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Meeting of the Ecosystem Approach Correspondence Group on Marine Litter Monitoring

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Agenda Item 3: Data Standards (DS) and Data Dictionaries (DD) for IMAP Ecological Objective 10 (EO10) Common Indicator 24

Regional Operational Strategy for Monitoring IMAP Candidate Indicator 24

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Meeting of the Ecosystem Approach Correspondence Group on Marine Litter Monitoring (CORMON Marine Litter)

Videoconference, 30 March 2021

Agenda item 5: Regional operational strategy for monitoring IMAP Candidate Indicator 24

Regional Operational Strategy for Monitoring IMAP Candidate Indicator 24

UNEP/MAP
Athens, 2021

Note by the Secretariat

During COP 19 (Athens, Greece, 9-12 February 2016), the Contracting Parties to the Barcelona Convention adopted the Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP).

Marine litter is addressed under the 10th Ecological Objective (E010) of IMAP. It is monitored through two Common Indicators (i.e. CI22[1] and CI23[2]) and a Candidate Indicator (CI24). IMAP Candidate Indicator 24 addresses the "Trends in the amount of litter ingested by or entangling marine organisms, focusing on selected mammals, marine birds and marine turtles" (Decision IG22/7, COP19).

UNEP/MAP and its MED POL Programme, together with the Regional Activity Centre for Specially Protected Areas (SPA/RAC) implemented several activities in the framework of the EU-funded Marine Litter MED Project (2016-2019) to develop IMAP Candidate Indicator 24. The report "Defining the most representative species for IMAP Candidate Indicator 24" (UNEP/MED WG.464/5), puts forward *Caretta caretta* as a reliable bio-indicator for monitoring the ingestion of, and entanglement in marine litter. It recommends developing methodologies and networks to collect standardized data. Workshops on marine litter impacts and special training sessions on collecting samples and data on litter ingestion by sea turtles were organized over the two-year period of 2017–2018 in collaboration with the EU-funded INDICIT project (2017–2019). These efforts culminated in the development of a common protocol for monitoring marine litter ingested by sea turtles and impacts of this litter on their health.

The aim of the present document is to provide practical guidelines to the Contracting Parties in designing and developing monitoring programmes to collect standardized data on marine litter ingested by sea turtles, with the aim of achieving GES. The document provides the following information:

a) A presentation of the tools required to collect and record standardized data on litter ingestion by sea turtles;
b) A review of the existing networks and methods for collecting specimens, processing samples, and collecting and analysing the data;
c) An evaluation of the current impact of litter on sea turtles, as well as the indicator criteria and proposed methods to assess GES targets; and
d) An assessment of the implementation costs and recommendations to help operationalize a monitoring programme.

The document is submitted for review by the Meeting of the Ecosystem Approach Correspondence Group on Marine Litter Monitoring for endorsement and further submission to the Meeting of the MED POL Focal Points planned in May 2021.

[1] *"Trends in the amount of litter washed ashore and/or deposited on coastlines, i.e. Beach Litter"*
[2] *"Trends in the amount of litter in the water column, including micro-plastics, and on the seafloor"*

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List of Abbreviations/Acronyms

CI	Candidate Indicator
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
COP	Meeting of the Contracting Parties
CORMON	Ecosystem Approach Correspondence Group on Monitoring
EC	European Commission
EcAp	Ecosystem Approach to the management of human activities
EO	Ecological objective
GES	Good Environmental Status
IMAP	Integrated Monitoring Assessment Programme
INDICIT	Implementation of Indicators of Marine Litter on Sea Turtles and Biota in Regional Sea Conventions and Marine Strategy Framework Directive Areas project
INSTM	National Institute of Marine Sciences and Technologies (Tunisia)
ISPRA	Italian Institute for Environmental Protection and Research (Italy)
LBS Protocol	Protocol for the Protection of the Mediterranean Sea against pollution from Land-Based Sources and activities
LRSE	Environmental Monitoring Network Laboratory (Algeria)
MAP	Mediterranean Action Plan MED POL Programme for the Assessment and Control of Marine Pollution in the Mediterranean Sea MED QSR Mediterranean Quality Status Report
MEDPOL	Mediterranean Pollution Assessment and Control Programme
MSFD	Marine Strategy Framework Directive
QSR	Quality Status Report
RPML	Regional Plan on Marine Litter Management
SPA/RAC	Regional Activity Centre for Specially Protected Areas
UNEP/MAP	United Nations Environment Programme / Mediterranean Action Plan

1. INTRODUCTION

1. Marine litter causes major harm to the environment. This is especially the case in the Mediterranean Sea, where items accumulate due to its semi-enclosed configuration and the high rate of coastal urbanization around the basin (MED QSR, 2017; Mansui et al., in press). Marine litter, mainly consisting of plastics, is known to severely impact fauna, particularly as a result of ingestion and entanglement (INDICIT consortium, 2018a). Interaction with marine litter can lead to direct mortality, but more generally results in an individual's decreased capacity to reproduce and survive by weakening body condition and altering feeding and moving behaviour.

2. Marine litter has been an issue of concern since the 1970's for the Barcelona Convention, so that Mediterranean countries adopted the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-Based Sources and Activities (LBS Protocol) since 1980 and amended it in 1996. During the 18th Meeting of the Contracting Parties (COP 18) in 2013, United Nations Environment Programme (UNEP)/ Mediterranean Action Plan (MAP) adopted the legally-binding Regional Plan on Marine Litter Management (RPML; Decision IG.21/7) with the aim of tackling the threat caused by marine litter. RPML establishes a set of programmes of measures along with implementation timetables in order to monitor, prevent and reduce the adverse effects of marine litter on the marine and coastal environment.

3. In 2016, the COP 19 adopted the Integrated Monitoring and Assessment Programme (IMAP) of the Mediterranean Sea and Coast and Related Assessment Criteria (Decision IG 22/7) and in 2017, the COP 20 approved the revised reporting format for the implementation of the Barcelona Convention and its Protocols.

4. Since 2013, UNEP/MAP and its Mediterranean Pollution Assessment and Control Programme (MED POL) and the other MAP components have implemented numerous actions for supporting the Contracting Parties' efforts for setting up a series of measures which will progressively evolve till 2025.

5. All Contracting Parties to the Barcelona Convention have established or are currently in the final phase of finalizing the development of marine litter monitoring programmes, and also identifying the designated competent authorities responsible for monitoring the different marine litter IMAP indicators. The Contracting Parties to the Barcelona Convention are supported by UNEP/MAP and MED POL for implementing their respective national marine litter monitoring programmes, e.g. through capacity-building workshops and the development of practical tools (monitoring protocols and assessment methods, metadata and reporting templates, national monitoring schemes and guidance factsheets).

6. In the Mediterranean, the loggerhead turtle *Caretta caretta* (Linnaeus,1758) has been proposed as the most appropriate species for this indicator, taking into account the mature monitoring methodologies and protocols. Indeed, the occurrence and quantities of marine litter ingestion are particularly high in the loggerhead turtle and would be highest in the Mediterranean Sea (Dell'Amico and Gambaiani, 2013; Darmon, INDICIT consortium, Miaud, 2019). In addition, its wide distribution and the extensive existing networks for collecting specimens and data on litter ingestion by this species make it a good candidate for evaluating the impacts of litter in various marine compartments and at a large spatial scale. In a lesser extent, the green turtle *Chelonia Mydas* being also regularly entountered in the Mediterranean, can also be used for IMAP Candidate Indicator 24, the networks and standard methodologies being the same than those employed for the loggerhead turtle. The aim of the present document is to provide practical guidelines to the Contracting Parties to the Barcelona Convention to support them in designing and developing monitoring programmes to collect standardized data on marine litter ingested by sea turtles, and to assess how GES can be achieved. It

provides details concerning the necessary steps and methods to implement a monitoring programme and includes: (1) a presentation of the tools required to collect and record standardized data on litter ingestion by sea turtles; (2) a review of the existing networks and methods for collecting specimens, processing samples, and collecting and analysing the data: (3) an evaluation of the current impact of litter on sea turtles, as well as the indicator criteria and proposed methods to assess GES targets; and (4) an assessment of the implementation costs and recommendations to help operationalize a monitoring programme.

2. MONITORING STRATEGY

2.1. Protocol for collecting standardized data on litter ingestion by sea turtles

7. Several tools exist to support the collection of standardized data on marine litter ingestion by sea turtles. These explain the procedure to assess the occurrence of marine litter ingestion or entanglement in marine litter, as well as the impact on the individual's health.

8. The first methodologies for extracting marine litter ingested by dead or live individuals were developed in Italy and incorporated into the European Marine Strategy Framework Directive (EU MSFD) guidelines (Matiddi et al., 2011; MSFD-TG ML, 2013) and later applied along the Italian, Spanish and French coasts (Camedda et al., 2014; Darmon & Miaud, 2016; Matiddi et al., 2017; Domenech et al., 2018). The protocol was consolidated in the framework of the European INDICIT project and harmonized with the protocol developed by SPA/RAC in the framework of the EU-funded Marine Litter MED project. The consolidated document (SPA/RAC-UNEP/MAP, INDICIT, 2019) is available in both English and French and is the most up-to-date protocol on the subject.

