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

National Strategy for Monitoring IMAP Candidate Indicator 24 in Tunisia

UNEP/MAP
Athens, 2022

Note by the Secretariat

The Marine Litter MED II project, funded by the European Commission, DG Environment (EC-DG ENV), further supports the implementation of the updated Regional Plan on Marine Litter Management in the Mediterranean approved by COP 22 (Antalya, Turkey, 7-10 December 2021) at national, sub-regional and regional level with a particular focus on southern Mediterranean countries namely Algeria, Egypt, Israel, Lebanon, Libya, Morocco, and Tunisia. The project builds on the outcomes of the EU-funded Marine Litter MED project (2016-2019).

The project contributed to the implementation of the UNEP/MAP 2016-2021 Mid-Term Strategy (MTS) and the 2020-2021 Programme of Work, and is now providing an important contribution to UNEP/MAP Mid-Term Strategy (MTS) 2022-2027 and 2022-2023 Programme of Work, and of several COP Decisions related to the implementation of the Regional Plan on Marine Litter Management in the Mediterranean, the region-wide Marine Litter Guidelines, the Ecosystem Approach and Integrated Monitoring and Assessment Programme (IMAP), with a focus on supporting enhanced marine litter management in the region and a litter-free Mediterranean.

Within its specific objectives to expand the implementation of the selected measures both in terms of geographical scope and impact as well as to further contribute to the development of IMAP Candidate Indicator 24 towards making it operational at country level, the project supported the preparation of the national operational strategy for monitoring IMAP Candidate Indicator 24 in Tunisia, developed in line with the respective regional operation strategy.

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List of Abbreviations / Acronyms

CI	Common Indicator
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CNVZ	Centre National de Veille Zoosanitaire
COP	Meeting of the Contracting Parties
CORMON	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 and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria
INDICIT	Implementation of Indicators of Marine Litter on Sea Turtles and Biota in Regional
INSTM	National Institute of Marine Sciences and Technologies (Tunisia)
MAP	Mediterranean Action Plan
MED POL	Programme for the Assessment and Control of Marine Pollution in the Mediterranean Sea
MSFD	Marine Strategy Framework Directive
QSR	Quality Status Report
RPML	Regional Plan on Marine Litter Management
SPA/RAC	Regional Activity Center for Specially Protected Areas
UNEP/MAP	United Nations Environment Programme/Mediterranean Action Plan

1. INTRODUCTION

1.1 Justification

Regional level:

1. Floating and stranded Plastics are ubiquitous (Andrady, 2011; Barnes et al., 2009). their damage in the marine environment is alarming, leading to possible effects on the renewal of natural resources in addition to economic and sanitary repercussions (Hardesty et al., 2015). This is especially the case in the Mediterranean Sea, due to its semi-enclosed configuration and the high rate of coastal urbanization (MED QSR, 2017).

2. Marine litter affects various compartments of the marine environment and monitoring its impacts on marine organisms is of growing importance.

3. Whatever temporal and spatial scale are considered, marine litter (mainly plastics) interact with a vast range of marine species. The different types of impact of marine litter on these organisms can be classified according to the modes of action such as entanglement, ingestion and transportation of species that may be colonized on them.

4. 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.

5. 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.

6. 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).

7. Marine turtles, in particular the loggerhead turtle *Caretta caretta*, are considered to be good biological indicators to measure the impacts of debris on marine fauna (Matiddi et al., 2017) (1) they tend to ingest marine debris (2) they have a great distribution (3) they use all the ecological compartments of the sea (From the bottom to the surface of the oceans). In addition, in most areas a sufficient number of stranded or accidentally captured turtles are observed and can provide information as part of a monitoring program. In a lesser extent, the green turtle Chelonia Mydas being also regularly encountered 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.

8. One of the steps identified in the Regional Plan was linked to the implementation of the Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coasts and Related Assessment Criteria (IMAP) and its 10th Ecological Objective on Marine litter "*Properties and quantities of marine litter do not cause harm to the coastal and marine environment*", partly based with the Candidate Indicator 24 "Trends in the amount of litter ingested by or entangling marine organisms focusing on selected mammals, marine birds, and marine turtles".

National level:

9. Tunisia's commitment to the conservation of sea turtles in its national waters was underlined in particular by the ratification of the Barcelona Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean in 1977. This convention is associated with seven protocols, including the Protocol on Specially Protected Areas and Biodiversity in Mediterranean (1995) and its annexes. Tunisia has also adopted the Action Plan for the Conservation of Marine Turtles in the Mediterranean and its updates (UNEP-PAM SPA / RAC, 2007, 2013).

10. Following the recommendation of the Action Plan for the Conservation of Marine Turtles in the Mediterranean for the development of National Action Plans (NAPs) to strengthen marine turtle conservation measures, Tunisia has adopted its national action plan in 2020 (UNEP-MAP-SPA/RAC, 2020).

