INDICIT
INDICATOR•IMPACT•TURTLE

IMPLEMENTATION of INDICATORS of MARINE LITTER IMPACTS on SEA TURTLES AND BIOTA

in Regional Sea Conventions and Marine Strategy Framework Directive Areas

FINAL REPORT

1st February 2018 - 31st January 2019

Darmon G., INDICIT Consortium and Miaud. C

Grant DG ENV. Number: 11.0661/2016/748064/SUB/ENV.C2

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Acknowledgements

The INDICIT consortium gratefully acknowledges the following persons and institutions for their precious support in the achievement of the project objectives, thanks to their participation in the collection of data, their advice and their improvement of the documents and tools.

Our heartfelt thanks to:

- Our collaborators, for the support in the collection of specimens and samples and for sharing scientific expertise:

France: Florence Dell'Amico (CESTM Aquarium La Rochelle, France), Delphine Gambaiani (CESTMED/EPHE, France), Jean-Baptiste Senegas (CESTMED), Sidonie Catteau (RTMMF), Jacques Sacchi (RTMMF), Cathy Cesarini (RTMMF), Jo Befort (Laboratoire vétérinaire du Gard), Jean-Marie Péricard, fishermen involved in recovering sea turtles, all volunteers of stranding networks, and Mélanie Nouen for communication tasks.

Greece: Reggina Stefanatou (ARCHELON, The Sea Turtle Protection Society of Greece).

Italy: Sandra Hochscheid (Stazione Zoologica Napoli); Cristiano Cocumelli (Istituto Zooprofilattico Sperimentale Lazio e Toscana); Ludovica Di Renzo (Istituto Zooprofilattico Sperimentale Abruzzo e Molise); Santo Caracappa (Istituto Zooprofilattico Sperimentale Sicilia), Giorgio Massaro, Roberto Brundu (Amp "Penisola del Sinis – Isola di Mal di Ventre), Stefania Coppa, Luca Palazzo (National Research Council - CReS – Centro di Recupero del Sinis), Angelo Ruiu (Istituto Zooprofilattico della Sardegna, Oristano), Paolo Briguglio (Clinica veterinaria "due mari" Oristano), Flavio Gagliardi (Acquario di Cala Gonone), Maria Ledda, Sergio Masala (Assessorato per la Difesa Ambiente della Regione Sardegna), Corpo Forestale e di Vigilanza Ambientale, Coast Guard and all the Sardinian Regional Network (Marine Protected Areas and National Parks), all the citizens and fishermen who contributed to the recover and conservation of sea turtles around Sardinian coast.

Spain: Francesc Domènech and the members of the Marine Zoology Unit of the Cavanilles Institute (UVEG); Conselleria de Medio Ambiente, Agricultura, Canvio Climático y Desarrollo Rural de la Generalitat Valenciana; A. Casquet-Pérez (CRAM Foundation), Carolina Fernández-Maldonado (CEGMA), Eva M. Morón-Manchado (EQUINAC); M. J. Gens-Abujas and Fernando Escribano-Cánovas (CR El Valle); Gloria Fernández (Palma Aquarium); J. L. Crespo J. and V. Marco (Oceanografic Foundation); J. J. Castillo-Martín (CREMA).

Portugal: RACA, Flying Sharks, Thomas Dellinger (University of Madeira), Cláudia Moreira (University of Madeira) and all voluntaries of stranding networks.

Tunisia: Garde nationale tunisienne, Agence de protection et d'aménagement du Littoral, Centre national de veille zoologique, Association jeunes sciences kerkennah (Dr Sami Karaa), Association Notre Grand Bleu

Turkey: EKODOSD (Ekosistemi Koruma ve Doğa Sevenler Derneği); AKUT Bodrum Association, Bodrum Branch Office.

- The members of the External Advisory Board, for their advice:

Benjamin Guichard (AFB, France), Filipe JM Porteiro (DRAM), Gilberto MP Carreira (DRAM), Marco Aurelio Santos (DRAM), Ana Tejedor Arceredillo (EEA), Jesus Gago (IEO), François Galgani (TG ML, IFREMER, France),

Marta Martínez-Gil Pardo de Vera (MAGRAMA), Isabelle Terrier (MTES, France), Oliviero Montanaro (MATTM, Italy), Roberto Giangreco (MATTM, Italy), John Mouat (OSPAR), Philip Stamp (OSPAR), Lobna Ben Nakhla (RAC/SPA), Stefanie Werner (TG ML), Tatjana Hema (UNEP/MAP), Christos Iokeimidis (UNEP/MAP).

- Mr Michail Papadoyannakis (European Commission, Marine Environment and Water Industry, Directorate General Environment), Policy Officer, for scientific advice and recommendations for a better coherence with MSFD requirements,

The INDICIT consortium also warmly thanks the administrative teams for their precious support in the coordination of the project:

France (CNRS) : Richard Joffre, Chloé Richard, Elodie Vatonne, Mathilde Büchler, Marjorie Gadéa, Mélanie Dumeige

Greece (HCMR): Roula Frangou

Italy: Andrea Fabbri (financial manager ISPRA) Giuseppe Tedesco (ISPRA), Lucia Gigante (ISPRA) and Emi Morroni head of the Department Bio (ISPRA)

Spain: Ángeles Sanchís, Sebastián Torrijos, Pablo Aller and the staff of the OPER-UVEG

Portugal: (FRCT): Francisco Pinto, Natividade Machado, Fábio Vieira

European Commission, Marine Environment and Water Industry, Directorate General Environment: Cecile Leroy

Project Full Title	IMPLEMENTATION OF THE INDICATOR OF MARINE LITTER ON SEA TURTLES AND BIOTA IN RSC AND MSFD AREAS		
Project Acronym	INDICIT		
Grant Agreement n°	11.0661/2016/748064/SUB/ENV.C2		
Coordinator, institution	C. MIAUD and G. Darmon EPHE-CNRS		
Start date, duration	1st February 2017, 24 months		
Project website	https://indicit-europa.eu		
Deliverable n°	D1.11	**Deliverable Date**	31/03/2019
Status:	**Final (F)**	(F)	
	Draft (D)		
	Revised draft (RV)		
Dissemination level:	**Public (PU)**	(PU)	
	Restricted to other program participants (PP)		
	Restricted to a group specified by the consortium (RE)		
	Confidential, only for members of the consortium (CO)		

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AFB: French Agency of Biodiversity

CEMP: Coordinated Environmental Monitoring Programme

CleanAtlantic project: Tackling Marine Litter in the Atlantic Area, Atlantic Area Transnational Program 2014-2020

CNRS: French National Scientific Research Council

DG Env: Environment Directorate General Environment of the European Commission

DRAM: Regional directorate of Sea Affaires of Azores government (Direção Regional dos Assuntos do Mar)

EAB: External Advisory Board

EMODNET: European Marine Observation and Data Network

EU: European Commission

EPHE: French School of Higher Studies

FOO: Natural food for sea turtles (e.g., pieces of crabs, jellyfish, algae…)

FRCT: Regional Fund for Science and Technology

GES: Good Environmental Status

GTMF: Working Group Marine Turtle France

HCMR: Hellenic Centre for Marine Research

HELCOM: Convention on the Protection of the Marine Environment of the Baltic Sea Area (Helsinki convention)

IAMC-CNR: Institute for the Marine Coastal Environment of the National Research Council

ICES: International Council for the Exploration of the Sea of OSPAR

IEO: Spanish Institute of oceanography

IFREMER: French Research Institute for Exploitation of the Sea

IMAR: Institute of Marine Research

INDICIT: European project “Implementation of the indicator of marine litter on sea turtles and biota in RSC and MSFD areas (Indicator Impact Turtles)”. N° GA11.0661/2016/748064/SUB/ENV.C2

INDICIT-II: European project “Implementation of the indicator of marine litter on sea turtles and biota in RSC and MSFD areas (Indicator Impact Taxa). No. 110661/2018/794561/SUB/ENV.C2

IND PLA: Industrial plastic granules, usually cylindrical but also sometimes oval spherical or cubical shapes, or suspected industrial item, used for the tiny spheres (glassy, milky...)

INSTM: Tunisian National Institute of Sciences and Technologies of sea

ISPRA: Italian National Institute for Environmental Protection and Research

ISTS: International Sea Turtle Society

MAGRAMA: Spanish Ministry of Agriculture, Food and Environment

MATTM: Italian Ministry of Environment

MEDCIS: Support Mediterranean Member States towards coherent and coordinated implementation of the second phase of the Marine Strategy Framework Directive.