2.1.1. Technical requirements

9. Sea turtles are protected species, therefore handling live or dead animals or parts of these animals requires special authorization. If and animal is found dead or alive, its handling and recovery should be reported to and coordinated with the responsible authorities. A CITES permit is required if a specimen or sample has to be sent/received between countries. Moreover, sanitary precautions must be taken when handling dead or live wild animals to minimize the risk of infectious diseases, in particular zoonotic diseases. Thus, those responsible for data collection should be trained to handle sea turtles and extract ingested marine litter (see section 2.1.2. Guidelines).

2.1.2. Guidelines

10. For dead sea turtles, standardized procedures for collecting data are exposed in SPA/RAC-UNE/MAP, INDICIT protocol (2019). For living sea turtles, a protocol is also proposed in this document for evaluating the marine litter excreted through the faeces from individuals monitored at rescue centres is also. However, it is less uniform as it requires to consider constraints in the process of data collection and should also depend on the level of competence of the rescue centre. It is nonetheless recommended to collect data from living individuals in order to refine the procedure and acquire more knowledge on the impact of marine litter ingestion on health, so that the GES can later be evaluated with the aid of this approach.

11. The protocol recommends collecting a set of so-called 'basic' and 'optional' parameters. The basic parameters are the minimum data fundamental to assess the occurrence and quantity of marine litter ingestion in sea turtles. The optional parameters provide more knowledge regarding the characteristics of the ingested marine litter and the impacts of its ingestion on an individual's health. It is highly recommended to collect these optional parameters in order to better understand the factors leading to marine litter ingestion, which will later allow a more accurate assessment of the indicator's biological constraints. It is also recommended to take pictures regularly, throughout all the steps of the

procedure, with a reference of measurement to indicate the order of magnitude (e.g. a measuring tape).

12. To allow reuse and easy cleaning, the protocol can be printed out and laminated. The SPA/RAC-INDICIT protocol (SPA/RAC-UNEP/MAP, INDICIT, 2019) provides an 'Observation sheet' for recording data during handling. The checklists of materials are provided at the end of this document (for handling in the field or in the laboratory or rescue centre) (see Appendix 1 - Checklists of materials).

13. The protocol details each step for recording data on marine litter ingestion:

i) Recovering the animal: describing the location and circumstances of the discovery and the initial assessment of the individual's body condition.
ii) Extracting the marine litter ingested by the animal:
    a. dead individuals: performing a necropsy in an authorized service centre and extracting the digestive tract. marine 2019);
    b. live individuals: collecting the faeces excreted by an individual for at least 1 month and ideally 2 months from the individual's arrival at the rescue centre (individuals living the rescue centre before 1 month are excluded from the analyses).
iii) Evaluating the possible impacts of marine litter on the individual's health and body condition through external observation, as well as an internal diagnostic during a necropsy (on a dead individual).
iv) Classifying and quantifying the marine litter found ingested (same procedure for dead and live individuals).
v) Recording the data in a standardized database (Excel file) with a specific tab for data extracted from necropsies and another tab for live individuals.

14. When an animal is recovered, it should be attributed with an identifier. The protocol recommends using a standard identifier in order to facilitate research and the potential sharing of data or samples between different institutions. It suggests using a two-letter country code, followed by a two-letter region/institution code, followed by the year, the month, the day, the individual's arrival number, as well as the part of the digestive tract analysed (Oeso/Stom/Intest/Faeces), each separated with an underscore (e.g. FR_GR_2017_03_12_9_Oeso, for the oesophagus of the 9th individual arrived in the Grau du Roi rescue centre in France on 12 March 2017). This ID should be written on all samples before storing them for later analysis in a freezer at -20°C.

15. The body conservation status of individuals will determine possible handling. The status should be attributed on an initial assessment of body condition as one out of five levels: live individuals (1), and then from recently dead/little decomposed (2) to mummified or missing part of the skeleton or body (5). It is considered that marine litter can be extracted and described for levels 2 and 3. Level 4 allows biometric data and the presence/absence of ingested plastic to be measured (to evaluate the frequency of marine litter ingestion) and possibly entanglement, but it may be not useful for determining GES. In level 5 individuals, which have usually lost their gastrointestinal material, the detailed analysis of marine litter ingestion is not possible. If a living turtle (level 1) dies during the care period at a rescue centre, the marine litter already excreted and collected should be included in the dataset for necropsied turtles.

16. The aim of the protocol is to evaluate the occurrence of marine litter ingestion and the characteristics of the ingested marine litter. Optional parameters related to entanglement are also provided, to collect standardized data that would allow the development of a specific indicator for impacts related to entanglement (Claro et al., 2018). This data should describe the type of marine litter involved and consider the impact on the individual's health, which would enable accurate analyses for

defining this indicator. If the marine litter is related to fishing activity, passive entanglement caused by fishing gear discarded at sea should, if possible, be differentiated from bycatch resulting from active fishing.

17. The protocol for the description of ingested marine litter is the same for dead and live turtles. All marine litter fragments over the size of 1 mm should be collected. This was originally based on the guidelines for fulmar seabirds in the OSPAR area, which recommended evaluating marine litter fragments over 1 mm, and this definition was retained for sea turtles in the EU MSFD guidelines (Matiddi et al., 2011; MSFD guidelines, 2013). The new protocol recommends separating marine micro-litter (from 1 to 5 mm) from marine macro-litter (> 5 mm).

18. In this analysis, both natural (remains from the turtle's diet) and manmade items in the digestive tract should be collected after rinsing them with running water over two filters (a 1-mm filter and a 5-mm filter). Marine litter is defined as items that have been made or used by people and deliberately discarded or unintentionally lost at sea or on beaches (Commission Decision 2010/477/EU). It includes any persistent, manufactured or processed solid material, but does not include semi-solid remains of, for example, mineral and vegetable oils, paraffin or chemicals, which should be included in the category 'Other'. For further evaluation, these items can be described in detail in the 'Notes' column.

19. The EU MSFD guidelines (2013) provide a standard classification for marine litter ingested by fulmars and sea turtles. This list was then simplified by the INDICIT consortium in collaboration with stakeholders involved in the collection of specimens and data to focus specifically on the plastic categories most often found ingested by sea turtles (Table 1). The marine litter should be categorized visually or with the help of a binocular loupe or magnifying glass in case of uncertainty. Fishing hooks, which are regularly found ingested by sea turtles, should not be classified as 'marine litter' as these individuals are considered longline victims (bycatch). Nevertheless, the presence of a hook should be recorded in the 'Notes' section. In this column it is also recommended to specify the type of ingested marine litter for potential further evaluation by programmes monitoring specific types of plastic (Darmon, INDICIT consortium, Miaud, 2019).

20. The abundance of marine litter by category should be assessed, as well as categories of natural items (food remains and non-food items; Table 1). The dry mass per category should be measured, as well as totalled for all plastic items, to a precision of 0.01 g. Other measures such as the number of fragments (i.e. all pieces counted), the number of items (i.e. after assembling the fragments of a single object) and the volume are proposed as optional parameters.

Table 1: Classification of marine litter items, food remains and natural non-food remains (from the SPA/RAC-UNEP/MAP, INDICIT, 2019)

TYPE	CODE	DESCRIPTION
Industrial plastic	**IND PLA**	Industrial plastic granules: usually cylindrical but also sometimes oval, spherical or cubical shapes
Used sheeting	**USE SHE**	Remains of sheeting, e.g. from bags, cling film, agricultural sheeting, rubbish bags
Used thread	**USE THR**	Threadlike materials, e.g. pieces of nylon lines, net fragments, fibres from woven clothing
Used foam	**USE FOA**	Foam plastics, e.g. polystyrene, foam/sponge rubber (as in mattress filling, etc.)
Used fragments	**USE FRAG**	Broken pieces of thicker plastics: may be a bit flexible, but not thin, sheet-like materials
Other used plastics	**USE POTH**	Any other type of plastics: elastics, dense rubber, cigarette filters, balloon shreds, airgun pellets, etc. (specify in the column 'Notes')
Marine litter other than plastic	**OTHER**	All non-plastic rubbish and pollutants

TYPE	CODE	DESCRIPTION
Natural food items	FOO	Natural foods for sea turtles (e.g. pieces of crab, jellyfish, algae, etc.)
Natural non-food items	NFO	Anything natural that is not considered a normal nutritious food for sea turtles (stone, wood, pumice, etc.)

2.1.3. Video tutorials for collecting data from a necropsy

21. A video tutorial[3] (in French) describes all the steps of a necropsy of a loggerhead turtle with the objective of collecting data on ingested marine litter and its impacts. It includes an external and internal evaluation of the turtle's body condition, the extraction of the three sections of the digestive tract, and the evaluation of its digestive capacity (Darmon, Raymond, Miaud, 2017). The video also shows the extraction of other samples (e.g. liver, blood collected in the heart cavity, etc.) for possible complementary analyses.

22. 23. Matiddi et al. (2019) provides an even more complete video tutorial[4] (in English, with subtitles available in 17 languages) for all detailed procedures for the collection and dissection of a dead sea turtle, including the categorization and analysis of ingested marine litter. The article accompanying the video explains the methodology, from the recovery of the animal to the collection of standardized data on marine litter ingestion and provides proposals for evaluating GES thresholds.