11. The National Action Plan (UNEP-MAP-SPA/RAC, 2020) recommends, in the marine litter issue, for the next five years:

- Assessment of ingestion and entanglement rates of marine turtles with marine litters;
- Analysis of spatio-temporal variations in the presence rate of litters;
- Simultaneous censuses of litter and turtles in Tunisian waters, making it possible to assess the relevance of marine turtles as indicators of pollution and good sea health; and
- Indicators of success will be estimation of ingestion and entanglement rates and proposed Good Environmental Status (GHG) scenario.

12. Tunisia and namely the "*Institut National des Sciences et Technologies de la Mer* » (INSTM) participated in the INDICIT and INDICIT II projects "Implementation of Indicators of Marine Litter on Sea Turtles and Biota in Regional Sea Conventions and Marine Strategy Framework Directive Areas" and COMMON project (COastal Management and MOnitoring Network for tackling marine litter in Mediterranean Sea) funded by the European Union. Many scientists acquired expertise on the issue in this frame and also in the framework of the EU-funded Marine Litter MED project.

13. In Tunisia, three species of sea turtles are known: the loggerhead *Caretta caretta*, the green turtle *Chelonia mydas* and the leatherback turtle *Dermochelys coriacea*. The first species is common and nests on many beaches. The green turtle is rarely reported, while the leatherback turtle is regularly observed (Bradai and Jribi, 2010).

14. International conventions on marine turtle conservation were ratified by Tunisia and were sustained by a national decree which forbids the catch of sea turtles and their eggs (decree of Minister of Agriculture of 28 September 1995). Legislation on the conservation of vulnerable species is under amendment to consider recommendations of conventions ratified by Tunisia and those of GFCM and ICCAT.

15. The gulf of Gabes in south Tunisia is likely to be one of the most important areas for marine turtles in the Mediterranean. It is considered as an important foraging and wintering area. Studies on fishery interactions, stranding and tagging confirm this importance (Laurent and Lescure, 1994; Bradai et al., 2009; Karaa et al., 2016).

16. The nesting activity of the Loggerhead turtle *Caretta caretta* occurs principally in Kuriat islands and Chebba beaches which are monitored. The first site is the most important in Tunisia. Although the smallness of the two nesting sites, Kuriat islands and Chebba, at the Mediterranean scale, the nesting activity is regularly registered, and the nests number increases since respectively 1997 and 1994.

17. In the frame of the project "Conservation of sea turtles in the Mediterranean region" coordinated by SPA/RAC and financially supported by MAVA, exploration of sandy beaches, along Tunisian coasts, looking for new and potential sea turtles nesting sites, was done. Primarily results show that about 20 sites were identified as nesting sites for the loggerhead turtle (SPA/RAC - ONU Environnement/PAM, 2020; Bradai and Karaa, 2017; Chaieb et al., under press). An exceptional nesting event of green turtle was recorded in the summer of 2019 in Rejch beach (Mahdia – Eastern coasts), Tunisia representing the western most nesting record of the green turtle.

18. The study of stranded cetaceans and sea turtles was reinforced in 2004 with the creation of a national stranding network (RNE). This program is part of the activities of the marine biodiversity laboratory of the National Institute of Science and Technology of the Sea (INSTM). Three teams have been set up for this purpose, the first based in the North (from the border with Algeria to Kelibia), the second in the center (from Kelibia to the Chebba) and the third to the south (from the Chebba to the border with Libya). Strandings are reported by appropriate forms or transmitted online via the RNE website.

19. A marine turtle rescue centre was created in 2004 at INSTM - Monastir. Its mission is conservation through rescuing and rehabilitation of suffering turtles, education, awareness and research. Moreover, the centre plays a key role in monitoring stranded turtles. Necropsies are performed to identify the potential causes of death and to collect samples for the tissue bank of the National Standing Network. A new center of first aids was launched recently (2021) in the university of Sfax.

1.2 Objective of the Marine Litter MED II Project

20. The MED MARINE Litter project II supports the implementation of the Regional Plan on Marine Litter Management in the Mediterranean at national level for some countries including Tunisia. The project builds on the outcomes of the EU-funded Marine Litter MED project (2016-2019).

21. The overall objective of the Regional Plan on Marine Litter Management in the Mediterranean is to reduce and prevent the generation of marine litter in the Mediterranean. Its main specific objective is to further contribute to the development of IMAP Candidate Indicator 24 towards making it operational at country level.

22. On the basis of the work undertook during 2016-2019 in the framework of the previous Marine Litter MED project regarding the development of the regional operational strategy for IMAP Candidate Indicator 24; SPA/RAC will provide support to Tunisia to further advance and operationalize monitoring of IMAP Candidate Indicator 24, with a focus on the ground implementation of the said operational strategy.

23. The aim of the present National Operational Strategy for the monitoring of Candidate Indicator 24, based on the regional operational strategy, is to provide practical guidelines to develop monitoring programmes to collect standardized data on marine litter ingested by sea turtles, and to assess how GES can be achieved.