MEDPOL: Programme for the Assessment and Control of Pollution in the Mediterranean region of UNEP-MAP

MedSeaLitter: Interreg MED Project co-financed by the European Regional Development Fund, for the development of protocols to monitor marine litter abundance and impacts at basin and local scale for the Mediterranean Marine Protected Areas

MEEM: French Ministry of Environment, Energy and Sea

MISTIC SEAS (I & II): Macaronesia Islands standard indicator and criteria. Reaching common ground on monitoring marine biodiversity in Macaronesia

MNHN: French National Natural History Museum

MPA: Marine Protected area

MSFD: Marine Strategy Framework Directive

MSFD TG ML: Target Group Marine Litter of the Marine Strategy Framework Directive

NFO: Anything natural, but which cannot be considered as normal nutritious food for sea turtle (stone, wood, pumice, etc.)

OSPAR convention: Convention for the Protection of the Marine Environment of the North-East Atlantic (Oslo-Paris convention)

PAU-DEKAMER: Turkish Sea Turtle Rescue Centre

POM: Program of Measure

RAC/SPA: Regional Activity Centre for Specially Protected Areas

RSC: Regional Sea Convention

ULPGC: University of Las Palmas de Gran Canaria

UNEP-MAP: Mediterranean Action Plan of the United Nations Environment Programme

USE FOA: All foamed plastics e.g. polystyrene foam, foamed soft rubber (as in mattress filling)…

USE FRAG: Fragments, broken pieces of thicker type plastics, can be a bit flexible, but not like sheet like materials.

USE POTH: Any other plastic type of plastics, including elastics, dense rubber, balloon pieces, soft air gun bullets… Specify in the column “Notes”.

USE SHE: Remains of sheet, e.g. from bag, cling-foil, agricultural sheets, rubbish bags…

USE THR: Threadlike materials, e.g. pieces of nylon wire, net-fragments, woven clothing…

UVEG: University of Valencia

Context

This report presents the outputs of the two-years INDICIT project (1st February 2017 – 31st January 2019), which aimed to support the Regional Sea Conventions (RSC) OSPAR, Barcelona and HELCOM and the Marine Strategy Framework Directive (MSFD) in the implementation of litter impact on marine fauna indicators. These indicators belong to the Criteria D10C3 and D10C4, according to the New Commission Decision 2017/848/EC. This document corresponds to the deliverable n° D1.11, the last of the project (deliverables listed in Appendix 1).

Introduction

Marine litter is defined as "any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment". Anthropogenic marine litter consists of items that have been made or used by people and deliberately discarded or unintentionally lost into the sea and on beaches including such materials transported into the marine environment from land by rivers, draining or sewage systems or winds (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 and chemicals (see general definitions in Appendix 2).

Anthropogenic marine litter, 60 to 80% of which being plastic, is an issue of global concern (Derraik, 2002). Ubiquitous in the marine environment (Cózar et al., 2014; Derraik, 2002; Eriksen et al., 2014), huge amounts of items are continuously entering the seas and oceans (Jambeck et al., 2015). Due to its semi-closed configuration, the Mediterranean Sea is particularly concerned (Galgani et al., 2015). More than 62 million floating litter items are evaluated to enter this sea (Suaria and Aliani, 2014).

In addition to aesthetic and financial problems (Hardesty et al., 2015), litter pollution has a major ecological impact on marine habitats and fauna (Andrady, 2011a; Thompson et al., 2014; Vegter et al., 2014). The number of species known to be impacted, especially because of the ingestion of litter or entanglement (Gall and Thompson, 2015), increases with studies that invest on this issue: From 247 species listed in Laist's review of 1997, 663 species have been counted in 2014 and 690 in 2015 (Gall and Thompson, 2015; Laist, 1997; Thompson et al., 2014). All sea turtle species, about half of mammal species and one-fifth of sea bird species, but also fish and invertebrates, have been observed with impacts related to marine litter (Cole et al., 2013 ; Schuyler et al., 2014; Thompson et al., 2014; Nelms et al. 2015), number of those concerned species being already classified as vulnerable or endangered on IUCN red list (e.g. sea turtle species).

Various reasons may explain why animals are entangled in or ingest litter. They may confound litter with natural preys (e.g., Schuyler et al., 2014), or ingest such items by inadvertence, since litter could be mixed in the food bowl (Anastasopoulou et al., 2013). Plastic durability and fragmentation will result in an increase of (secondary) micro-plastics (legacy items), defined as items below 5 mm (Barnes et al., 2009; Cole et al., 2011; Law and Thompson, 2014; MSFD GES TG ML, 2017; Thompson et al., 2004). This fragmentation will inevitably facilitate the ingestion of plastics by a greatest part of the food chain (Botterell et al., 2019; Vroom et al., 2017).

The impacts of plastic ingestion on health are still poorly understood. Direct mortality, caused by perforation or obstruction of the digestive system seems relatively rare. Sub-lethal effects are more likely, such as pathologies or changes in feeding and movement behaviours. Upon consumption of plastic items, the chemical

components constituting them, and the chemicals and metals they absorbed in the environment, may diffuse into the marine organisms' body (Rochman, 2015; Rochman et al., 2013a, 2013b; Tanaka et al., 2013). Mechanistic (e.g., obstruction, lack of an essential quantity of nutrients) and toxicological impacts related to plastic ingestion may lead to a decrease in body condition and may then affect individuals' chances of survival and reproduction. Possible repercussions on populations and trophic chain are conceivable, but difficult to prove quantitatively.

The characteristics and quantity of litter ingested by sentinel organisms can reflect the spatial and temporal trends of litter in the environment, and made available to organisms. Plastic ingested by the fulmar *Fulmarus glacialis* is a bio-indicator for the OSPAR Regional Sea Convention (RSC) (EcoQO 3.3; OSPAR, 2009) and the Marine Strategy Framework Directive 'MSFD) (indicator 10.2.1, EC 2010, see Appendix 2). A long term monitoring has shown the relevance of this indicator to assess the effectiveness of restoration measures, since the frequency of litter ingestion and the abundance of ingested litter in Fulmar populations varies in space and time with litter in the environment (van Franeker et al., 2011). Nonetheless, the absence of the Fulmar species in the Southern OSPAR areas and in the Mediterranean prevent the use of this indicator outside the OSPAR northern zones.

Sea turtles show a global distribution, occupying multiple habitats throughout their life cycle (Casale et al., 2008) and showing a propensity to ingest debris (Schuyler et al., 2014) in particular in the OSPAR (Darmon et al., 2014; Pham et al., 2017) and the Barcelona RSCs (Camedda et al., 2014; Matiddi et al., 2017). These characteristics make them a relevant indicator for MSFD and RSCs (Matiddi et al., 2011; MSFD Technical Subgroup on Marine Litter, 2013). They may also be relevant for monitoring impacts related to entanglement (Duncan et al., 2017; Nelms et al., 2015). Highly exposed to litter (Darmon et al., 2017a, and references therein), sea turtles can testify the spatial variations in litter abundance. Therefore, they have been proposed to evaluate the impact of litter according to the secondary Criteria D10C3 of Descriptor 10 "Marine Litter" of MSFD (see Appendix 2). The loggerhead turtle *Caretta caretta* being the most abundant species of sea turtle in the Mediterranean and commonly seen in the surrounding Atlantic waters, is the target species. Specimens are regularly collected by existing rescue centres and stranding networks, which enables the collection of data useful for acquiring knowledge on the species' biology and on the factors leading to litter ingestion or entanglement. The leatherback species *Dermochelys coriacea* has also been recommended especially for the OSPAR zones III, IV and V (Claro et al., 2014; Darmon and Miaud, 2016).

While sea turtles are relevant indicators for litter impacts related to ingestion (D10C3), with litter being considered superior to 1mm (Mattidi et al., 2011; MSFD Technical Subgroup on Marine Litter, 2013), a specific indicator should address distinctly the impacts of micro-litter items below 5 mm. Likewise, an indicator should also specifically concern impacts related to entanglement (D10C4). For these two new litter impact indicators, target taxa/species have to be identified.

The INDICIT project.

Among the projects funded by European Commission for supporting the implementation of MSFD, the project "Implementation of indicators of marine litter impacts on sea turtles and biota in Regional Sea Conventions and Marine Strategy Framework Directive Areas", with acronym INDICIT for "Indicator Impact Turtle", targeted the implantation of indicators of litter impacts for Descriptor 10 (Criteria D10C3 and D10C4). The project fitted into

three of the four marine regions covered by MSFD: Baltic Sea (HELCOM), North-east Atlantic Ocean (OSPAR) and Mediterranean Sea (Barcelona convention) (see Appendix 2).