Figure 1. Examples of marine litter categories.(a) IND PLA: plastic pellets and granules, (b) USE SHE: thin plastic materials, such as plastic bags, agricultural sheeting or cling film, (c) USE THR: ropes, filaments, fibres and other threadlike materials, (d) USE FOA: polystyrene foam or foam/sponge rubber, (e) USE FRA: fragments of hard plastics, (f) USE POTH: any other plastic items, including elastics, dense rubber, balloon shreds or airgun pellets, (g) OTHER: any non-plastic marine litter such as cigarette butts, newspapers, rubbish and hard pollutants, (h) FOO: remains of the turtle's natural diet (from Matiddi et al., 2019).

[3] https://www.canal-u.tv/video/ephe/examen_externe_et_interne_d_une_tortue_caouanne_caretta_caretta_film_tutoriel.52557
[4] https://www.jove.com/video/59466/data-collection-on-marine-litter-ingestion-sea-turtles-thresholds-for

2.2. Sampling

2.2.1. Spatial coverage

23. The sampling effort should target the largest spatial area possible, or representative areas should be selected if rescue/stranding networks do not cover the entire national waters. Dead sea turtles are generally collected on beaches (from stranding) or at sea, typically after accidental mortality from longline fishing (bycatch) or boat collisions.

2.2.2. Survey frequency

24. Continuous opportunistic sampling is required. A minimum sample size of 50 turtles per year and per country is recommended to obtain annual averages for a particular area, although a larger standard dataset is required to accurately assess the minimum sample size for detecting significant variation in the impact of marine litter on sea turtles in response to a specific measure (Matiddi et al., 2019). For a reliable assessment of variations in the quantity of ingested marine litter and an evaluation of the effectiveness of monitoring programmes, data over periods of 3 to 6 years is needed.

2.2.3. Cost of and recommendations for data collection

25. It is very important to work with trained experts, and it is recommended to involve veterinarians and perform necropsies in adapted centres in order to respect sanitary precautions (see section 2.1.1. Technical requirements).

26. The SPA/RAC-UNEP/MAP, INDICIT protocol (2019) provides the list of materials required for:

a. Recovering living or dead individuals in the field and taking the initial measurements;
b. Performing a necropsy and extracting the sections of the digestive tract;
c. Collecting the faeces of live individuals; and
d. Collecting standardized data on ingested marine litter and natural food remains (see Appendix 1 - Checklists of materials).

27. The distribution of a field and/or lab tool kit including the minimum materials required for safely handling the specimen should be envisaged. To respect sanitary precautions, certain materials should be disposed after use, while others could be reused if carefully cleaned with appropriate hygiene products (e.g. washable suits, boots, laminated checklists, etc.).

28. The cost for the entire procedure of monitoring marine litter in sea turtles depends on the country, the network organization, the local cost of the material, as well as the local skills and salaries of the involved staff. To estimate this, calculate costs for an average of 8 hours for two employees for the collection and initial biometric measures of the individual in the field and the transport from the location where it was discovered to the authorized centre for handling. After this, about 5 hours should be considered for two handlers to collect data from a dead turtle brought to the laboratory (from the external examination of the body to the characterization of the marine litter ingested). The salary costs should thus be included in the cost estimation.

29. The intervention of an external specialized team to support stakeholders with low local capacities relative to the number of specimens to analyse could be considered. In this case, local stakeholders should carry out the necropsies and store the digestive samples in a freezer, and an external team could then help with data collection.

30. For live turtles, faeces must be collected every day. This requires a few minutes per individual turtle per day. The whole sample can then be analysed after between one to two months of

monitoring. A total of one day on average should be calculated to characterize the marine litter excreted by one individual for complete monitoring.

2.3. Data banking

2.3.1. Creation of a standard database

31. The data can be recorded in an Excel file, as in the spreadsheet proposed in the INDICIT project (https://indicit-europa.eu/protocols/), with columns for qualitative variables and a distinction between the so-called 'basic' and 'optional' parameters which appear in grey italics. Two separate sheets should be considered: one for the data collected from necropsies and the other for data collected from faeces. Each row corresponds to one individual and includes information on its characteristics, impacts related to entanglement and, in more detail, to ingestion. There is a column for other notes, e.g. for specifying the type of ingested marine litter or for more details on observed health impacts. This information is useful as it may help improve the protocol in the future.

32. The parameters in the observation sheet are presented in the SPA/RAC-UNEP/MAP, INDICIT (2019). The video tutorial in Matiddi et al. (2019) shows the procedure for recording data.

33. Following a period of data collection, data cleaning is a necessary step before statistical analyses to avoid errors. This is highly time consuming and should be included in the staff's schedule. Developing a specific platform that can be accessed by each stakeholder or by a reference body per country could facilitate data cleaning and sharing. In this case, an agreement that states the specific rights for visualizing, downloading, or using data should be signed.

2.3.2. Quality assurance/quality control

34. Due to the lack of long-term monitoring programmes previously, quality assurance/quality control (QA/QC) has only been fulfilled for the scientific results obtained in recent years (Camedda et al., 2014; Darmon, INDICIT consortium, Miaud, 2019; Matiddi et al., 2011, 2017, 2019). Ensuring QA/QC requires specific long-term monitoring programmes. It is recommended that data be validated by an expert reviewer.

2.3.3. Recommendations to facilitate data banking

35. The creation of a secure online platform is recommended. The structure could be standardized to collect data for the 'Marine litter ingestion by sea turtles' indicator between INFO-RAC, UNEP/MAP, OSPAR and MSFD. The cost evaluation should consider the time necessary for data cleaning and recording information in the database. A national reference body, trained to verify and centralize national data in an internal database, is recommended.

2.4. Capacity building for standardizing monitoring throughout the Mediterranean

36. For an accurate evaluation of the current situation and how it is changing over time, effective monitoring relies on the expertise, the extent and the reliability of the networks to collect standardized data over the entire Mediterranean basin. The capacity of these networks was evaluated in a report by the SPA/RAC (SPA/RAC UNEP/MAP, 2018) and then supplemented by the INDICIT project (Darmon, INDICIT consortium, Miaud, 2019). Other stakeholders have been contacted to complete the information on the human and material resources available in each country, as well as to evaluate local knowledge on the prevalence and level of impact related to marine litter ingestion. Details are shown in the tables in Appendix 2 – Capacity for monitoring litter impacts on sea turtles in Mediterranean countries.

2.4.1. Existing networks

37. Sea turtles recovered by Mediterranean networks are generally found stranded on beaches or as bycatch (Darmon, INDICIT consortium, Miaud, 2019). Hence, the collection of living and dead specimens relies on interaction between stranding networks and rescue centres, along with close collaboration by fishermen and coast guards. To accurately evaluate individual health and assess mortality or indirect effects caused by marine litter ingestion or entanglement, the involvement of veterinarian centres is essential. The extraction, characterization and analysis of the ingested marine litter is generally performed by research institutes.

38. As all seven species of sea turtles are listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), only authorized personnel can handle live and dead animals or parts of these. A questionnaire sent to stakeholders to evaluate their capacity to monitor the ingestion of marine litter by sea turtles reported a lack of a structured network for the collection of such data. Most countries have a network for observing/recovering stranded turtles (Appendix 2 – Capacity for monitoring litter impacts on sea turtles in Mediterranean countries), but the network is not always recognized as a national point of reference (SPA/RAC-UNEP/MAP, 2018), and many do not actively monitor marine litter impacts on sea turtles (SPA/RAC-UNEP/MAP, 2018; Appendix 2 – Capacity for monitoring litter impacts on sea turtles in Mediterranean countries). Moreover, in most cases, the networks' activities are limited geographically, and thus part of the territory is not monitored. In some countries, there may not be relationships between different centres or they may not be aware of each other's existence (SPA/RAC-UNEP/MAP, 2018). The regional/national reference centre and the SPA/RAC contact(s) are also not always known. This clearly highlights a need for more systematic organization of the networks and the development of information-sharing tools.

39. Unlike necropsies, which can be carried out by qualified personnel in veterinary laboratories, the observation of marine litter in faeces can only be done by rescue centres (Camedda et al., 2014). In the framework of the Sea Turtle Rescue Map project launched in 2016, the Mediterranean Association to Save Sea Turtles (MEDASSET) has produced a distribution map of rescue centres across the Mediterranean (Figure 2); the map was completed during the MedSeaLitter project (2016–2019) (E. Touloupaki, personal communication). As highlighted in the SPA/RAC-UNEP/MAP report (2018), there is a lack of rescue centres, especially in the southeast Mediterranean.

40. With the aim of collecting data in a more standardized way, the INDICIT consortium has created a network of 43 institutions in the Barcelona Convention area, including: (i) stranding networks, responsible for the observation and recovery of dead or live turtles (alerting the relevant centres of a discovered turtle, taking the first measurements and recovering the specimen); (ii) rescue centres, responsible for the medical care of live individuals brought in by stranding networks; (iii) transit centres, which hold recovered individuals temporarily before rescue centres take charge of them; (iv) veterinarian institutes, responsible for external examinations and necropsies, and (v) research laboratories, some of which have several roles. Other existing institutions could also be involved, especially in countries where monitoring is not yet implemented (i.e. southern and eastern Mediterranean sub-basins, Appendix 2 – Capacity for monitoring litter impacts on sea turtles in Mediterranean countries).