2. MONITORING STRATEGY

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

24. The first methodologies for extracting marine litter ingested by dead or live marine turtles were incorporated into the European Marine Strategy Framework Directive (EU MSFD) guidelines in 2013 (Matiddi et al., 2011; MSFD-TG ML, 2013). 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 (SPA/RAC-UNEP/MAP, INDICIT, 2019). The protocol was tested with satisfaction as part of a sub-regional training session on marine litter (ingestion and entanglement by marine turtles) (Monastir, Tunisia November 1-2, 2018) (SPA/RAC- UNEP/MAP, 2019). The consolidated document (SPA/RAC-UNEP/MAP, INDICIT, 2019) was disseminated in 5 languages (Arabic, English, French, Greek, Spanish) along with a video-tutorial detailing the manipulations (Matiddi et al., 2019).

25. The protocol is intended to respond to the MSFD requirements for the indicator 10.2.1 "Trends in the amount and composition of litter ingested by marine animals". Marine turtles are proposed as an indicator species to study marine litter ingestion on biota through the development and the implementation of one major indicator "Litter ingested by sea turtles".

26. Both "basic" and "optional" parameters are proposed to be collected. The basic parameters correspond to the minimum parameters fundamental to determine the indicator criteria. The optional parameters allow acquiring more knowledge on loggerheads' behavior and probability to ingest debris and better specify the indicator criteria in development. The optional parameters can also help to better assess the relevance of two new indicators on litter impacts for which pilot studies are in process ("Entanglement with marine debris by biota" and "Micro-plastic debris ingested by sea turtle and fish").

27. An INDICIT Spreadsheet for recording standard data template is provided (https://indicit- europa.eu/protocols/) in order to facilitate banking and statistical analysis, data must be filled in standardized table, by respecting the units and proposed menu choices, and specifying remarks or other proposals in the last column "Note". All boxes must be filled, either by the information (data) or by 0 or "NA" (information not available or not evaluated).

28. The present national strategy for monitoring IMAP CI 24 litter ingested by sea turtles is based principally on this consolidated document (SPA/RAC-UNEP/MAP, INDICIT, 2019)

2.1.1 Notes

29. International conventions on marine turtle conservation were ratified by Tunisia and were sustained by a national decree which forbids the catch of sea turtles and their eggs (decree of Minister of Agriculture of 28 September 1995). therefore, handling live or dead animals or parts of these animals requires special authorization. If an 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.

30. Sanitary precautions must be taken when handling dead or live wild animals to minimize the risk of infectious diseases, in particular zoonotic diseases. Handling necessitates to wear a protective suit with glasses, gloves and rubber boots, then carefully separated and disinfected or thrown.

2.1.2 Procedures for collecting data on marine litter ingestion

31. 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. The minimum size of litter items considered for the indicator "Litter ingested by sea turtles" is 1 mm, thus including both micro (1-5 mm) and macro-plastics (> 5 mm).

32. The SPA/RAC-INDICIT protocol (SPA/RAC-UNEP/MAP, INDICIT, 2019) provides an 'Observation sheet' for recording data during handling (https://indicit-europa.eu/protocols/). For handling marine turtles in the field or in the laboratory or rescue centre, some materials are needed. Checklists of these materials are provided at the annex I of this document.

33. 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; for dead individuals performing a necropsy in an authorized service centre and extracting the digestive tract.) and for live individuals collecting all the faeces excreted by an individual for at least 1 month and ideally 2 months from the individual's arrival at the rescue centre. 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.

34. When an animal is recovered, it should be attributed with a standard identifier. the identifier must provide information on the country and site where the animal was found, the institution, the date (at least the year), the species, the part of the digestive tract analyzed (Oeso/Stom/Intest) or from Faeces and the number of the turtle in the series. Example: for the 1st sample in the faeces of the 1st individual of Caretta caretta collected in Tunisia in Sfax by INSTM in 2017, the sample code will be Tu- Sf-INSTM-2017-Cc-01-feces01 (ou Oeso01, ou Stom01 ou Intest01). This ID should be written on all samples before storing them for later analysis in a freezer at -20°C.

35. The status of individuals should be attributed on an initial assessment of body condition as one out of five levels (Table 1). It is considered that marine litter can be extracted and described for levels 2 to 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 not be useful for determining GES. In level 5, 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.

36. 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). A protocol on this issue is under finalization in the framework of INDICIT II project. If the marine litter is related to fishing activity, entanglement caused by passive fishing gear discarded at sea should, if possible, be differentiated from bycatch resulting from active fishing.

Table 1 : Description of the animal's decomposition level

Assessment of body condition	
Levels	Description
Level 1: ALIVE	live individuals 
Level 2: FRESH	Recently dead/little decomposed (turtle in good conditions)  
Level 3: PARTIAL	Internal organs still in good conditions. Autolysis (swollen). Bad smell. Colour changes in skin
Level 4: ADVANCED	Skin scales raised or lost. Still possible mensuration and presence of ingested plastic (only FO%) & entanglement. 
Level 5: MUMMIFIED	Mummified or missing part of the skeleton or body 

37. The protocol for the classification 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, separating marine micro-litter (from 1 to 5 mm) from marine macro-litter (> 5 mm).