INDICIT major objectives addressed three indicators of litter impact:

1) The "**Litter ingested by sea turtles**" indicator, belonging to D10C3 criteria (previously called indicator "10.2.1" associated with "Trends in the amount and composition of litter ingested by marine animals"). This indicator concerns litter from 1 mm, thus combining the micro-items under 5 mm (here from 1 to 5 mm) and the so-called meso-litter (from 5 to 25 mm) and macro-litter (>25 mm) (Andrady, 2011b; MSFD Technical Subgroup on Marine Litter, 2013).). This currently does not respect the Definition of the New Decision Commission 2017/848 which entails to consider litter size > 5 mm and micro-litter size < 5 mm. . Indeed, INDICIT project took over from MSFD guideline which provided a first protocol for monitoring litter ingestion in sea turtles based on the Fulmar protocol (Matiddi et al., 2011; MSFD Guideline, 2013). The OSPAR ecological indicator EcoQo for Fulmar, which was produced many years before the definition of micro- and macro-litter, took into consideration all litter items from 1 mm. The EcoQO was adopted by the TG ML group for the GES definition of marine litter indicators in the Northern European countries. When INDICIT project started, some stakeholders already collected data in sea turtles, especially in Italy, Spain and France, generally considering litter size >1 mm. Considering the difficulty to change habits and regarding the duration of the INDICIT project, the INDICIT consortium, together with the stakeholders in charge of specimen and data collection, have worked to propose a standard protocol including the differentiation of micro- and litter in data collection as an optional parameter (see §2.3). The relevance of a specific indicator for micro-litter ingested by sea turtles will be further assessed. The INDICIT proposal focused on this indicator, already candidate for both OSPAR and Barcelona RSCs, and in order to respond to the needs expressed by the 2010/477/EU Commission Decision for further development (see Appendix 2). For this reason, the specific objectives of the project were to develop tools for supporting the harmonization of monitoring programmes across MSFD marine regions, by:
    (i) identifying, enlarging and empowering the networks for the standard collection of data in both OSPAR-Macaronesia and Barcelona RSCs;
    (ii) providing and disseminating protocols and tutorials for the collection of standard data;
    (iii) analysing the standard data to define the indicators' possible biological constraints, sample size and spatial and temporal units;
    (iv) evaluating GES and Contracting Parties' distance to GES, which GES, regarding D10C3, corresponds to "the amount of litter and micro-litter ingested by marine animals that is at a level that does not adversely affect the health of the species concerned" (see Appendix 2).

2) The "**Entanglement with debris by marine biota**" indicator.
INDICIT proposal was to evaluate the feasibility of this new litter impact indicator at the levels of OSPAR, Barcelona and HELCOM RSCs, by assessing, thanks to a literature review and questionnaires to experts, the prevalence of entanglement in several taxa (cetaceans, fishes, birds, turtles, invertebrates), the possible target species, the network and survey methods, either already existing, or the potential for the implementation of a monitoring.

3) The "**Micro-plastic ingestion in fish and sea turtles**" indicator.
INDICIT proposal was to evaluate the feasibility of this new litter impact indicator. Sea turtles appeared relevant for a concrete application of such an indicator, due to existing networks and data, and the potential to specifically differentiate the size range 1 to 5 mm from data collected in the framework of indicator "Litter ingested by sea turtles". Fish was also evaluated, because of their exposure to particles inferior to 5 mm, enabling to integrate HELCOM (where the indicator "sea turtle" can be used) in addition to OSPAR and Barcelona RSCs. A literature review and analysis of data already available were done in order to evaluate the target species and the existing survey methods to collect samples and data.

The next chapter of this report presents INDICIT structure and approach. The INDICIT outputs are detailed in the following chapters, first the detailed results concerning the indicator "Litter ingestion in sea turtles": tools for networking, tools for the collection and reporting of standard data, knowledge acquired thanks to the data collected and gathered by INDICIT consortium and collaborators, then the GES scenarios for the implementation of the indicator, with recommendations and perspectives provided for each section. The two feasibility study reports on Entanglement and Micro-litter ingestion indicators can be found in the INDICIT website in the section Documents. They are summarized in the third chapter of this report.

1. INDICIT project: Structure and approaches

1.1. General presentation of the project

INDICIT project started the 1st February 2017 and finished the 31st January 2019. The total budget represented 1 328 119 euros, considering an allocation of DG Environment of 999 955 Euros added to the 20% of partners' co-funding.

INDICIT consortium was composed by a consortium of 10 partner institutions from public sector, belonging to 5 European countries (France, Greece, Italy, Portugal, Spain) and 2 non-European countries (Tunisia, Turkey), all contracting parties of Barcelona and/or OSPAR RSC (Figure 1): FRCT (Regional Fund for Science and Technology), ULPGC (The University of Las Palmas de Gran Canaria), UVEG (University of Valencia), MNHN (French National Natural History museum), CNRS (French National Centre of scientific Research) with EPHE (practical school) of advanced studies) as affiliated entity, ISPRA (Italian National Institute for Environmental Protection and Research), INSTM (Tunisian National Institute of Sciences and Technologies of sea), IAMC-CNR (Institute for the Marine Coastal Environment of the National Research Council), HCMR (Hellenic Centre for Marine Research), PAU-DEKAMER (Turkish Sea Turtle Rescue Centre). FRCT partner subcontracted IMAR (Institute of marine research), in order to obtain their scientific support in the follow up of the project. Both FRCT and IMAR participated in the INDICIT internal meetings.

Figure 1. Distribution of partners represented in INDICIT consortium (FRCT, ULPGC, UVEG, MNHN, CNRS, ISPRA, INSTM, IAMC-CNR, HCMR, PAU-DEKAMER. FRCT subcontracted IMAR to provide scientific expertise.

The project was structured in 5 inter-related "Act ivities" (Figure 2), all leaded by one partner.

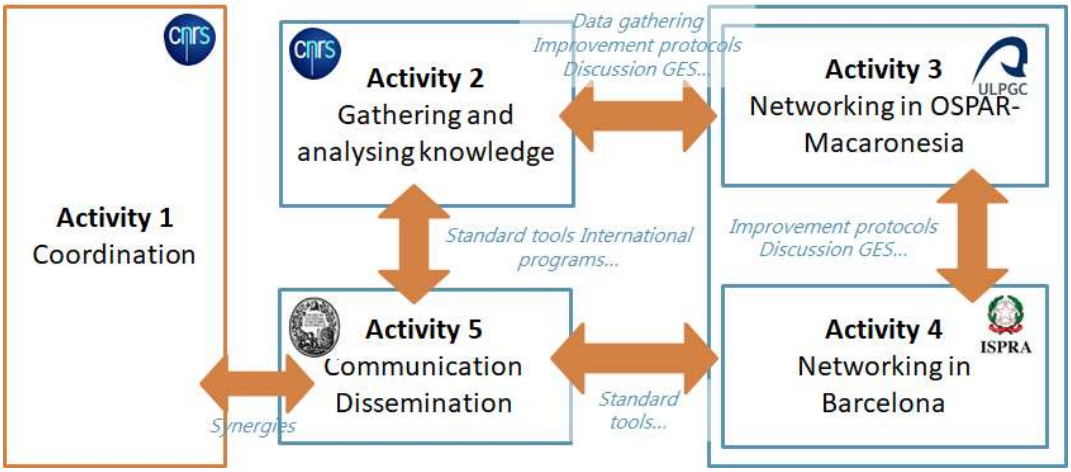

Figure 2. Inter-relationships among the five Activities

- **Activity 1 - Management and coordination**, leaded by CNRS/EPHE (Claude Miaud, Gaëlle Darmon).

The objective was to ensure the proper implementation and management of the project with respect to its objectives, time frame and budget constraints and to allow a smooth communication between the consortium members. This activity, involving all the members of the INDICIT Consortium, was divided in two tasks:

1) Task 1.1. "Technical coordination of the project", which aimed at ensuring an efficient technical coordination of the project,

2) Task 1.2. “Administrative, legal and financial management of the project” which coordinated the administrative, legal and financial management of the project in accordance with the articles of the Grant Agreement and of the Consortium Agreement signed by all project partners.