41. Most of the contacted stakeholders reported regularly observing the impacts of marine litter on sea turtles, either from ingestion or entanglement (Appendix 2 – Capacity for monitoring litter impacts on sea turtles in Mediterranean countries). Some also reported marine litter pollution on nesting beaches (Casale and Margaritoulis, 2010). Although not all stakeholders are familiar with the IMAP 24 indicator, most expressed interest in being part of a monitoring programme. Some stakeholders are involved in projects not directly related to marine litter, but which could provide means for working on marine litter impacts or for recovering specimens (Appendix 2 – Capacity for monitoring litter impacts on sea turtles in Mediterranean countries; e.g. DeFishGear, a derelict fishing

gear management system in the Adriatic). Several stakeholders have asked to be involved in international projects on marine litter impacts or to be connected with experts on this topic (RAC/SPE-UNEP/MAP, 2018). Aside from those already involved in a network (e.g. the INDICIT project), several institutions asked for more human and material resources for conducting regular monitoring of marine litter impacts on sea turtles, and almost all stakeholders contacted reported a need for trained experts in this (Appendix 2 – Capacity for monitoring litter impacts on sea turtles in Mediterranean countries). These institutions were often not aware of existing and available tools such as video tutorials.

42. The sampling capacity of institutions was sometimes difficult to assess, as several may take charge of different samples from the same specimen. The ability to take a specimen and conduct the entire monitoring procedure and then to extract and share standardized data is highly variable among institutions. The capacities are more developed in the northwest Mediterranean basin, where stranding networks, rescue centres, research and veterinarian laboratories are organized to recover specimens, generally have links with each other, and have been collecting data for a relatively long time.

43. Across the Mediterranean, there are gaps in the spatial coverage of monitoring capacities and in existing data on marine litter ingestion. Specifically, more knowledge needs to be acquired in the southern and eastern sub-basins. There is a lack of data from Libya to Syria, in the central Mediterranean Sea and the Levantine basin, and a need for more precise data in the Aegean and in the Adriatic. This could be achieved by creating new networks (SPA/RAC-UNEP/MAP, 2018).

44. Some institutions are already involved in monitoring marine litter ingestion by sea turtles, reporting this information to local, regional or national authorities. Furthermore, some countries are developing National Monitoring Programmes (led by national institutes) for the Marine Strategy Framework Directive, taking into account marine litter ingestion by Caretta caretta (Criteria D10C3). Other institutions began this monitoring through the INDICIT project (2017–2019), which provided training sessions on collecting and sharing data. The INDICIT-II project (2019–2021) aims to reinforce and extend these networks, considering the conditions cited by stakeholders during the first round of the project that are prerequisites for their involvement in monitoring marine litter ingestion and entanglement (e.g. sharing tools and information, increasing human and material resources, training). Similar to the map produced by MEDASSET (https://www.medasset.org/our-projects/sea-turtle-rescue-map/, INDICIT produced a Google map aimed at stakeholders showing institutions already involved in monitoring marine litter ingestion in sea turtles (Figure 3. Snapshot of INDICIT Google map (in progress) aimed at stakeholders involved in monitoring marine litter ingestion by sea turtles (from the INDICIT project).). This map shows the spatial distribution of the networks, highlighting gaps in spatial coverage. The objective is to complete it with all institutions working across the Mediterranean basin.

Figure 2. Snapshot of MEDASSET's map of sea turtle rescue and first aid centres in the Mediterranean (https://www.medasset.org/our-projects/sea-turtle-rescue-map/)

Figure 3. Snapshot of INDICIT Google map (in progress) aimed at stakeholders involved in monitoring marine litter ingestion by sea turtles (from the INDICIT project).

45. To consolidate these networks, SPA/RAC has recommended that more institutions be involved in such projects (SPA/RAC-UNEP/MAP, 2018). The workshop on marine litter impacts organized by SPA/RAC in partnership with the INDICIT project and the Archipelagos Institute of Marine Conservation during the MedTurtle Conference in Croatia in 2018 assembled over a hundred participants. Such events are valuable to disseminate tools and share knowledge.

46. Guidelines for the design of a regional network for Monitoring and assessment of IMAP CI24 in the Mediterranean have been elaborated within the framework of the Marine Litter MED Project,

where the main steps for the implementation of Mediterranean Regional Network have been developed.

2.4.2. Organization of the networks

47. Network organization is highly variable between countries, which sometimes have a reference centre or advisor at the regional and/or national level, and sometimes not. In some countries, an official stranding network does not exist, but a local organization, generally a research laboratory or a rescue centre, may act to alert relevant bodies and recover dead or live individuals in the field. The SPA/RAC questionnaire (SPA/RAC-UNEP/MAP, 2018) as confirmed by INDICIT project, showed that institutions are not always connected at the national level. This highlights the need to create an organizational diagram of the institutions within a country that are in charge of specimen and data collection. Specimens are sometimes managed by several institutions, which take charge of different samples or different steps of the procedure for collecting data on marine litter ingestion. The use of a national-level database to manage information about the specimens and samples and to share the data between institutions would support monitoring efficacy. A nationally coordinated network of all those involved in sea turtle rescue and monitoring activities in the country is recommended; this network should oversee a database that is considered the unique point of reference at the national level.

48. On a Mediterranean-wide scale, a network of all Contracting Parties to the Barcelona Convention should be developed. As a starting point, this could include the institutions that responded to the SPA/RAC questionnaire (SPA/RAC-UNEP/MAP, 2018), the partners and stakeholders involved in the INDICIT project, and the contacts compiled in this document (Appendix 2 – Capacity for monitoring litter impacts on sea turtles in Mediterranean countries).

49. Another important topic is how to maintain and strengthen networks. Two main critical needs have been reported by stakeholders (Darmon, INDICIT consortium, Miaud, 2019): the co-signing of sharing agreements stipulating the conditions for data use, especially regarding scientific publications or conservation reports, and the need for sufficient and ongoing financial means for equipment and staff. Stakeholders often also have more specific requirements, such as (i) training for standardized monitoring, (ii) receiving a summary of the results found locally, particularly results arising from the data they collected, (iii) being involved in research projects as partners or invited to workshops as experts, and (iv) receiving financial assistance to meet personnel and material needs. Moreover, as in addition to stranding, turtles are mainly recovered from bycatch in most countries (Appendix 2), collaboration with fishermen is essential. Specific workshops aimed at involving these stakeholders in the study of marine litter impacts on marine fauna are recommended.

2.4.3. Cost of and recommendations for standardizing and maintaining the networks

50. Creating organizational diagrams of participating institutions would facilitate contacts locally and the reporting of information to relevant bodies (e.g. SPA/RAC), thus helping to identify any gaps. This would also assist links between CORMON experts (Ecosystem Approach Correspondence Group on Monitoring) and sea turtle experts in each country.

51. To fill the knowledge gaps within the Mediterranean basin, developing new local networks and extending the capacity of existing stranding networks are both necessary. Some stakeholders reported that they are unable to manage a high number of specimens (Darmon, INDICIT consortium, Miaud, 2019). The selection of representative areas should be considered in such cases, considering criteria such as carrying capacity, field accessibility and number of specimens. These areas and, as far as possible the related institutions and data collectors, should remain the same over the years in order to collect comparable data and reveal accurate temporal trends so that effective restoration measures can be determined.

52. The SPA/RAC report (SPA/RAC-UNEP/MAP, 2018) recommends supporting the participation of stakeholders in existing projects and training courses. The costs for this must be taken into account in organizing training sessions and events, considering the following:

- The reference centre responsible for data cleaning and centralizing data on a national platform.
- The experts from rescue centres, stranding networks and laboratories responsible for recovering specimens in the field and performing necropsies with precise data collection and respecting all sanitary precautions.
- The biologists from rescue centres responsible for collecting information on individuals and on the marine litter excreted in faeces, respecting sanitary precautions and avoiding risks of plastic contamination. Specific workshops bringing together experts from rescue centres and research laboratories may improve the protocol for living individuals.
- Fishermen, professionals from the maritime sector and conservation managers, who could be better involved in recovering specimens, in providing feedback on marine litter impacts and in conservation projects.
- The costs of translation, the dissemination of protocols and video tutorials, and the tool kits necessary for monitoring (see section
- 2.1.3. Video tutorials for collecting data from a necropsy Appendix 1 - Checklists of materials).

53. One-day workshop could be held every two years depending on the level of the participants (to transfer skills for collecting data on marine litter impacts), in English and French and, if necessary, a translator for other languages. The translation of protocols and the tool kit should be considered so these can be disseminated during training sessions.

54. In addition to these events, a dedicated mailing list and an online platform for sharing documents, tools and a photo gallery should be created to support those involved in data collection. For stakeholders that lack the capability to process samples (e.g. lack of time or resources), cost estimates should take into account the support of a specialist team (experts in marine litter ingestion) that could come to the local site (e.g. for a 6-month period) to conduct laboratory analyses (Darmon, INDICIT consortium, Miaud, 2019).