38. 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), these items can be described in detail in the 'Notes' column of the protocol.

39. The marine litter should be categorized visually or with the help of a binocular loupe or magnifying glass in case of uncertainty. Table 2 gives a standard classification for marine litter ingested by sea turtles as well as categories of natural items (food remains and non-food items) (Figure 1). This list adopted by the INDICIT consortium focuses specifically on the plastic categories most often found ingested by sea turtles.

40. Fishing hooks, which are regularly found ingested by sea turtles, should not be classified as 'marine litter' as these individuals are considered longline bycaught. Nevertheless, the presence of a hook should be recorded in the 'Notes' column. 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).

Table 2: Classification of marine litter items, food remains and natural non-food remains (SPA/RACUNEP/MAP, INDICIT, 2019)

TYPE	CODE	DESCRIPTION
Industrial plastic	IND PLA	Industrial plastic: granules of variable shapes and colors.
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., Bits of fishing line, nets, rope, cloth.
Used foam	USE FOAM	Foam plastics, e.g., polystyrene, foam/sponge rubber (as in mattress filling, etc.)
Used fragments	USE FRAG	Fragments 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 (e.g., tar blobs)
Natural food items	FOO	Remains of usual Natural food for sea turtles (e.g., pieces of crab, shells, jellyfish, algae, etc.).
Natural non-food items	NFO	Anything natural non-food items for sea turtles (pebbles, wood, tree bark, feathers, pumice, etc.).

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,

41. For identification of the species, Biometric measurements, necropsy procedure and health status of turtles to refer also to annexes III to VI.

2.1.3 Video tutorials for collecting data from a necropsy

42. A video tutorial (in French[1]) 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.

43. A second video tutorial (in English[2]) (Matiddi et al., 2019) details 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.

1 https://www.canalu.tv/video/ephe/examen_externe_et_interne_d_une_tortue_caouanne_caretta_caretta_film_tutori el.52557

2 https://www.jove.com/video/59466/data-collection-on-marine-litter-ingestion-sea-turtles-thresholds-for

2.2 Sampling effort

2.2.1 Spatial coverage

44. The sampling effort should target the largest spatial area possible, rescue centers and mainly stranding networks should cover the entire national waters. Dead sea turtles are generally collected on beaches (from stranding) or at sea (dead specimens floating or bycaught individuals).

45. The national stranding network should be represented, for this strategy, by more than the three current intervention teams along the Tunisian coast.

2.2.2 Survey frequency

46. Continuous opportunistic sampling is required. A sample size of 50 turtles per year is recommended, 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 (SPA/RAC–UN Environnent/MAP, 2019).

2.2.3 Recommendations for data collection and needs

47. It is very important to work with trained expert teams (involving veterinarians) available currently in Tunisia and to perform trainings for new scientists to cover well Tunisian coasts and to increase number of samples.

48. Necropsies should be performed in adapted centres in order to respect sanitary precautions: Veterinarian laboratory of INSTM-Salammbô, marine turtle rescue center in the INSTM-Monastir and the centre of first aids in the university of Sfax. Teams/stakeholders involved in the national stranding network in other zones should carry out the necropsies and store the digestive samples in a freezer, and an external team (above centers) could then help with data collection.

49. 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 analyzed 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.

50. Lists of Materials required are provided in Annex I. They concern:

- A1.1. Examination of the animal and sample collection at the discovery site;
- A1.2. Sample collection from dead individuals and the extraction of ingested marine litter from the digestive tract; and
- A.1.3. Sample collection from live individuals and the extraction of ingested litter in faeces.

51. 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.).

52. The cost for the entire procedure of monitoring marine litter in sea turtles include

- the local network organization;
- the cost of the material,
- salaries of the involved staff.

53. For this consider 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).

2.3 Data banking

2.3.1 Creation of a standard database

54. 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 feces. 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.

55. The parameters in the observation sheet are presented in the SPA/RAC-UNEP/MAP, INDICIT (2019). The tutorial videos in Matiddi et al. (2019) and in Darmon, Raymond and Miaud (2017). show the procedure for recording data.

56. Following a period of data collection, data cleaning is a necessary step before statistical analyses to avoid errors. This can be highly time consuming and should be included in the staff's schedule.

2.3.2 Quality assurance/quality control

57. Ensuring quality assurance/quality control (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

58. 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. Involving in a regional platform to collect standardized data for the 'Marine litter ingestion by sea turtles' indicator between INFO-RAC, UNEP/MAP, OSPAR and MSFD, as recommended in Regional Operational Strategy for Monitoring IMAP Candidate Indicator 24 (SPA/RAC–UN Environment/MAP, 2019), is also very recommended.