- **Activity 2 - Acquiring and using scientific knowledge to develop the indicators of litter impact at the sub-regional and the whole MSFD spatial scale**, leaded by CNRS/EPHE (Gaëlle Darmon, Claude Miaud)

This activity aimed to fill the gaps of knowledge needed to define GES and criteria for the indicator “Litter ingested by sea turtles” and to evaluate the relevance of two new impacts indicators “Entanglement with debris by marine biota” and “Micro-plastic ingestion by marine biota”. The objectives were:

(i) to propose standard tools to collect samples and data (designed and shared by Activity 5, see below), which are evaluated and improved by stakeholders in charge of data collection (Activities 3 and 4);
(ii) to gather and standardize data collected in Activities 3 and 4 (see below);
(iii) from a literature review and statistical analyses of data, to evaluate the possible biological constraints of the Indicator and evaluate GES scenarios for indicator “Litter ingestion by sea turtles” from a literature review and statistical analyses of data.

For the other two indicators, this Activity aimed to record the prevalence of the considered impact (entanglement / micro-plastic ingestion) per taxa/species, in order to evaluate the relevance of the indicator and, for proposing recommendation for the implementation of the indicator, to assess:

(i) the relevant indicator taxa/species;
(ii) the methodologies to sample individuals and collect data;
(iii) the existing networks and datasets.

The Activity 2 was divided into 6 tasks:

1) Task 2.1 “Establishment of a state-of-the-art on the biological constraints which can influence the indicators’ criteria”, coordinated by CNRS/EPHE, ULPGC and ISPRA and which aimed to provide a literature synthesis on the biological constraints which can influence the criteria of the 3 indicators;

2) Task 2.2 “Pilot study for the indicator “Entanglement with debris by marine biota” and “Micro-plastic ingestion by fish and sea turtles”, which aimed to provide a feasibility study of the potential implementation of the 2 new impact indicators at the OSPAR, HELCOM and Barcelona RSCs levels;

3) Task 2.3 “Improvement and standardization of the monitoring for the indicator 10DC3”, the objective being to develop a feasible set of procedures to collect data for the estimation of the indicator “Litter ingested by sea turtles”;

4) Task 2.4 “Establishment of common databases in order to gather existing and new data for indicator “Litter ingested by sea turtles”, which aimed to closely work with Activities 3 and 4;

5) Task 2.5 “Evaluation and/or revision of GES initial assessment, distance to GES and indicator criteria”, which aimed to provide/update GES scenarios and the criteria for the implementation of Indicator 1 (i.e., possible biological constraints, spatial and temporal units, sample size);

6) Task 2.6 “Evaluation of the influence of practical restoration measures implemented in pilot areas on the evaluated distance to the target of GES(s)”, which aimed to evaluate the changes in the indicator “Litter ingestion by sea turtles’ with litter management in pilot areas.

- **Activity 3 - Implementation of the indicator “Impacts of marine litter on sea turtle and biota” in RSC OSPAR/Macaronesia regions**, leaded by ULPGC (Ana Liria Loza),

This activity intended to implement a perennial and sustainable monitoring programme for Indicator 1 “Litter ingested by sea turtles” in OSPAR, Macaronesia being included for more coherence. The main objectives consisted in:

(i) networking and training stakeholders to collect standard data from the tools elaborated in Activity 2;
(ii) collecting and gathering data with Activity 2;
(iii) participating in the improvement of standard tools for the implementation of the indicator.

The Activity was divided into 5 tasks:

1) Task 3.1 “Identification of the local stakeholders to be mobilized in France, Spain and Portugal”, which aimed to network the stakeholders (rescue centres, stranding networks, laboratories, universities, etc.) who will be in charge of the collection of dead and/or live sea turtles and/or of the laboratory analyses to collect the litter ingested by these individuals;

2) Task 3.2 “Local training on the established protocols for data collection to the identified stakeholders”, which aimed to improve the tools for monitoring (protocol) and empowering the stakeholders thanks to the local dissemination and training sessions;

3) Task 3.3 “Sharing of already available data in the common databases”, which aimed to share stakeholders’ available literature and data on litter ingested (micro and macro-items) by dead and live individuals and on entanglement in the RSC area with Activity 2;

4) Task 3.4 “Collection of new data and uploading to the common databases”, which aimed to support the determination and the constraints of the indicator by collecting and gathering data;

5) Task 3.5 “Validation and implementation of the GES (baseline or trends) and indicators’ criteria with the stakeholders at the RSC scale, and evaluation of each Member states/sub-regions’ distance to the target of GES” which aimed to validate with Activity 2, the GES produced thanks to the results of data analysis.

- **Activity 4 - Implementation of the indicator “Impacts of marine litter on sea turtle and biota” in UNEP/MAP EcAp process (Barcelona convention)**, leaded by ISPRA (Marco Matiddi, Cecilia Silvestri)

In parallel to Activity 3, this Activity aimed to implement a sustainable monitoring programme for the indicator “Litter ingested by sea turtles” at the Barcelona RSC level. The main objectives were the same than in Activity 3, but targeting MSFD Contracting Parties and countries outside EU belonging to the Barcelona convention for a greater coherence in the assessment of GES and harmonization of monitoring for evaluating restoration measures, the dissemination at a large level being facilitated by Activity 5. The Activity 4 was divided into 5 tasks, with the same objectives than the tasks in Activity 3:

1) Task 4.1 “Identification of the local stakeholders to be mobilized in Italy, Greece, France and Spain”;

2) Task 4.2 “Local training on the established protocols for data collection to the identified stakeholders”;

3) Task 4.3 “Sharing of available data in the common databases”;

4) Task 4.4 “Collection of new data and uploading them into the common databases”;

5) Task 4.5 “Validation and implementation of the GES (baseline or trends) and indicator s’ criteria with the stakeholders at the RSC scale, and evaluation of each Member states/sub-regions’ distance to the target of GES”.

- **Activity 5 – Communication and Dissemination**, leaded by MNHN (Françoise Claro, Judicaëlle Moussier).

This activity aimed to communicate about the project and its outcomes:

(i) by providing technical tools for the implementation of a monitoring on debris impacts to stakeholders within or outside INDICIT partners’ area;
(ii) by communicating about the project context, objectives and results towards a general audience in order to raise public awareness.

This Activity was divided into 2 tasks:

1) Task 5.1 “Dissemination of the developed tools for the monitoring of litter impacts on sea turtles”, which aimed to share the project’s outputs between consortium partners and to widely disseminate tools and results to stakeholders belonging to other countries either included in the MSFD area or other RSCs (e.g., HELCOM) or not (e.g., Southern Mediterranean countries);

2) Task 5.2 “Communication activities”, which aimed to communicate about the program’s context and results to the large audience for awareness about litter impacts on marine ecosystems.

One of the major goals of Activities 3 and 4 was to establish a network of stakeholders in charge of the sustained monitoring of litter ingestion by sea turtles, respectively in OSPAR-Macaronesia and Barcelona RSCs. The close collaboration with stakeholders was fundamental to achieve INDICIT objectives. The data they collected was shared with Activity 2, in which the data was compiled, cleaned and analysed in order to evaluate the indicator’s possible constraints and GES assessments. At the same time, the protocols produced in the framework of Activity 2 were reviewed and improved thanks to stakeholders involved in Activities 3 and 4, who implemented them in the field and for some of them, also in laboratory. Finally, the documents were compiled in the framework of Activity 5, for standard design and wide dissemination.

1.2. Coordination and internal organization

The consortium greatly benefited from the advice of its Policy Officer, Mr Michail Papadoyannakis and an External Advisory Board composed of representatives of MSFD, RSCs and Member States (EAB, Table 1), in order to ensure that INDICIT outcomes will allow a harmonized and sustained monitoring of litter impacts at the MSFD and RSCs levels, with coherence among national and regional cooperation processes. The Steering Committee,

who could vote the project's strategies, validated and completed the EAB composition during the project (Table 2).