2.5. Data analysis

2.5.1. Assessment

55. The purpose of the collected data is to allow an evaluation of the total abundance of marine litter and the identification of the main marine litter categories ingested by sea turtles. This involves calculating the arithmetic mean and the standard error of:

- The occurrence frequency of marine litter ingestion by considering the presence/absence of litter found in the entire digestive tract of necropsied individuals or in the faeces of living individuals (the value is the percentage of individuals having ingested marine litter out of all samples at the considered spatial and temporal scale).
- The dry mass (in grams) of marine litter ingested by marine litter category (accurate to two decimal places).
- The number of marine litter fragments by marine litter category (optional).
- The dry mass (in grams) of food remains (accurate to two decimal places).

56. One critical point during the analysis of the number of items is that multiple pieces may be derived from the fragmentation of one object inside the digestive tract or as a consequence of direct

ingestion. In this case, a subjective interpretation of whether it is a single item or multiple separate pieces could result in potential bias in the recorded number. For this reason, threshold values should be calculated using data only on ingested mass, to minimize misinterpretation and discrepancies due to methodology between stakeholders.

57. As plastics are the items most often found ingested by sea turtles, it was recommended to consider only the plastics to establish the MSFD Good Environmental Status (Camedda et al., 2014; Matiddi et al., 2017; Domènech et al., 2018). This is calculated from the sum per individual of the dry mass of each plastic category (USE PLA, USE SHE, USE FRAG, USE THR, USE FOR, USE POTH; Table 1). The INDICIT consortium also recommends comparing the dry mass of food remains (FOO) with the dry mass of ingested plastic per individual as a proxy of the individual's health (see section 2.2.3.).

58. Other information such as the colour of marine litter items (especially the categories white/transparent, dark or light), the volume, the prevalence of different marine litter types, and the incidence and abundance per digestive section (oesophagus, stomach, intestines) are useful for research and impact analysis. The sub-division of plastic items according to their shape (USE SHE, USE THR) is useful to identify the source of marine litter. This can help policymakers evaluate the success of conservation measures, providing evidence of their efficacy. For example, bans on plastic bags in shops should correspond to a reduction of USE SHE category items found ingested by sea turtles (Matiddi et al., 2019).

59. An example of data analysis and methodology can be found in Darmon, INDICIT consortium, Miaud (2019) and Matiddi et al. (2019). As more data is required to get an accurate assessment of the current situation across the Mediterranean, these documents recommend evaluating the current state of play and trends per country and at the regional scale (OSPAR and Barcelona conventions) in terms of biological constraints and GES.

2.5.2. Constraints

60. Habitat use by sea turtles, the mode of turtle recovery (stranding/bycatch, the involvement of fishing gear), growth stage and body condition are all factors that may influence the probability of marine litter ingestion in sea turtles. In this case, these factors must be considered as constraints when interpreting the indicator (Claro et al., 2014).

61. Previous Mediterranean data suggested that the prevalence of marine litter ingestion (measured as the occurrence or quantity of ingested marine litter) varied according to the parameter considered, including parameters considered a proxy of an individual's age (stage, carapace length, weight) and body condition (injuries, fat reserves, etc.). There is no clear evidence of an influence of these factors, even the circumstances of discovery (generally bycatch or stranding, the latter sometimes the result of the former) (Darmon, INDICIT consortium, Miaud, 2019). Thus it is suggested not to perform data stratification at this stage; however, stakeholders are encouraged to collect more data – including data considered optional in the SPA/RAC-INDICIT protocol (2019) – for more powerful analyses. Additionally, more knowledge about the parameters for evaluating sea turtle health, and especially the impact of marine litter ingestion on health, is required for better assessing thresholds.

2.5.3. Data assessment to propose targets for Good Environmental Status

62. As reported in Matiddi et al. (2019), there is a difference between analysing the ingestion of plastic by sea turtles as an indicator of the impact on the population with consequences for species conservation and analysing this as a bio-indicator of the impact on the coastal and marine environment. In the context of a monitoring programme, the data related to marine litter ingestion should be useful for the latter by allowing an evaluation of a system's environmental status and the availability of marine litter to marine organisms. The aim of the SPA/RAC-UNEP/MAP, INDICIT

protocol (2019) is to support effective data collection to better understand the impact of plastic on the marine environment, at either a global or a local scale – standardized data that can then be compared between neighbouring countries.

63. Currently, there is insufficient data available to allow an accurate analysis of GES in the Mediterranean. Some preliminary analyses of GES targets have been tested from a dataset collected in six Mediterranean countries (Turkey, Greece, Italy, France, Spain and Tunisia) (Darmon, INDICIT consortium, Miaud, 2019; Matiddi et al., 2019) based on necropsies only. Examples of the general calculations used to determine this threshold are provided in Matiddi et al. (2019) and Darmon, INDICIT consortium, Miaud (2019). To make more accurate assessments of GES targets, further data needs to be acquired so more powerful tests can be applied. In particular, more data related to the 'optional parameters' (SPA/RAC-UNEP/MAP, INDICIT, 2019) is needed to better take into account marine litter impact on an individual's health.

64. To date, the environmental quality threshold value put forward by different authors has been defined as the proportion of dead turtles that exceed a specific limit of plastic mass (average dry weight) in their gastrointestinal tract (Matiddi et al., 2019; Van Franeker et al., 2011). Therefore, the first GES proposal was based on the fulmar indicator used as an Ecological Quality Objective in the OSPAR area (van Franeker et al., 2011; MSFD Technical Subgroup on Marine Litter, 2013): "*There should be less than X% of sea turtles with more than Y g of plastic in the digestive tract in a minimum sample size of 50 dead turtles from each sub-region*", where Y is the average dry mass of ingested marine litter and X% is the percentage of sea turtles with a mass (in grams) of plastics higher than Y.

65. The second proposed GES was the following: "*There should be less than X% of sea turtles with a higher dry mass of plastics (in grams) than the dry mass of food remains in the digestive tract in a minimum sample size of 50 dead turtles from each sub-region*". This modification was put forward because the INDICIT consortium considered the proportion of food remains versus ingested plastics as a proxy of individual health. In this case, individuals with a completely empty gastrointestinal tract (i.e. containing neither marine litter nor natural materials) are excluded to avoid the possibility that the individual is not feeding during a reproductive period or due to illness. Otherwise, it is assumed that in a clean, healthy environment and when able to eat, an individual should not eat plastics but only natural food.

66. Another challenge is that in order to ascertain an area's distance to GES, the current situation must be compared to a reference. Theoretically, the benchmark should be a pristine environment with no marine litter, but such a situation does not exist in reality. As a substitute, the reference could be based on the minimum occurrence and dry mass of plastics ingested by sea turtles either in the prospected area or in all studied areas worldwide. Further analyses are needed in order to define the most constructive baseline and threshold.

2.5.4. Sample size

67. The accuracy of marine litter impact evaluations depends on the amount of data collected, which depends on sample size – that is, the number of dead sea turtles found and/or the number of live sea turtles recovered by rescue centres. Therefore, understanding the spatial variations in marine litter impacts relies on the number and extent of institutes involved in the collection and analysis of animals. Each country should involve the maximum number of institutes possible in order to obtain a representative number of samples. A sample size of 50 sea turtles per country per year has been proposed (Matiddi et al., 2019). For a trend analysis over 6 years, a minimum of 250 turtles is required for the entire Mediterranean. This could be achieved if local experts are trained to recover dead specimens and process the samples (Darmon, INDICIT consortium, Miaud, 2019).

2.5.5. Temporal scale

68. Historical data is currently insufficient to show significant temporal trends. To allow an assessment of the trends every 3 or 6 years, it is recommended to collect data as soon as a specimen is found (opportunistic sampling) and regularly report the data (by semester) so the averages can be calculated per year. The exact temporal unit of the indicator, which corresponds to the temporal window at which significant trends can be detected, will be refined when more data is acquired.

2.5.6. Spatial scale

69. The proposed GES covers the entire Mediterranean basin, with a single reference to which each Contracting Party of the Barcelona Convention can evaluate its distance to this GES. However, oceanographic features suggest that separate analyses should be carried out at least for the western and eastern Mediterranean. This has been reinforced by the results of the MedSeaLitter (2016–2019) project, which modelled floating marine litter in the Mediterranean and suggested a structure of two or more sub-regions (Mansui et al., in press). To specify the most effective spatial unit to assess GES, more data needs to be collected on the discovery locations of sea turtles and the related management units.

3. PERSPECTIVES AND RECOMMENDATIONS FOR IMPLEMENTING MONITORING

70. The Mediterranean region is ready to start implementing the monitoring programme on marine litter ingested by sea turtles. Several tools (a protocol, video tutorials and guidelines) for collecting and recording standardized data are available and can be widely disseminated via workshops, mailing lists or a dedicated online platform.

71. Most of the stakeholders contacted consider that training sessions on performing necropsies and extracting standardized data from dead individuals are necessary. Specific training sessions for rescue centres could also be organized in order to improve the protocol for collecting data from live individuals.

72. Some countries are already – or have recently become – involved in monitoring marine litter impacts on sea turtles. First analyses have been performed on a database collected by Tunisia, Spain, France, Italy, Greece and Turkey, with more accurate results obtained in the north-western Mediterranean due to the availability of historical data in that region. The preliminary statistical analyses enabled a baseline of marine litter ingested by sea turtles (occurrence and mean quantity) to be defined for these countries (Darmon, INDICIT consortium, Miaud, 2019), but the GES threshold value and the distance of each country from the threshold will need to be further evaluated after more data is collected from all the Contracting Parties to the Barcelona Convention.