2.4 Capacity building for monitoring throughout the Tunisian coasts

59. 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 Tunisian coasts.

60. The national action plan for the conservation of marine turtles (UNEP-MAP-SPA/RAC, 2020) recommends, among other things:

- Restructure and strengthen the existing national stranding network;
- Prepare a national program under the responsibility of INSTM;
- Extend the network over the entire Tunisian coast and improve the flow of communication and information;

2.4.1 Strengthening of the existing network

61. Currently, the national stranding network activities cover all Tunisian coasts but new monitoring teams should be established to enhance data collection in some areas not well monitored. These monitoring teams will include necessary biologists and veterinarians framed by the INSTM and CNVZS.

62. Records of marine turtles stranding by NGOs and other stakeholders, generally posted on Facebook, should be organized under the umbrella of the INSTM in the framework of participatory sciences.

63. Annex II provides current Capacity for monitoring litter impacts on sea turtles in Tunisia: Contacts and organizations involved (or potentially involved) in monitoring marine litter ingestion in sea turtles.

64. Collection of living and dead specimens relies on interaction between stranding network and rescue centres, along with close collaboration by fishermen, coast guards and other stakeholders. 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.

65. As marine turtle species 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 (Coordination commission of the national stranding network appointed by the annual order of the Minister of Agriculture).

66. 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 centers (Camedda et al., 2014). In Tunisia, a rescue centre, in Monastir, launched in 2004 achieves such activities. A new first aid centre was recently created (2021) in Sfax will contribute to enhance such data collection.

67. For collecting data in a more standardized way, Tunisia has (i) a national stranding network, responsible for the observation and recovery of dead or live turtles (alerting the relevant monitoring centers of a discovered turtle, taking the first measurements and recovering the specimen); (ii) a rescue center, responsible for the medical care of live individuals brought in by stranding networks or fishermen; (iii) A new first aid centre in Sfax, giving medical care of live individuals; (iv) veterinarian institute, responsible for external examinations and necropsies, and (v) research laboratories, some of which have several roles. Other existing institutions could also be involved.

68. More synergy should be established with institutes involved in projects related to marine litter (e.g. Medbycatch, COMMON, INDICIT (previously), laboratory programme…).

69. 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 (monitoring of nesting sites, fishery programmes…)

70. several institutions and stakeholders need more human and material resources and trained experts in this for conducting regular monitoring of marine litter impacts on sea turtles. These institutions should be aware of existing of available tools such as protocol of data collection and tutorial videos.

71. In Tunisia, national stranding network, 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.

72. INSTM and CVZS were involved in monitoring marine litter ingestion and entanglement by sea turtles, through the INDICIT project (2017–2021), which provided training sessions on collecting and sharing data.

73. The national network could be involved in the Mediterranean-wide scale network of all Contracting Parties to the Barcelona Convention which was recommend to be developed (SPA/RAC– UN Environment/MAP, 2019).

74. To consolidate networks in Mediterranean, SPA/RAC has recommended that more institutions be involved in such projects (SPA/RAC-UNEP/MAP, 2018). Participation at events such as workshops on marine litter impacts should be encouraged. Such events are valuable to disseminate tools and share knowledge.

2.4.2 Organization of the network

75. 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, coordinated by the INSTM, that is considered the unique point of reference at the national level.

76. Two main critical needs are necessary for 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, specific workshops aimed at involving these stakeholders in the study of marine litter impacts on marine fauna are recommended.

2.4.3 Recommendations for Capacity building

77. The SPA/RAC report (UNEP/MAP- SPA/RAC, 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 or other institution, 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

78. One meeting year for the national partners to discuss the progress of the program on the data collection and eventual improving of the procedure. This meeting can also discuss the participation to the two-year workshop at the Mediterranean scale proposed by the Regional Operational Strategy for Monitoring IMAP Candidate Indicator 24 (SPA/RAC–UN Environment/MAP, 2019).

2.5 Data analysis

2.5.1 Assessment

79. 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 frequency of occurrence 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 (all observed pieces).
- The dry mass (in grams) of food remains (accurate to two decimal places).

80. 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., 2019). This is calculated from the sum per individual of the dry mass of each plastic category (IND PLA, USE SHE, USE FRAG, USE THR, USE FOA, USE POTH) (Table 2). 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 is also recommended following The INDICIT consortium recommendation.

81. Other information such as the color 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.

82. 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). For this, it is recommended to specify the type of item in the column Note when the identification was possible.

83. Darmon, INDICIT consortium, Miaud (2019) and Matiddi et al. (2019) provide an example of data analysis and methodology. These documents recommend evaluating the current state of play and trends at national level in terms of biological constraints and GES. As more data is required to get an accurate assessment of the current situation across the Mediterranean, Tunisian data will improve such assessment.

2.5.2 Constraints

84. 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).