Table 1: Members of INDICIT External Advisory Board (as presented in the proposal; in grey, new members added during the project)

Organization	EAB members
OSPAR Commission	John MOUAT, replaced in July 2018 by Philip STAMP (Deputy secretary, London, UK)
HELCOM Commission	Stefanie WERNER (UBA, German Environment Ministry)
Barcelona convention secretary	Tatjana HEMA, Christos Ioakeimidis
Barcelona convention secretary	Lobna BEN NAKHLA
MATTM (Italian Ministry of Environment)	Oliviero MONTANARO/ROBERTO GIANGRECO
MEEM : French Ministry of Environment, Energy and Sea	Isabelle TERRIER
MPAs: French Marine Protected areas	Benjamin GUICHARD
MAGRAMA (Ministry of Agriculture, Food and Environment)	Marta MARTÍNEZ-GIL PARDO DE VERA
IEO (Spanish Institute of oceanography)	Jesús GAGO
IFREMER	François GALGANI
DRAM, Azores government	Gilberto MP. CARREIRA / Marco A. SANTOS

Table 2. Members of INDICIT Steering Committee (as presented in the proposal; in grey, new members involved during the project)

Institution	Representative	Substitute
CNRS	Claude MIAUD *(male)*	Gaëlle DARMON (female)
MNHN	Françoise CLARO *(female)*	Judicaëlle MOUSSIER (female)
ISPRA	Marco MATIDDI *(male)*	Cecilia SILVESTRI (*female*)
CNR-IAMC	Giuseppe DE LUCIA *(male)*	Andrea CAMEDDA (*male*)
HCMR	Eleni KABERI *(female)*	Catherine TSANGARIS (*female*)
UVEG	Jesús TOMÁS *(male)*	Ohiana REVUELTA (*female*)
ULPGC	Ana LIRIA LOZA *(female)*	Ricardo J. HAROUN TABRAUE (*male*)
PAU-DEKAMER	Yakup KASKA *(male)*	Dogan SOZBILEN (*male*)
FRCT	Maria Luz PARAMIO MARTIN *(female)*	Francisco Pinto (*male*)
INSTM	Mohammed Nejmeddine BRADAI (*male*)	Olfa CHAIEB (*female*)

CNRS managed the inception report as well as the INDICIT consortium agreement signed by beneficiaries, which referred to the rules and respective obligations among partners and towards EC. An amendment was added, in order to consider the conditions asked by some stakeholders, that the data shared by them could be used only for evaluating the constraints and GES of litter impact indicators (see §2.1.3). CNRS also managed the 6-month intermediate reports, compiling the outputs of each Activity sent by each Activity leaders (with ULPGC, ISPRA and MNHN), at each Reporting Period, as well as the financial reports.

Regular meetings and workshops were organized among INDICIT consortium (Table 3). EAB was invited by CNRS/EPHE to the intermediate meeting in February 2018 and by MNHN, HCMR and CNRS/EPHE to the Dissemination meeting in December 2018. The minutes of the regular meetings (Deliverables D1.2 to D1.6, see Appendix 1) and Dissemination meeting (Deliverable D5.12) are available in INDICIT website.

Table 3. Internal meetings for INDICIT partners

Date	Location	Type of meeting and participants	Objectives
Month 1	Brussels (Belgium)	Kick-off meeting (all partners and DG representatives)	Mutual presentation of partners + presentation of the project to DG representatives
		1st meeting (all partners + External Advisory Board)	Facilitation exercises for collective decisions on the programme's orientation
Month 6	Rome (Italy)	Intermediate meeting n°1 (all partners)	Progresses presented by each Activity Leader
		Workshop n° 2 (all partners)	Sharing technical skills + training session
Month 13	Montpellier (France)	Intermediate meeting n°2 (all partners)	Progresses presented by each Activity Leader
		Workshop n° 3 (all partners + EAB)	(parallel sessions on Activities 2,3,4,5)
Month 20	Sao Miguel, Azores (Portugal)	Intermediate meeting n°3 (all partners)	Progresses presented by each Activity Leader
		Workshop n° 4	Decisions on data classification and analyses
Month 24	Brussels (Belgium)	Final meeting	Final results

Meanwhile, partners communicated remotely by regular emails and videoconferences, in particular Activity leaders as soon as a specific organizational question was raised. For the second year of the project, ULPGC and FRCT organized a one-month videoconference for partners involved in Activity 3.

MNHN, regularly supported by all partners, shared news within Activity 5, which facilitated the exchange of news, both among partners and with EAB, by providing regular newsletter sent by email, on the website and INDICIT Facebook. Each partner also regularly communicated with EAB. EAB members sent several feedbacks, especially to comment on the news, helped in the improvement of the feasibility studies, and provided general and specific advice during the project meeting in Montpellier and the Dissemination meeting in Athens.

Partners also regularly interacted with other experts and other projects in which they were actively involved; e.g., CNRS/EPHE, ISPRA, UVEG and HCMR involved in MedSeaLitter, HCMR and ISPRA in MEDCIS, FRCT in MISTIC

SEAS II. As far as possible, partners participated in other meetings to share expertise (WG GES, TG ML, RSCs meetings, other DG Env funded meetings, etc.). They also shared expertise and INDICIT outputs in international conferences (MedTurtle conference, the symposium of the International Sea Turtle Society [ISTS], etc.) in order to disseminate INDICIT tools and possibly enlarge the network and collect a greater dataset. In that respect, the INDICIT protocol was shared with international expert communities in a peer-reviewed journal (JOVE, video-article managed by ISPRA, published) and other articles are planned.

The Project Management Team (CNRS/EPHE) regularly communicated with the PO and DG ENV. All draft deliverables were submitted to the PO, who greatly helped improving the documents for an applicable implementation of the indicators in keeping with MSFD requirements.

1.3. Communication outputs

The Communication outputs are presented in the Final report on Communication and in the Final report on Dissemination provided by MNHN (deliverables D5.7 and D5.9, available on INDICIT website).

The tasks related to Communication were treated in Activity 5 and coordinated by MNHN. INDICIT consortium decided to limit the plastic in the activities as far as possible (e.g., preference for "useful" goodies (USB keys, porcelain mug, cloth bag); no paper pamphlet produced (but printable upon request online); glassware used during meetings, etc.).

Several tools were elaborated to communicate within and outside INDICIT, such as:

- Specific email addresses for coordination managed by CNRS/EPHE and communication managed by MNHN, respectively coordination@indicit-europa.eu and communication@indicit-europa.eu;
- The logo and the graphic chart,
- The website https://indicit-europa.eu/ managed by MNHN with some support from CNRS/EPHE and regularly fed by partners, in which:
    - (i) news was fed by each partner in the section "Latest announcements" (56 news);
    - (ii) a public agenda announcing next events where INDICIT would participate in;
    - (iii) general presentations of the project for general audience in the tab "About INDICIT";
    - (iv) a presentation of INDICIT consortium, EAB and collaborators (rescue centres, stranding networks, etc.) in the tab "Participants". Partners' logos were presented on a map and videos elaborated by MNHN present each partner's activities;
    - (v) a tab "Documents" for downloading the INDICIT documents (feasibility and pilot studies, protocols and translations, peer-reviewed publications), and other relevant peer-reviewed publications by other experts on marine litter impacts or sea turtles;
    - (vi) other documents for monitoring litter impacts downloadable in the tab "Tools" in INDICIT website (MSFD guidance (MSFD Technical Subgroup on Marine Litter, 2013), INDICIT protocol and a tutorial);
    - (vii) documents for awareness and education (see below);
    - (viii) a photo gallery on litter ingestion, entanglement in litter, and people in action;
    - (ix) links of other relevant initiatives, directives and projects;
    - (x) Link to INDICIT Facebook account, INDICIT private area (see below) and web designer contact.

- A Facebook account to share regular news on partners and stakeholders' activities related to the project (67 posts gathered with 280 persons);
- Templates of documents (e.g., feasibility reports, protocols, with logo and graphic chart, managed by MNHN;
- A private area on a Share point platform hosted and managed by CNRS, where partners having signed the consortium agreement can share (deposit, download and modify) working documents, notably data collected in partnership with stakeholders. In accordance with the Consortium agreement and its amendment (see §1.2), such datasets cannot be shared freely.

Activity 5 also aimed to simultaneously propose technical tools to stakeholders who will be in charge of monitoring litter impacts in RSCs/MSFD areas, to favour synergies with international experts, and to raise awareness.

For standard monitoring of litter ingestion and entanglement, INDICIT partners developed a tool kit to be disseminated to stakeholders. This tool kit contains:

- a standard protocol for monitoring litter ingestion and entanglement in litter by sea turtles (available on INDICIT website);
- translations of the standard protocol in Arabic (leaded by INSTM), Greek (leaded by HCMR), French (leaded by CNRS/EPHE) and Spanish (leaded by UVEG and ULPGC) for a wider dissemination;
- a video tutorial (Deliverable D5.11), led by ISPRA, published in a peer-reviewed journal (Matiddi et al., 2019).