73. To create an effective monitoring programme, the main priorities are the establishment of an extensive, coordinated Mediterranean network for collecting and processing samples and for recording, cleaning and analysing data, and then comparing the results with neighbouring countries and with the GES value at the regional scale. To make the monitoring programme operational, national networks need to be developed that are strongly linked to SPA/RAC policymakers and CORMON delegates. Each network should be coordinated nationally to monitor all rescue activities occurring in their territory and should be equipped with a database (connected to the INFO-RAC system) considered as the unique point of reference for all national institutions.

74. Training sessions for national coordinators should be held by international experts (Appendix 2; Table 1) for French and English speakers (or with a translator), and then be conducted at a local level by the national coordinators. These workshops should be organized regularly, depending on the level of the participants and the improvements that may need to be made to the protocols. For stakeholders lacking sample processing capability, international reference institutes could be identified to conduct the laboratory analyses.

75. Lastly, in order to (i) assess the potential biological constraints more accurately, (ii) specify the temporal and spatial scales of the GES, and (iii) validate the GES indicators, it is recommended to collect more standardized data following the SPA/RAC-UNEP/MAP, INDICIT protocol (2019) protocol. In particular, the data corresponding to “optional parameters” would allow a better understanding of the impact of plastic ingestion on individual health. The statistical approach used to select the GES reference and threshold values also needs to be validated. Workshops on this topic should be integrated in the procedure for implementing the monitoring programme, inviting experts and representatives from member countries of the Barcelona Convention, the EU MSFD and other regional sea conventions to standardize the approach. Analyses based on marine litter category will allow specific conservation measures (for example, related to plastic bans) to be evaluated (Matiddi et al., 2019). As raw data is needed to test such approaches, sharing agreements will need to be signed between stakeholders to specify the conditions for sharing and using the data

ANNEXES

Annex I
Checklists of Materials

Annex I: Checklists of materials

These checklists from the SPA/RAC-INDICIT protocol (SPA/RAC-UNEP/MAP, INDICIT, 2019) could be part of the tool kit provided to stakeholders during training sessions.

A1.1. Examination of the animal and sample collection at the discovery site:

Rope (to mark off the zone)	
Integral protective suit	
Safety glasses and protective mask or shield	
Cut-resistant gloves	
Gloves	
Boots	
Camera	
Measuring tape	
Pen	
Observation sheet for recording data	
Bottle/zipper storage bags	
Cooler	
Permanent marker	
Transport bins or containers for the turtle	
Garbage bag	

A1.2. Sample collection from dead individuals and the extraction of ingested marine litter from the digestive tract:

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

A.1.3. Sample collection from live individuals and the extraction of ingested litter in faeces:

Freezers (-20°C)	
Proofer (not mandatory)	
Garbage bags	
Safety glasses and protective mask (optional)	
Gloves	
Camera	
Pen	
Observation sheet	
Permanent marker	
Measuring tape	
Sliding caliper	
Permanent marker	
Containers for samples (tubes/zipper storage bags)	
Sieve with 1 mm mesh	
Sieve with 5 mm mesh (optional – for the study of ingested micro-plastics: 1–5 mm)	
Measuring cylinders (10 ml, 25 ml, 50 ml) (optional)	
Decimetre (optional)	
Precision balance (0.01 g)	
Binocular loupes (optional)	
Filtration grids with 1 mm mesh (where water arrives and is discharged)	
Landing net with 1 mm mesh	

ANNEX II

Capacity for monitoring litter impacts on sea turtles in Mediterranean countries

Annex II: Capacity for monitoring litter impacts on sea turtles in Mediterranean countries

Table 2. Leaders for EU MSFD National Monitoring Programme (Criteria D10C3)

Country	Institute	Representative	Contact	Location
FRANCE	MNHN	CLARO Françoise	claro@mnhn.fr	Paris
GREECE	HCMR	ZERI Christina	chris@hcmr.gr	Athens
ITALY	ISPRA	MATIDDI Marco	marco.matiddi@isprambiente.it	Rome
SPAIN	Not organised. For the moment Ministry for the Ecological Transition	MARTÍNEZ-GIL Marta	Mmgil@miteco.es	Madrid

Table 3. INDICIT and INDICIT-II Mediterranean partners

Countries	Institute	Representative	Contact	Location
CYPRUS	Exeter University	GODLEY Brendan; BRODERICK Annette; DUNCAN Emily, NELMS Sarah	B.J.Godley@exeter.ac.uk; A.C.Broderick@exeter.ac.uk; ed291@exeter.ac.uk; S.Nelms@exeter.ac.uk	Exeter
FRANCE	Centre for Evolutionary and Functional Ecology (CEFE)- School for Higher Studies (EPHE)/ French National Centre for Scientific Research (CNRS)	MIAUD Claude; DARMON Gaëlle	claude.miaud@cefe.cnrs.fr; gaelle.darmon@cefe.cnrs.fr	Montpellier
	National Museum of Natural History (MNHN)	CLARO' Françoise	claro@mnhn.fr	Paris
ITALY	Higher Institute for the Protection and Environmental Research (ISPRA)	MATIDDI Marco; SILVESTRI Cecilia	marco.matiddi@isprambiente.it; cecilia.silvestri@isprambiente.it	Rome
	National Research Council (CNR)	DELUCIA Giuseppe Andrea. ; CAMEDDA Andrea	giuseppe.delucia@cnr.it; andrea.camedda@iamc.cnr.it	Oristano
GREECE	Hellenic Centre for Marine Research (HCMR)	KABERI Helen; TSANGARIS Catherine	ekaberi@hcmr.gr; ctsangar@hcmr.gr	Athens
SPAIN	University of Valencia (UVEG)	TOMAS Jesus	jesus.tomas@uv.es	Valencia
TURKEY	Sea Turtles Research and Application Centre (PAU/DEKAMER)	KASKA Yakup	yakupkaska@gmail.com	Dalaman
TUNISIA	National Institute of Science and Technology of the Sea (INSTM)	BRADAI Mohammed; CHAIEB Olfa	mednejmeddine.bradai@instm.mrt.tn; offachaieb@yahoo.fr	Tunis; Monastir

Table 4. Contacts and organizations involved (or potentially involved) in monitoring marine litter ingestion in sea turtles for each country (from literature and responses to questionnaire).

Country	SPA/RAC contact	Contact number	Sea turtle monitoring contact	Role of institution	Location
ALBANIA	DEDEJ Zamir (NAPA); KLODIANA Marika; ALSHABANI Silvamina	1	KORRO Kastriot (Agriculture University of Tirana and NGO)	Laboratory (wildlife diseases)	
		2	SELMANI Jula (National Agency of Protected Areas, AKZM)	Regional administration of protected areas (Vlorë) + turtle first aid centre	Karaburun-Sazan Marine National Park
ALGERIA	TAOUS Farida Moulai; ROUF HADJ Aissa	3	RAC/SPE-UNEP/MAP, 2018	Research laboratory (University of Oran)	Province of Oran
BOSNIA & HERZEGOVINA	KUPUSOVIĆ Tarik; ALADŽUZ Admir	4	RAC/SPE-UNEP/MAP, 2018		
CROATIA	UROŠ Jelena; KOBAŠLIĆ Ana; literature (Lazar in Casale & Margaritoulis, 2010)	5	Feitoumatt Lematt Ghrib; Dean Karaica (Institute for Medical Research and Occupational Health); Casale and Margaritoulis (2010); Lazar and Gracan (2011)	Research organization	45°49'28.8"N 16°01'37.5"E (45.824663, 16.027080)
CYPRUS	ARGYROU Marina	6	Fuller et al., in Casale and Margaritoulis (2010)	Conservation NGO, research laboratory	Northwest
		7	Demetropoulos & Hadjichristophorou, in Casale and Margaritoulis, 2010		Southeast
		8	Emily Duncan, Robin Snape, Exeter University	Research laboratory in collaboration with stranding network	North of Cyprus between Famagusta in the east to Yeşilırmak/Limnitis in the west
EGYPT	ABDELWARITH Mohamed Said; FOUDA Moustafa	9	SPA/RAC (2019) SPA/RAC 19wg464_inf_03		
		10	Nahla Nagib, Egyptian Environmental Affairs Agency	Government organization	
		11	Nada & Casale, in Casale and Margaritoulis (2010); Alexandria Turtle and Wildlife Rescue Team	Conservation NGO	
FRANCE	WOLFF Anastasia (MTES), GUICHARD Benjamin (AFB), CLARO Françoise (MNHN), VERMOT Jean, DECKERT Nadia, RODRIGUES Benoit	12	C. Miaud, G. Darmon, D. Gambaiani, CEFE-EPHE/CNRS	Research laboratory, veterinarian laboratories, NGOs, rescue centre, observatory networks	
GREECE	NIKOKAVOURAS Charilaos, ALVANOPOULOS George	13	Guido Pietroluongo, Archipelagos Institute of Marine Conservation	Conservation research, veterinarian	Northeastern Aegian sea (Samos, Lipsi, Ikaria, Agathonisi, Marathi)
		14	Ministry of Agriculture, HCRM, MEDASSET, ARCHELON, WWF Greece, Katelios group, Turtle first aid station of Amvrakikos Bay (Kopraina), Turtle first aid station of Pangalohori (Rethymno)	Research laboratory, rescue centre, conservation NGOs	
ISRAEL	YAHEL Ruth, NEMTZOK Simon, ROSEN Ayelet	15	Yaniv Levy - Israel sea turtle rescue centre (Israel National Nature and Parks Authority)	Conservation, management, research	Mevoot Yam - Mikhmoret
ITALY	TUNESI Leonardo	16	Marco Matiddi (ISPRA)	Research institute	Western Med. Sea, Adriatic Sea, Central Med. Sea.
		17	de Lucia G.A (CNR/IAS)	Research institute	Sardinia
LEBANON	SAMAHA Lara	18	Aureggi and Khalil In Margaritoulis and Casale (2010)		
LIBYA	AYAD ELAGIL Elmaki	19	Hamza in Margaritoulis and Casale (2010)		
MALTA	STEVENS Darrin T.	20	Carmen Misfud	Governance	
MONACO	SIMONET Raphaël	21	Olivier Brunel, Alexandra Beal (Institut Oceanographique Fondation Albert Ier Prince de Monaco)	Rescue centre	