85. 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 parameters considered, including parameters considered a proxy of an individual's age (stage, carapace length, weight) and body condition (injuries, fat reserves, etc.). At this stage, there is no clear evidence of an influence of these factors in the Mediterranean basin, even the circumstances of discovery (generally bycatch or stranding, the latter sometimes the result of the former) (Darmon, INDICIT consortium, Miaud, 2019; Darmon et al., no published data). Thus, it is suggested not to perform data stratification at this stage; however, collect of more data – including data considered optional in the SPA/RAC- INDICIT protocol (2019) – for more powerful analyses is encouraged. 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 Good Environmental Status

86. In the context of a monitoring programme, data related to marine litter ingestion should be analyzed as a bio-indicator of the impact on the coastal and marine environment (Matiddi et al.,2019) 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.

87. 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., 2017) 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) and improved in the frame of INDICIT II project.

88. 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 or Z pieces of ingested plastic", 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. Z is the number of plastic pieces ingested.

89. The second scenario-INDICIT approach- (Matiddi et al., 2017; INDICIT consortium (Miaud, Darmon) 2019) 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". 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 are excluded to avoid the possibility that the individual is not feeding during a reproductive period or due to illness.

90. Data obtained during INDICIT II project based on more than 1100 necropsied loggerhead turtles from the 1988 do not provide a precise knowledge of the health of individuals and especially the impact of litter on health (Darmon et al., to be submitted). At this stage, an OSPAR type approach based on the abundance of ingested litter pieces would provide the more powerful models. The INDICIT II project recommends continuing to collect accurate health information and recording the quantity of natural food ingested in order to deepen the link between litter ingestion and health (Darmon et al., unpublished data).

91. 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. Based on the average occurrence and quantity of ingested litter calculated per country, the minimum appears in the Eastern Mediterranean (Fig. 2), which was considered by INDICIT II project as a reference for comparing GES scenarios and distance to GES. As a substitute, the reference could be based on the minimum occurrence and dry mass of plastics ingested by sea turtles in the prospected area. Further analyses are needed in order to define the most constructive baseline and threshold.

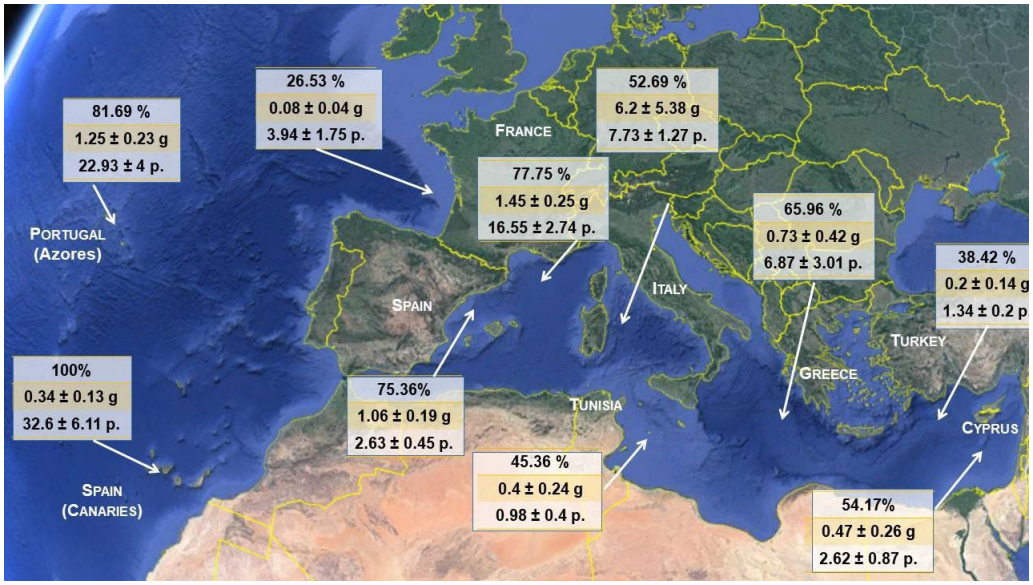


Figure 2: Litter ingestion by the necropsied loggerheads in each country and façade: Occurrence (%), mean dry mass of ingested plastics (g), mean abundance of plastic (number of pieces, p.) (Darmon et al., to be submitted).

2.5.4 Sample size/ Temporal scale

92. 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. A sample size of 50 sea turtles per year has been proposed (Matiddi et al., 2019) for a trend analysis over 3-6 years, is required for the entire Mediterranean. For Tunisia and on the basis of some experiences sample size > 30 sea turtles per year over 3-6 years will be acceptable.

3. RECOMMENDATIONS FOR IMPLEMENTING MONITORING

93. Tunisia is ready to implement a 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.

94. 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.

95. Tunisia is already 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. 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). The GES threshold value and the distance of each country from the threshold is being further evaluated and should be specified after more data is collected from all the Contracting Parties to the Barcelona Convention.