For awareness, various documents were elaborated:

- A story of a turtle which was found entangled at sea and who excreted litter in a rescue center. This story, elaborated by MNHN, showed the anthropogenic threats to which turtles are at risk, in particular caused by litter, and the process to care for and release live individuals. The story was translated in Arabic (INSTM), French (MNHN), Greek (HCMR), Portuguese (FRCT) and Spanish (ULPGC);
- An educational activity sheet for children, proposed by CNRS/EPHE and ULPGC, possibly modified by all partners, in order to create a turtle from plastics;
- An educational activity sheet for children, proposed by MNHN, for fishing litter and learning on litter impacts on sea turtles.

An INDICIT challenge for the Ocean World Day and the Sea turtle World Day in June 2018, was proposed by CNRS/EPHE and MNHN, and realized by all partners. The challenge mobilized 697 participants. The objective was to raise awareness about plastic impacts on the environment, by proposing simultaneously and as wider as possible, various activities to children, based on the INDICIT pedagogical tools. Some partners proposed other activities, such as the release of a turtle at sea (PAU-DEKAMER, CNR-IAMC). This challenge was also proposed by ULPGC at the occasion of training sessions.

A documentary was elaborated by ISPRA with the support of all partners. It presented the way all partners worked on the conservation of sea turtles and on the analysis of litter impacts, as well as how they worked together for supporting MSFD and RSCs in the implementation of litter impact indicators. This documentary was greatly appreciated, both by the general public and by the experts. It was diffused during a public conference

organized by DG Environment and disseminated on partners' websites (e.g., official websites, Facebook or Twitter accounts) as well as ISPRA's Youtube channel.

In parallel, INDICIT consortium did a big work on communication with other experts notably involved in other programs or in representative institution. Synergies with other programs (detailed in INDICIT communication report available in INDICIT website) was favored by (i) participating to other projects' meetings where INDICIT approaches and results were presented, (ii) having regular remote meetings by video-conferences, (iii) discussing by emails. The consortium organized or participated in various events, such as:

- Fourteen training sessions proposed to stakeholders, both to competent authorities and biologists in charge of the collection of samples, belonging to rescue centers, stranding networks or veterinarian laboratories for example. The workshop organized by ISPRA in Naples (Month 6) involved UNEP/Map delegates of the Contracting Parties of the Barcelona Convention;
- A dissemination meeting (Deliverable D5.12, Minutes available in INDICIT website), in December 2018, organized by HCMR and MNHN in Athens (Greece). In the morning, CNRS/EPHE and ULPGC presented INDICIT results and the standard protocol for litter ingestion by sea turtles with the presentation of the video-tutorial. During the afternoon, two panel discussions gathering representatives from EU – DG ENV, MEDPOL, OSPAR / HELCOM, MSFD TGML, French National Authority (MTES), Spanish National Authority (MAPAMA), Azores Regional competent authority (DRAM), as well as NGOs (MEDASSET) discussed the implementation of INDICIT outputs for national and international policies and the possible articulation among RSCs and MSFD. The event was attended by stakeholders from 9 countries, 47 persons were present in Athens and more than 20 persons were connected on the livestreaming;
- Six international and 3 national conferences where 14 posters and 14 oral presentations were provided;
- Four workshop (co)-animated, like a workshop on Marine litter impacts, proposed and realized by CNRS/EPHE, MNHN and ULPGC in Poreč (Croatia) during the MedTurtle conference; a workshop on ingested marine litter at the GTMF (Working Group Marine Turtle France) annual meeting.

Various documents were elaborated from INDICIT outputs, such as a draft Coordinated Environmental Monitoring Programme (CEMP) guideline document for the candidate indicator "Litter ingested by sea turtle" for OSPAR written by CNRS/EPHE supported by MNHN and IFREMER, presented by France to OSPAR commission in April 2019 and which participating to change the indicator's status to "common" (see Appendix 2).

INDICIT consortium also provided a consolidated document for the collection of standard data on litter ingestion and entanglement in sea turtle, between the INDICIT protocol and the RAC/SPA protocol leaded by CNRS/EPHE for RAC/SPA (L. Ben Nakhla). This document (RAC/SPA INDICIT, in press) aimed at harmonizing the approaches between MSFD and as far as possible, the whole Mediterranean level.

1.4. Achievement of expected deliverables

INDICIT proposed 53 deliverables (see Appendix 1). Most of them were considered as fully achieved. Only for Deliverables 2.7 ("Final Report on set of procedures for a standard monitoring using the Indicator "Debris ingestion by sea turtles""), D3.7 ("Final report on the validated GES and clarified indicator criteria for the standard monitoring at the RSC scale") and D4.7 ("Final report on the validated GES and clarified indicator

criteria for the standard monitoring at the RSC scale"), the level of achievement was not judged at 100% but 90%. These deliverables aimed at providing the criteria for using indicator "Litter ingested by sea turtles" thanks to the analysis of standard data (Activity 2) and the discussion of results among stakeholders and partners (Activities 3 and 4). The "basic" and "optional" data collected throughout the project (see §2.3.2) enables assessing most of the biological constraints on litter ingestion, but they did not allow assessing the impact on health accurately, regarding the definition proposed by the new Commission Decision (New Commission Decision 2017/848/EC) for D10C3 GES ("the amount of litter and micro-litter ingested by marine animals that is at a level that does not adversely affect the health of the species concerned", see Appendix 2). Nonetheless, several GES scenarios have been discussed and 2 scenarios were proposed by the INDICIT consortium (see §2.4.4). The data collected during the INDICIT project should be further studied and completed with other data which will be collected during INDICIT-II project (February 2019-January 2021, No. 110661/2018/794561/SUB/ENV.C2). To reach a 100% of achievement, new knowledge is needed to provide a better assessment on how litter affects the health of individuals, allowing the use of health or proxies for GES scenario. Moreover, INDICIT project proposed to evaluate the influence of practical restoration measures on the performance of the indicator (i.e., capacity to detect changes in occurrence and quantity in different litter categories and decrease in the distance to GES) in specific pilot areas (Task 2.6 in the framework of Activity 2). While this task was not completed during the project, the data already collected and the data which will be collected by the (now) experimented networks will enable achieving this task after the project (objectives of INDICIT-II project; see §4). The pilot areas will be selected by considering the risky areas evaluated from INDICIT results, litter accumulation areas (synergy with MEDSEALITTER programme), and the capacity of networks to collect samples in the field.

2. Implementation of indicator "Litter ingested by sea turtles"

The main goal of INDICIT project was to support the implementation of the indicator "Litter ingested by sea turtles" at the MSFD scale in coordination with RSCs OSPAR-Macaronesia and Barcelona. To achieve this goal, after performing a pilot study of the implementation of the indicator (§2.1), INDICIT consortium has identified and trained stakeholders (§2.2) to collect data in a harmonized way (§2.3). Common experiences and techniques were shared to complement and improve the INDICIT standard tools for monitoring litter ingestion in sea turtles (§2.3). Data collected with collaborators were compiled in a standard Excel file and gathered in the private platform available with login and password (§2.4). The data was analyzed in order to fill the gaps of knowledge necessary for the definition of the indicator's constraints and GES (§2.5).

2.1. A pilot study for refining the action plan

A literature review was conducted during the first months of the INDICIT project in order to establish a strategy and action plan for supporting the implementation of the indicator "Litter ingested by sea turtles" (Darmon and INDICIT consortium (2018), available in INDICIT website). The objective was to identify:

1) The state of knowledge on i) the prevalence of litter ingestion in sea turtles (occurrence and quantities), ii) the intrinsic, environmental and logistic factors that might influence litter ingestion in sea turtles, and which may be considered as constraints to consider during the monitoring (e.g., stage, body condition, methods),

2) the stakeholders who already collect or may collect specimens and/or information on impacts related to litter ingestion in sea turtles and their available material and human means.

A review of published and grey literature was conducted, targeting all sea turtle species, more specifically the loggerhead and the leatherback turtles. The stakeholders' contacts were found thanks to each INDICIT partner's previous collaborations, advised by members of the EAB or found from the literature review.