Country	SPA/RAC contact	Contact number	Sea turtle monitoring contact	Role of institution	Location
MONTENEGRO	BATAKOVIĆ Milena	22			
MOROCCO	AMHAOUCH Zouhair, TAHARI Sabah, ENDICHI Mohammed	23	Moustafa Aksissou, Wafae Benhardouze	Research laboratory	Morocco
SLOVENIA	TURK Robert	24	Lazar and Žiža in Casale and Margaritoulis (2010); Lazar and Gracan (2011)		
SPAIN	ALONSO RODRIGUEZ Jorge, GARCIA-BELLIDO CAPDEVILLA Elvira, ESCOBAR PAREDES Victor	25			
SYRIA	SAAD Mayada, ALHAYEK Belal	26	Adib Saad, Syrian Society for Aquatic Environment protection (SSAEP)	Stranding network	
TUNISIA	ALID BEN TEMESSIK Mohamed	27	M N Bradai, O. Chaieb and H. Attia El Hili	Research laboratory (M.N. Bradai and O. Chaieb, Institut National des Sciences et Technologies de la Mer (INSTM), Veterinarian (H. Attia Hil, Centre National de Veille Zoosanitaire)	Tunisia
TURKEY	ERGÜN Güner	28	PAU-DEKAMER		

Table 5. Available means and needs for collecting living or dead sea turtle specimens and recording data on marine litter impacts, as reported by the stakeholders contacted in each Mediterranean country

Country	Contact number	Existence of a stranding network	Existence of a rescue centre	Personnel for monitoring	Existing material				Involvement in another project
					EU MSFD guidelines	SPA/RAC-INDICIT protocol	Familiar with IMAP CI24	Video tutorial	
ALBANIA	1	Yes	First aid centres in Vlorë and Patok (UNDP projects); proposal to build a rescue centre not yet approved	Yes (2 employees, ~500 volunteers)	No	No	No	No	
	2		Yes (new)	Yes (part- and full-time employees, and summer seasonal employees, as well as volunteers, notably a veterinarian)	No	No	Yes	No	UNDP project (not related to marine litter)
ALGERIA	3	LRSE (Laboratoire Réseau Surveillance Environnemental)	No		No	No	Generally no (71.4%)	No	
BOSNIA & HERZEGOVINA	4	No	No						
CROATIA	5		Yes (Blue World Institute - island of Lošinj; Aquarium Pula)	Yes (6 employees, no volunteers)	No	Yes	No	No	
CYPRUS	6	Yes? Through teams monitoring nesting activities							
	7	Yes, Meneou (under Department of Fisheries and Marine Research)							
	8	Yes	Yes (Meritta, located near Kyrenia, http://www.meritta.org/	Yes (4 permanent, 80 volunteers)	Yes	Yes	No	Yes (Matid di et	

Country	Contact number	Existence of a stranding network	Existence of a rescue centre	Personnel for monitoring	Existing material				Involvement in another project
					EU MSFD guidelines	SPA/RAC-INDICIT protocol	Familiar with IMAP CI24	Video tutorial	
			eng; also collaborate with PAU-DEKAMER in Turkey)					al., 2019)	
EGYPT	9	No	No				Yes		
	10	No	No	Yes (10 employees, 15 volunteers)	Yes	Yes	Yes	No	
	11								
FRANCE	12	Yes (RTMMF, including Cari in Corsica)	Yes (CestMed in Grau du Roi, CRFS in Antibes)	Yes (stranding networks with volunteers, rescue centres with temporary staff)	Yes	Yes	Yes	Yes (Matiddi et al., 2019)	
GREECE	13		First aid						
	14	Yes (ARCHELON)	Yes (ARCHELON)	Yes	Yes	Yes		Yes (Matiddi et al., 2019)	
ISRAEL	15	Yes	Yes	Yes (5 employees, ~50 volunteers and 250 volunteers and 50 rangers during the nesting season)	No	No	No	No	
ITALY	16	Yes	Yes	Yes	Yes	Yes	Yes	Yes (Matiddi et al., 2019)	INDICIT 2, MEDREGION, Plastic Buster
	17	Yes	Yes	Yes	Yes	Yes	Yes	Yes (Matiddi et al., 2019)	INDICIT 2
LEBANON	18	No?	No?	No?					
LIBYA	19	No, information received by MBRC and EGA	Yes (Marine Biology Research Centre)	No?					
MALTA	20	Yes (24H call system under Environment Protection Directorate supported by NGO and University of Malta; Armed forces of Malta, Malta Maritime Authority and Administrative Law Enforcement provide sea craft when needed)	Yes (Small first aid and rescue centre coordinated by Veterinary, Fisheries Conservation and Control Division (VRFCC) and Environment Protection Directorate of Malta Environment and Planning Authority, in Fort Saint Lucian M'Xlokk Department premises)				Yes	No	Life Migrate project (surveys at sea)
MONACO	21	Yes (related to RTMMF, France)	Yes	Yes (with 2 employees working in rescue centre; and possible volunteers)	No	Yes	No	EPHE, 2017	
MONTENEGRO	22								

Country	Contact number	Existence of a stranding network	Existence of a rescue centre	Personnel for monitoring	Existing material				Involvement in another project
					EU MSFD guidelines	SPA/RAC-INDICIT protocol	Familiar with IMAP CI24	Video tutorial	
MOROCCO	23	Yes	Yes	Yes (employees)		No	No	No	
SLOVENIA	24								
SPAIN	25	Yes	Yes		Yes	Yes	Yes	Yes (Matiddi et al., 2019)	
SYRIA	26	Yes		Yes (5 employees, 42 volunteers)	No	No	No	No	
TUNISIA	27	Yes (INSTM)	Yes (INSTM)	Yes (employees and volunteers)	Yes	Yes	Yes	Yes (Matiddi et al., 2019)	
TURKEY	28	Yes	Yes		Yes	Yes	Yes	Yes (Matiddi et al., 2019)	

Table 6. Means available in each Mediterranean country for rescue centres welcoming living turtles and recording data on marine litter impacts, and first observations reported on marine litter impacts related to ingestion and entanglement in live sea turtles.

Country	Contact number	LIVING TURTLES			
		Number of tanks	Main means of observation/recovery of specimens	Collection of marine litter in faeces (Yes/No)	Observed impact of marine litter
ALBANIA	1	1	Bycatch, Found at sea	Yes	1/3?
	2	1 external (5 m^3), 1 internal, transport tanks (material must be completed)	Bycatch, Observation at sea, Stranding	No	
ALGERIA	3				No
BOSNIA & HERZEGOVINA	4				
CROATIA	5		Bycatch, Observation at sea, Stranding	No	
CYPRUS	6		Bycatch		
	7		Stranding		
	8			Yes	
EGYPT	9				
	10	0	Bycatch, Stranding	No	No
	11		Bycatch		
FRANCE	12	10	Bycatch, Stranding	Yes	Between 2013–2018: 48.53% (N=68); dry mass 0.21 ±0.06 g (N=60), mostly plastics (sheets, fragments, threadlike plastics) (Darmon, INDICIT consortium, Miaud, 2019)
GREECE	13				1 case in 2018
	14		Stranding	Yes (from 2017)	From 2017–2018: 100% (N=3) with 0.03 ±0.02 (N=3) (Darmon, INDICIT consortium, Miaud, 2019)
ISRAEL	15	40	Stranding or found at sea, recovered by fishermen and citizens	No	Yes (observed in 2 individuals)
ITALY	16		Stranding mostly, Bycatch, Found at sea	Yes	64.52% (N=93); 1.71 ±0.87 (N=93), Darmon, INDICIT consortium, Miaud, 2019
	17		Stranding mostly, Bycatch, Found at sea	Yes	64.52% (N=93); 1.71 ±0.87 (N=93), Darmon, INDICIT consortium, Miaud, 2019
LEBANON	18			No	
LIBYA	19				
MALTA	20	16 (Misfud, in Casale & Margaritoulis, 2010)		No	Not observed in living turtles
MONACO	21	5	Observation at sea	Not yet	Not yet studied
MONTENEGRO	22				
MOROCCO	23	1	Stranding	Yes	1 turtle per year
SLOVENIA	24				
SPAIN	25		Stranding, Bycatch	Yes	88.9% (N=18); 2.36 ±1.31 g (N=10); Darmon, INDICIT consortium, Miaud (2019)
SYRIA	26			No	No
TUNISIA	27	10	Stranding, Bycatch	Yes	Observed in 20 turtles (SPA/RAC, 2019); 47.1% (N=17), 0.2 g