96. National network should be coordinated nationally to monitor all rescue and stranding activities occurring in Tunisia and should be equipped with a database. Its connection to the INFO-RAC system is recommended. This System (IMAP (Pilot) Info System), supported by the EU funded EcAp- MED II Project, enables the Contracting Parties reporting data for selected 11 IMAP Common Indicators as requested by the Integrated Monitoring and Assessment Programme (IMAP), adopted by COP 19 (Decision IG.22/7) http://www.info-rac.org/.../infoma.../imap-pilot-platform...

97. 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). 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.

Annex I

Checklists of materials

Annex I: Checklists of materials (SPA/RAC-INDICIT protocol (SPA/RAC – ONU Environment/ PAM, INDICIT, 2019)

A-1: Materials for 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

A- 2. Materials for 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)

A-3 Materials for 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
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

Current Capacity for monitoring litter impacts on sea turtles in Tunisia: Available means and needs for collecting living or dead sea turtle specimens and recording data on marine litter impacts

Annex II: Current Capacity for monitoring litter impacts on sea turtles in Tunisia: Available means and needs for collecting living or dead sea turtle specimens and recording data on marine litter impacts

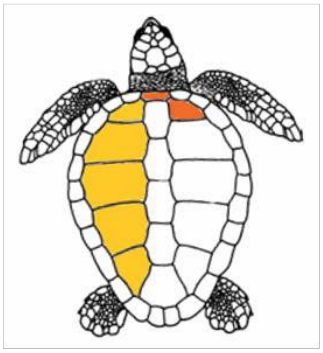
Available means
National stranding network managed by the INSTM along all Tunisian coasts Rescue centre managed by the INSTM (Monastir) First aid centre managed by the university of Sfax (FSS-Sfax) Research laboratory in the INSTM Sfax Veterinarian laboratory in the INSTM – Salammbô Veterinarian laboratory in the CNVZ - Tunis
Sea turtle monitoring contact / expertise
Mohamed Nejmeddine Bradai (Research laboratory, INSTM - Sfax) Olfa Chaieb (Research laboratory and Rescue centre, INSTM-Monastir) Kaouthar Maatouk (Veterinarian, INSTM-Monastir) H. Attia Hili (Veterinarian, CNVZ Tunis) Imed Jribi (First aid centre FSS- Sfax) Sami Karaa (Expert on marine turtles- Sfax)
Trained persons on marine debris for eventual contribution Sub-regional training session on marine debris and sea turtles (ingestion and entanglement) (Monastir, Tunisia November 1-2, 2018)
Idriss BEN NAILA (Veterinarian, marine biotoxin laboratory-Sfax) bennailaidriss@yahoo.fr Wiem KHALFAOUI (Veterinarian, CNVZ- Mahdia) wiemkhalfaoui80@yahoo.fr Walid ZROUD (Veterinarian, CNVZ- Mahdia) walidzroud@yahoo.fr Ahmed ZADDEM (APAL – Monastir) ahmedzaddem2@gmail.com Najet DIMASSI (APAL/NGB) dimassi.najet@hotmail.fr
Existing material
SPA/RAC-INDICIT protocol Video tutorial (Matiddi et al., 2019) Video tutorial (Darmon, Raymond and Miaud, 2017)

Annex III

Identification of the three common marine turtles in Tunisia

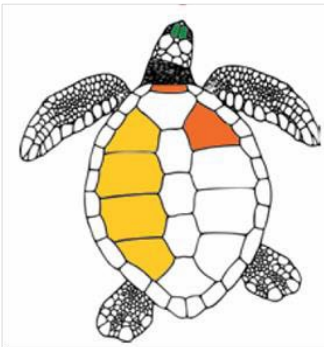
Annex III: Identification of the three common marine turtles in Tunisia

Carapace hard with scutes
5 costal scutes;
1st touches nuchal



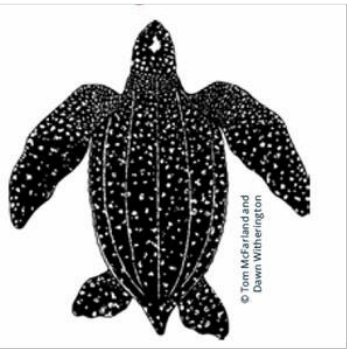
Loggerhead
Caretta caretta

Carapace hard with scutes
4 costal scutes ,
1st does not touch nuchal
One pair pre-frontal scales