The mean observed percentage of litter ingestion, evaluated from the means found during the literature review weighted by the sample sizes, was of 87.1% for *Dermochelys coriacea* and 39.3% for *Caretta caretta*. The published data was not accurate enough to evaluate the occurrence of litter ingested per species and per marine regions, nor the quantities per individual. These data did not allow proposing GES scenarios. The results published in literature and detailed in the report, were indeed hardly comparable, due to a disparity in the protocols used by the authors to collect the litter ingested by sea turtles. The influence of the methods for collecting specimens (e.g., stranding, bycatch, etc.), the protocols on dead and living turles and other intrinsic factors such as individual's size/stage for example, appeared highly contrasted according to studies and very variable among regions. Results found in the Mediterranean Sea were regularly different from those found in the oceans, maybe because of specific environmental processes in this smallest and almost closed area, where neritic resources might be more accessible to young turtle individuals compared to oceans, or because of the lack of studies, yet insufficient to well understand the factors leading to litter ingestion in sea turtles. This first study enabled to identify the lack of knowledge and local means to implement the monitoring, and to refine the project's working strategy, with a need to reinforce the networks.

2.2. Networking

2.2.1. Situation at the beginning of the INDICIT project

By assessing the institutions already collecting or able to collect information on litter impacts on sea turtles, their training level, the procedures employed, the available material and human means, the knowledge and data already acquired, this situational analysis enabled to plan the strategy for implementing the network. The results in date of July 2017 have been reported in the Pilot study (Deliverable 2.5 available in the tab "Documents" of the INDICIT website, Darmon et al., 2018: first report in the "Pilot and feasibility studies for the implementation of litter impacts indicators in the MSFD and RSCs OSPAR-Macaronesia, HELCOM and Barcelona" document – p.8-67).

Seventy-two institutions (32 involved in OSPAR RSC -Macaronesia and 43 in Barcelona RSC) were identified, some of them endorsing several roles. They were institutions aiming mainly at the collection of specimens:

(i) 13 stranding networks, in charge of the observation and recovery of dead or live turtles and organized for alerting, taking the first measures and recovering specimens;
(ii) 21 rescue centers, in charge of medical care of live individuals brought back by stranding networks;
(iii) 11 transit centers, recovering individuals temporarily before rescue centers take charge of them;
(iv) 3 veterinarian laboratories, in charge of the external exams and necropsies;
(v) 11 research laboratories, among which some were also veterinarian research center such as the University of Las Palmas, or also rescue centers, such as IAMC-CNR in Sardinia island in Italy or PAU-DEKAMER in Turkey. INSTM also managed a stranding network and a rescue center in Tunisia. HCMR

collaborated with the only Greek rescue center managed by the NGO “ARCHELON - The Sea Turtle Protection Society of Greece”.

Some of these institutions, especially in Spain, Italy and France, were already engaged in the monitoring of litter ingestion of sea turtles, reporting their information to local/regional/national authorities, in charge of linking stakeholders with ministries, MSFD and RSCs. The others were contacted for being involved in the monitoring.

2.2.2. Training sessions for collecting data on litter impacts on sea turtles

INDICIT consortium has organized 14 training sessions during the project, 2 at international levels, 5 at national level and 7 locally. These workshops aimed to present the INDICIT protocol and put it into practice by performing initially an external and internal exam of a dead loggerhead turtle, and afterwards the extraction, characterization and quantification of the ingested litter.

Training was especially needed in the Atlantic area of the project, where monitoring of litter impacts just started: At the beginning of the project, only the stakeholders of France and Azores shared data. Several training sessions were proposed by ULPGC and FRCT to local stakeholders in Spain and Portugal. In France, only one rescue center (CESTM hosted by Aquarium La Rochelle), which also manages the stranding network of the French Atlantic coast (RTMAE), collected data from a long time and did not really need to be trained. CNRS/EPHE and CESTM collaborated and discussed how to improve the INDICIT protocol either during necropsies performed together, or by phone or email. For Spain and Portugal, the spatial configuration in archipelagoes had to be considered for reaching people and propose training sessions.

In order to harmonize the procedures of monitoring throughout the whole Mediterranean, an international training session was organized by ISPRA for INDICIT partners and the Mediterranean stakeholders in July 2017, in the Marine Turtle Research Centre of Anton Dohrn marine station in Portici. Thanks to UNEP MAP MedPol Program and the RAC/SPA (regional activity center of Barcelona convention Special protected areas), representatives from Egypt, Tunisia, Lebanon and Israel were included.

2.2.3. Listing stakeholders already or possibly in charge of monitoring and conditions for involvement

All INDICIT partners continued to make contact with stakeholders throughout the project duration, either by contacting them individually (email/phone, etc.) or during specific events such as during the 11 conferences where INDICIT was represented (e.g., ISTS in February 2018 in Japan or XV Portuguese-Spanish Herpetology Congress and XIX Spanish Congress of Herpetology in Salamanca 2018), or thanks to the facilitation by EAB and national representatives. INDICIT consortium participated in 5 national and international workshops on the topic of Marine Litter, and co-organized some of them, e.g. ULPGC in MICRO 2018 international conference in Spain in November 2018. CNRS/EPHE, MNHN and UVEG organized a specific workshop on marine litter impacts in synergy with RAC/SPA of Barcelona Convention (Lobna Ben Nakhla) and Archipelagos Institute of Marine Conservation during the 6th MEDTURTLE conference in Croatia in October 2018. These events provided the opportunity to not only share INDICIT outputs, in particular the INDICIT protocol, but also enlarge the networking, especially with southern countries. This protocol was also sent by email to various stakeholders, especially by FRCT for the Atlantic area. Posting it on INDICIT website also enabled to favor interactions with new contacts.

ULPGC and other Activity leaders supported by partners, listed all already involved stakeholders (tab "Collaborators" of INDICIT website"), or possibly involved stakeholders, for monitoring and reporting on "indicator Litter ingested by sea turtles" (Table 4). CNRS and MNHN begun a Google map, which should be consolidated, referencing the stakeholders already involved in monitoring litter ingestion in sea turtles. This may show the spatial distribution of the networks and should facilitate contacts. According to the consortium agreement (see §1.2), this list and this map will be transmitted to DG Env. and national representatives but cannot be open access since they contain personal coordinates and thus cannot be shared in this report but they.

At the end of the project, 106 stakeholders were contacted (some of them may be referenced twice, due to the double activities or activities in different areas). In the Mediterranean, 68 stakeholders were involved, 43 in the Atlantic and 6 in both areas. Several institutions, the first role of which was the collection of specimens, were already involved or interested to be involved in monitoring litter impact on sea turtles and in sharing data. From those who provided information, there were:

(i) **36 stranding networks**, in charge of the observation and recovery of dead or live turtles, sometimes managed by a rescue center. Some institutions were structured in networks with voluntaries trained to carry out the specimens and do the first measures after having alerted a regional referent (e.g., RTMFF and RTMAE in France). Other institutions, not directly structured in a network, could report stranding events, such as NGOs (e.g. in Turkey), National parks in Greece or research and rescue centers in Italy.

(ii) **28 rescue centers**, in charge of medical care of live specimens, some also supervising stranding networks and performing themselves the necropsies of dead individuals. Several did a considerable work with fishermen to collect bycaught individuals.

(iii) **10 transit centers**, recovering specimens temporarily before rescue centers took charge of the individuals.

There were also laboratories or groups in charge of the acquisition or dissemination of knowledge:

(i) **3 veterinarian centers, 1 veterinary school and 1 veterinarian laboratory**, in charge of the necropsies of dead specimens and of the evaluation of health status before death. Some research centers could also be in charge of veterinarian analyses centers, such as Istituto Zooprofilattico Sicilia, Istituto Zooprofilattico Abruzzo/Molise and Istituto Zooprofilattico Sardegna (Oristano) in Italy.

(ii) **31 research laboratories**, in charge of the collection and analysis of samples and the acquisition of knowledge especially on sea turtles and litter impacts. Among them, some had their own rescue center, such as IAMC-CNR in Sardinia Island in Italy, PAU-DEKAMER in Turkey. INSTM also managed a stranding network and a rescue center in Tunisia.

(iii) **2 institutions in charge of networking experts and of sharing information**.

The sampling capacity per stakeholder was sometimes difficult to assess because several institutions may take in charge different samples from the same specimens. The organization of the networks is highly variable among countries, with sometimes a referent at the regional and national level, sometimes not. In some countries, an official stranding network did not exist, but there was a local organization by research or rescue centers to be alerted and recovering on the field of dead individuals or live individuals needing care. Manipulations of sea

turtles generally required an official training for authorization (e.g., “carte verte” in France, delivered by the French Environment Ministry).

