging stakeholders in Portugal and Spain mainland is recommended. Various stakeholders have already been trained to collect individuals and data on litter impacts in a harmonized way. Conditions for stakeholders to be involved permanently in a long term monitoring, i.e. data sharing agreements and long term funding availability are recommended to be examined by contracting Parties. Furthermore, the spatial configuration of certain areas like archipelagos and remote islands, may also be a constraint to collect samples that should be taken into consideration when building the monitoring strategy. The reinforcement of capacities, through training sessions, workshops and the development of web platforms, are recommended for supporting the dissemination of the standard procedures to local stakeholders.

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* INDICIT final report and final communication report should be edited in the coming months 2019.

APPENDIX 1 – OBSERVATION SHEET (From INDICIT-RAC/SPA, in press)

OBSERVATION SHEET

Llitter ingestion in sea turtles

UNEP SPA/RAC — INDICIT

COLLECTOR | **LOCAL CODE**

INSTITUTION | **Contact:**

Discovery circumstances:

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

INDIVIDUAL TAG: Tag number: | Electronic chip nº:

DATE OF DISCOVERY / / (dd mm yyyy) **INDIVIDUAL CODE** - - - - - (CC/DC/CM RR (Region) YY MM DD nº)

LOCATION (Precise locality, Region, Country) **X COORD** (WGS 84 or specify) **Y COORD** (WGS 84 or specify)

CIRCUMSTANCES OF DISCOVERY	BYCATCH ENGINE CAUSE	CAUSE OF DEATH / STRANDING	ENTANGLEMENT TYPE
☐ Bycatch/Fishery	☐ Longline	☐ By-catch/Fisheries	☐ Passive ☐ Bycatch ☐ NR
☐ Stranding	☐ Trawl	☐ Entanglement in debris	
☐ Dead at rescue centre	☐ Drift net	☐ Ingestion of litter	
☐ Found at sea	☐ Fishing rod	☐ Antrophogenic trauma	
☐ Other	☐ Other	☐ Natural trauma	
☐ NR	☐ NR	☐ Natural disease	
		☐ Oils	
		☐ Healthy	
		☐ Other	
		☐ NR	

LITTER CAUSING ENTANGLEMENT
☐ Fishing net (N)
☐ Monofilament lines (L)
☐ Rope/s ®
☐ Raffia (Rf)
☐ Plastic bag (Pb)
☐ Other (Ot)
☐ NR

PICTURES ☐ Yes ☐ No

Picture names:

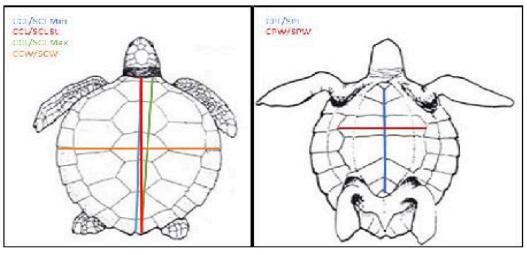
Animal body condition:

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



NOTES AND REMARKS (Discovery, animal conditions, severity of injuries, description of the material if entanglement, causes of death...):

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, perforation, presence of oil, etc.):	***ARRIVAL DATE*** / /
	DEPARTURE DATE / /
	DEAD DATE / /
DIGESTIVE TRACT (note the presence of any infection, suspect colour, fluid effusion, perforation, presence of oil, etc.):	***TURTLE BEHAVIOUR AND TREATMENTS:***

Capacities of digestive tract section and gut content

	Full				*Empty*			
	mass	*Vol (V1)*	*vol (V0)*	*V1-V0*	*mass*	*Vol (V1)*	*vol (V0)*	*V1-V0*
Oesophagous								
Stomach								
Intestines								
Total								

Marine debris measurements:

	OESOPHAGOUS		*STOMACH*		*INTESTINE*		*FAECES*	
	DRY MASS	*NUMBER*	*DRY MASS*	*NUMBER*	*DRY MASS*	*NUMBER*	*DRY MASS*	*NUMBER*
Ind. Plastic								
USE she								
USE thr								
USE foa								
USE frag								
Other (USE Poth)								
Non plastic								
Foo (nat. Food)								
Nfo (nat. no food)								
TOTAL								

TOTAL DEBRIS		*NUMBER OF PIECES*		*NUMBER OF PIECES*	
dry mass (0.01 g)		**micro (1-5mm)**		*white-transparent*	
number of pieces		*meso (5-25mm)*		*dark coloured*	
volume (0.01 ml)		*macro (>25mm)*		*light coloured*	

NOTES AND REMARKS (Necropsy, faeces collection and debris measurements; please describe plastic fragments (USE FRAG) items):

APPENDIX 2 - LIST OF MATERIAL (From INDICIT-RAC/SPA, in press)

I. For the examination of the animal and the collection of samples at the discovery site

This material could be part of the tool kit to provide to stakeholders during training sessions.

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	

II. For the collection of samples on dead individuals and the extraction of the ingested litter from the digestive tract

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

III. For the collection of samples on live individuals and the extraction of ingested litter in faeces

Freezers (-20°C)	
Proofer (not mandatory)	
Garbage bags	
Glasses and protective mask (optional)	
Gloves	
Camera	
Pen	
Observation sheet	
Permanent marker	
Measuring tape	
Sliding caliper	
Permanent marker	
Containers for samples (tubes/zipped bags)	
Sieve with 1 mm mesh	
Sieve with 5 mm mesh (optional – for the study of the ingested micro-plastics (1-5 mm))	
Measuring cylinders (10 ml, 25 ml, 50 ml) (optional)	
Decimeter (optional)	
Precision balance (0.01 g)	
Binocular (optional)	
Filtration grids with 1 mm mesh (at the levels of water arrival and discharge)	
Landing net with 1 mm mesh	