Green turtle
Chelonia mydas

Leathery; No scutes; 5 ridges



Leatherback
Dermochelys coriacea

Annex IV

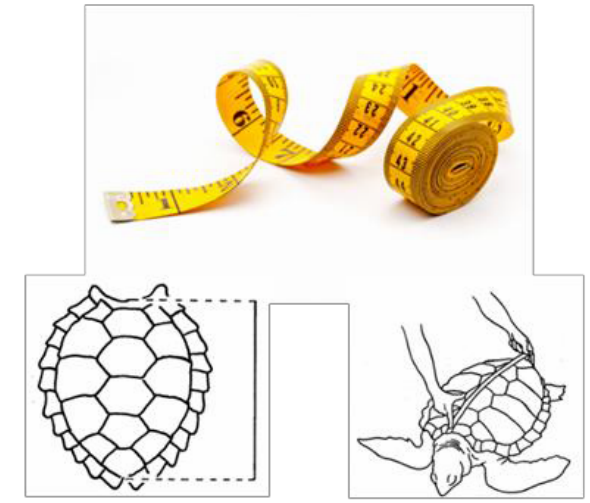
Assessing the status of a turtle from the concavity of its plastron. Drawings by V. Hergueta, modified after Thomson et al. (2009)

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

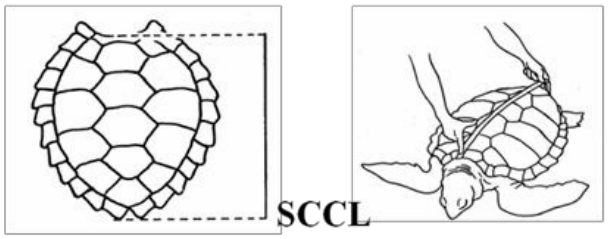
Annex V

Main biometric measurements

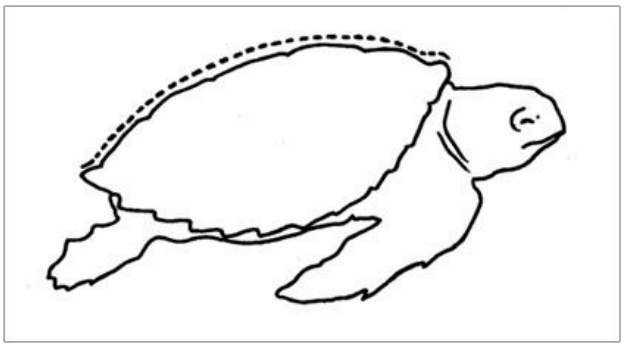
Annex V: Main biometric measurements

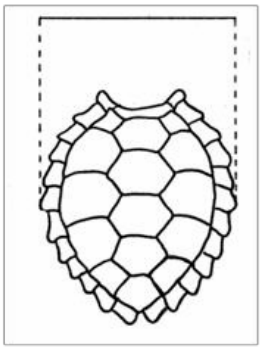


MCCL



SCCL



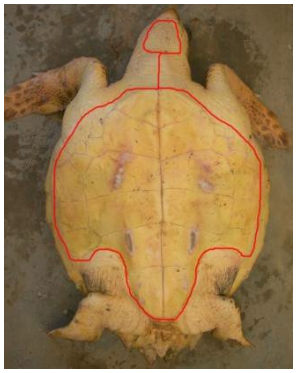


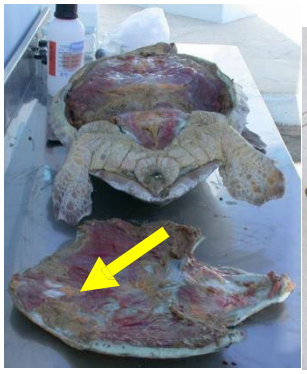
CCW

Annex VI

Necropsy

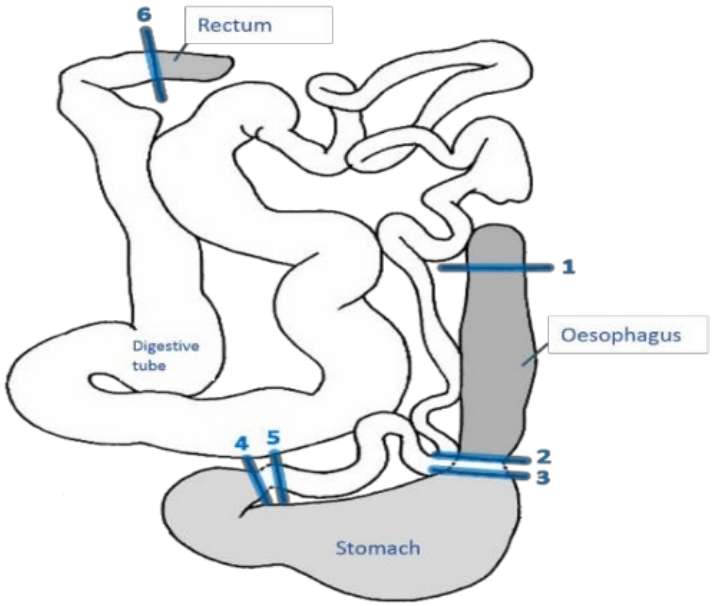
Annex VI: Necropsy





Cut line (red)

To release the plastron, the attachment to the Acromion process must be cut (arrow)



Separate the 3 parts of the GI (oesophagus, stomach, intestines) by adding a second strangling at the cut edge to prevent spillage of the contents. Drawing by V. Hergueta.

Annex VII

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