Country	Contact number	LIVING TURTLES			
		Number of tanks	Main means of observation/recovery of specimens	Collection of marine litter in faeces (Yes/No)	Observed impact of marine litter
ALBANIA	**1**	1	Bycatch, Found at sea	Yes	1/3?
	2	1 external (5 m^3), 1 internal, transport tanks (material must be completed)	Bycatch, Observation at sea, Stranding	No	
					±0.07 (N=8) from 2013–2018 (Darmon, INDICIT consortium, Miaud, 2019)
TURKEY	**28**		Stranding, Found at sea	Yes	Observed in 5 turtles (SPA/RAC, 2019); 25% (N=8), <<1 g (N=7; Darmon, INDICIT consortium, Miaud, 2019)

Table 7. Means available in each Mediterranean country for recovering dead turtles and recording data on marine litter ingestion, first observations reported on marine litter impacts related to ingestion and entanglement on dead turtles, and evaluation of monitoring capacity.

Country	Contact number	DEAD TURTLES						Data on entanglement
		Mean number recovered per year	Main means of recovery of specimens	Necropsies (Yes/No)	Collection of l marine itter in digestive tract (Yes/No)	Observed impact of marine litter	Use of a specific protocol	
ALBANIA	**1**	12	Stranding	Yes	Yes	32% (from clinic and faculty records)	No	Yes, however, no statistics have been calculated yet on the percentage of turtles impacted; plastics are the main type of marine litter involved
	2	6	Stranding	No	No		No	No (and not observed)
ALGERIA	**3**			Yes	Yes	From 2016, 27% of Caretta (N=18) and 100% of Dermochelys (N=1); fragments of yoghurt pots, fishing gear, glass	No	
BOSNIA & HERZEGOVINA	**4**							
CROATIA	**5**	27 dead (estimate based on the 6-year period from 2010–2015)	Bycatch, Observation at sea, Stranding	Yes	Depends on institution? Or planned	From 2001 to 2004 (Lazar and Gracan, 2011): 35.2% (N=54) of Caretta; soft plastic, ropes, styrofoam and monofilament lines; ingested dry mass from <0.01 to 0.71 g		No (planned)
CYPRUS	**6**		Bycatch					
	7		Stranding					
	8	65 Green; 58 Loggerhead (2019)	Stranding, Bycatch, Observation at sea	Yes	Yes	80–90% of green turtles (100% reported in Duncan et al., 2019, N=19); marine litter ingestion may be slightly lower in loggerheads	Yes	Yes
EGYPT	**9**			Occasional		33% (N=3) in 2017	Matiddi et al., 2017	
	10		Stranding mostly, Bycatch	Yes	No		INDICIT protocol and EU MSFD guidelines	No
	11		Bycatch			Yes (not quantified, but qualified as 'often', in loggerheads)		
FRANCE	**12**	20 (including 2 in Corsica)	Bycatch, Stranding	Yes	Yes	Between 2013–2018: 82.43% (N=76); 1.23 ± 0.27 (N=70), mostly plastics (sheets, threadlike, fragments) (Darmon, INDICIT	SPA/RAC - UNEP/MAP, INDICIT, 2019 (2019)	Yes, relatively low

Country	Contact number	DEAD TURTLES						Data on entanglement
		Mean number recovered per year	Main means of recovery of specimens	Necropsies (Yes/No)	Collection of l marine itter in digestive tract (Yes/No)	Observed impact of marine litter	Use of a specific protocol	
						consortium, Miaud, 2019)		
GREECE	**13**	10				5% of individuals necropsied per year. Evaluation of 60% occurrence of marine litter ingestion in sea turtles (expert knowledge); plastic bags, fragments, packaging, marine litter from fishing activities, balloons, pellets		Yes (Chelonia mydas), ~10%, with plastic bags and marine litter from fishing activities; could differentiate active/passive entanglement
	14	14	Stranding	Yes	Yes	From 2017–2018: 64.28% (N=28) with 0.13 ±0.06 (N=28) (Darmon, INDICIT consortium, Miaud, 2019); 9.82m² plastic in a leatherback (reported in Margaritoulis & Panagopoulo, in Casale & Margaritoulis, 2010)	SPA/RAC - UNEP/MAP, INDICIT, 2019 (2019)	Yes
ISRAEL	**15**	300 (85% loggerhead, 15% green)	Specimens not collected	No (occasionally)	No	In loggerheads: 0–7% of marine litter ingestion (expert knowledge); fragments and ropes. In 2001, 4 Dermochelys probably died due to blockage of the digestive tract (total N unknown; Levy in Casale and Margaritoulis, 2010)	No	Yes. From 1999–2005, 7.7% of loggerheads (N=104), 15% of greens (N=20) (no specification of the type of items causing entanglement; Levy in Casale and Margaritoulis, 2010). Marine litter involved: plastic sacks for holding rice, sand or gravel
ITALY	**16**	~25	Stranding mostly, Bycatch, Found at sea	Yes	Yes	62.01% (N=129), 0.92g ±0.19 (N=129), Darmon, INDICIT consortium, Miaud, 2019	SPA/RAC - UNEP/MAP INDICIT, 2019 (2019)	
	17	~25	Stranding mostly, Bycatch, Found at sea	Yes	Yes	62.01% (N=129), 0.92g ±0.19 (N=129), Darmon, INDICIT consortium, Miaud, 2019	SPA/RAC - UNEP/MAP, INDICIT, 2019 (2019)	~25
LEBANON	**18**				No	No	No	
LIBYA	**19**							
MALTA	**20**		Stranding	Yes (Support of a veterinarian in the rescue centre)		Mostly in leatherbacks, mostly involved nylon. Presence of	No	Yes (in loggerheads and leatherbacks;

Country	Contact number	DEAD TURTLES						Data on entanglement
		Mean number recovered per year	Main means of recovery of specimens	Necropsies (Yes/No)	Collection of l marine itter in digestive tract (Yes/No)	Observed impact of marine litter	Use of a specific protocol	
						loggerheads with tar, plastic and metal marine litter, transparent and white (Gramentz, 1988); Observations from 2001, but not reported		Marinelitter mostly from fishing activities, without possibility of differentiating passive and active entanglement; note of entrapment in reverse osmosis plants or in cooling intake systems of power plants (Misfud, in Casale and Margaritoulis, 2010)
MONACO	**21**	3 from 2015	Observation at sea	Yes	Yes (samples sent to CEFE, France, for laboratory analyses)	100%, dry mass of 6 to 12 g, plastic bags, fragments, threadlike materials (N=3)	Comparable to SPA/RAC - UNEP/MAP, INDICIT, 2019 protocol (partnership with CNRS in France from 2015–2018); INDCIT-SPA/RAC (2019) protocol	Yes
MONTENEGRO	**22**							
MOROCCO	**23**	5 loggerhead, 1 leatherback (up to 95?)	Stranding	Yes	Yes	5–50% according to expert (~20 individuals), bags, fragments, fishing activities	Own protocol (number of items)	Yes (8–10%; bags and materials from fishing activities involved, with possible differentiation between passive and active entanglement)
SLOVENIA	**24**					In Lazar and Gracan (2011), from 2001–2005, 35.2% (N=54) loggerheads with marine litter, heavy metals, organochlorine, contaminants (samples from Croatia and Slovenia)		
SPAIN	**25**	~14	Stranding, Bycatch	Yes	Yes	80.49% (N=85) 0.89 ±0.27g (N=70); Darmon,	SPA/RAC - UNEP/M	Yes

Country	Contact number	DEAD TURTLES						Data on entanglement
		Mean number recovered per year	Main means of recovery of specimens	Necropsies (Yes/No)	Collection of l marine itter in digestive tract (Yes/No)	Observed impact of marine litter	Use of a specific protocol	
						INDICIT consortium, Miaud (2019)	AP, INDICIT, 2019 (2019)	
SYRIA	**26**	22 green, 11 loggerhead		No	No		No	Yes
TUNISIA	**27**	~30 loggerhead and 4 leatherback	Stranding, Bycatch, Individuals died in rescue centre	Yes	Yes	In 2017, 30% (N=200, SPA/RAC 2019); 52.2% (N=46), 0.84 g± 0.7 (N=29) (Darmon, INDICIT consortium, Miaud, 2019)	SPA/RAC - UNEP/MAP, INDICIT, 2019(2019)	Yes
TURKEY	**28**	45	Stranding, Found at sea			From 2017, 35 from 2017% of N=80; <1 g, sheet (70%) and treated materials from fragments and foams (SPA/RAC 2017); 33.3% (N=93), 0.37 ±0.3 (N=93; Darmon, INDICIT consortium, Miaud, 2019)	SPA/RAC - UNEP/MAP, INDICIT, 2019 (2019)	Yes

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