The way to sustain networks was an important topic intensely discussed within the consortium as with other projects and representatives. INDICIT consortium participated in a discussion launched by emails by IDEM project on “Finding a common strategy for stakeholders’ involvement in DG-ENV funded projects”. The challenge and the conditions for involving stakeholders were also highly discussed with NGOs and representatives attending the INDICIT Dissemination meeting in December 2018 in Athens, since it was a high constraint sometimes underestimated.

Among the stakeholders contacted by the INDICIT consortium, some either did not reply or did not want to be involved in the project. The consortium reported all conditions for involvement shared by stakeholders. The co-signing of sharing agreements stipulating the conditions for using their data, especially regarding scientific publication or conservation reports, and the need for continuous and sufficient financial means for equipment and staff, were the two main critical needs they share. Stakeholders had also other requirements, such as:

(i) to be trained for standard monitoring;
(ii) to receive the results found locally, thanks to the data they collected;
(iii) to participate in the research projects as partners or be invited in workshops as experts.

An amendment into the INDICIT Consortium Agreement was signed by partners, in order to specify the rules for sharing and using stakeholders’ data, and testify their inclusion as co-authors, collaborators, or in acknowledgement, depending on their support and its objectives.

2.2.4. Recommendations for networking

In certain areas/countries, a better development of the network appears necessary, by e.g. creating/reinforcing stranding networks and rescue centers, ensuring constant means and training referents or coordinators at the local/regional level. Engaging stakeholders in certain zones would be necessary to provide accurate assessments of litter impacts and GES. This is especially the case in the Atlantic area where data is missing, in particular in Portugal and Atlantic Spain’s coasts. This is also needed for a better coherence between the North-Western Mediterranean basin and the South-Western and Eastern basins.

The conditions for involvement reported by INDICIT consortium could support engaging new stakeholders. Co-organizing workshops among stakeholders, with experts and representatives, could facilitate the task. These workshops should propose training sessions and sharing experience on e.g. methodologies to monitor litter impacts, which could still be optimized. Key-stakeholders, such as fishermen, who are essential to recover sea turtles in certain areas, should be involved. Including them in the monitoring of litter impact on sea turtles instead of using them for recovering sea turtles as accidental bycatch, could support the collaboration.

For a better reporting of information and data, a diagram of the networks should be built at both national and regional levels, considering specificities of each country. These diagrams should be disseminated to stakeholders in order to facilitate procedures and contacts. Some tools may accelerate the diffusion of information, in particular when a turtle is observed at sea or found stranded or bycaught, for example blogs or web applications,

such as the Red PROMAR application of the Canary islands government, allowing citizen to post pictures with GPS locations, or ObsEnMer which offers a collaborative platform (managed by Cybelle Planete, France).

2.3. Elaboration of a standardized protocol for monitoring litter impacts on sea turtles

2.3.1. Proposition of a common methodology

A first protocol for monitoring litter ingestion by sea turtles, based on the Fulmar approach, was proposed by Matiddi et al. (2011) and published in the MSFD guidance (MSFD Technical Subgroup on Marine Litter, 2013). It consisted in quantifying the litter found in the digestive tract extracted during necropsy of dead individuals. It also proposed the collection of the litter in the excrements of live individuals in rescue centers. The protocol has been adapted (Matiddi et al., 2017), then completed by INDICIT Activity leaders with the feedback from other partners and stakeholders in charge of collecting specimens and samples. The INDICIT protocol was addressed to the collection of the ingested litter, either in the digestive tract of dead individuals by performing a necropsy, or in the excrements of the live individuals who remain at least 1 month and maximum 2 months in the rescue center, the digestive transit time being evaluated from 4 to 6 weeks on average (Camedda et al., 2014; Darmon and Miaud, 2016; Valente et al., 2008). The INDICIT protocol aims to:

(i) Target especially the plastic categories (Table 4), the most often found ingested by sea turtles (Camedda et al., 2014; Pham et al., 2017). This should allow evaluating the programs of measures implemented in the framework of the Plastic Strategy;

(ii) Improve the observation sheet, in which the parameters are reported in the order of manipulations (see §2.2);

(iii) Collect basic information to evaluate the presence and the quantity of ingested litter in the individual (see §2.2);

(iv) Acquire knowledge on the factors leading to litter ingestion or entanglement, thanks to the so-called “optional parameters”.

Table 4. Categories of litter items (from INDICIT protocol, 2018)

CATEGORIES			CODE	DESCRIPTION
LITTER	PLASTIC LITTER	Industrial plastic	IND PLA	Industrial plastic granules, usually cylindrical but also sometimes oval spherical or cubical shapes, or suspected industrial item, used for the tiny spheres (glassy, milky...)
		Use sheet	USE SHE	Remains of sheet, e.g. from bag, cling-foil, agricultural sheets, rubbish bags…
		Use threadlike	USE THR	Threadlike materials, e.g. pieces of nylon wire, net-fragments, woven clothing…
		Use foam	USE FOA	All foamed plastics e.g. polystyrene foam, foamed soft rubber (as in mattress filling)…
		Use fragment	USE FRAG	Fragments, broken pieces of thicker type plastics, can be a bit flexible, but not like sheet like materials.
		Other Use plastics	USE POTH	Any other plastic type of plastics, including elastics, dense rubber, balloon pieces, soft air gun bullets… Specify in the column "Notes".
	Litter other than plastic		OTHER	All non-plastic rubbish and pollutant
OTHER ELEMENTS		Natural food	FOO	Natural food for sea turtles (e.g., pieces of crabs, jellyfish, algae…)
		Natural no food	NFO	Anything natural, but which cannot be considered as normal nutritious food for sea turtle (stone, wood, pumice, etc.)

The "basic" parameters correspond to information fundamental for determining the GES based on this indicator. While the GES scenario has still to be voted (see § 2.4.4), the minimum number of parameters to record may change in the next future, for example if the objective is to target the evaluation of Programs of measures (PoMs) related to a specific litter category. As the measurement of the other parameters is time-consuming, they were considered as "optional". However, this information is also highly important since it aims to better understand the factors which may influence litter ingestion by sea turtles. Their analysis should help to specify the indicator's possible constraints and better anticipate the risks related to litter exposure.

The "basic" parameters were:

- The observer's coordinates, in case that further precisions are needed;
- The circumstances of discovery of the sea turtle specimen, with a standard identification code which should be notified on each sample;
- The conservation status of the body of animals found dead, and for both live and dead individuals, the standard curved and straight carapace lengths;
- The main description of live individuals' behavior or of the internal organs of dead individuals in order to globally assess the individual's health status;
- The presence/absence of ingested litter;
- The dry mass of each litter category (as in Table 5).

The "optional" parameters concerned a precise external and internal exam, aiming at better understanding the role of litter ingestion and entanglement on the health status or possible cause of death. They also aimed to give a first assessment of the relevance and constraints of other litter impact indicators, related to (i) micro-plastic ingestion by sea turtles, for the specific size category 1-5 mm and (ii) entanglement of sea turtles in marine litter. The "optional" parameters concerned:

- A better description on the possible cause of death, in particular the type of fishing gear in case of bycatch;

- The observed impact of the entanglement, specifying the category of litter and if entanglement in litter was passive or rather active, which could be the consequence of active fishing;
- A characterization of the body condition, described thanks to fat reserves, injuries, measures of carapace and plastron, and weight;
- The digestive capacity, measured from the mass and volume of each section of dead individuals' digestive tract;
- The total volume of ingested plastics;
- The differentiation of ingested plastic by size class (micro: 1-5 mm, meso: 5-25 mm, macro: >25 mm, according to (Andrady, 2011b; MSFD Technical Subgroup on Marine Litter, 2013) and color class: As color might be difficult to assess, especially for multi-colored or worn and faded items, three main categories was proposed: white-transparent, light (light pink, light green, etc.), and dark (black, dark green, etc.).

Several modifications were brought all along the project thanks to the experience shared among partners and with stakeholders. Among the major ones, the quantity in terms of number appeared to be confusing, some counting the number of fragments (of the same item) and other, the number of items (all fragments considered as coming from the same item, e.g. several fragments of white plastic coming from the same white plastic bag were counted as 1). The two parameters may give a useful but different information, the first enabling more to assess the impact of ingestion on health and being maybe less subjective, the second informing more on the type of litter causing impacts. Number of fragments and number of items were thus differentiated and proposed as optional parameters.

Pictures showing the order of magnitude of the object were highly recommended for possible verification of some details or for further research, in the field as in the laboratory.

2.3.2. Dissemination of the standardized methodology for monitoring litter impacts

The INDICIT protocol and the "Observation sheet" in which data have to be handwritten, can be found in the INDICIT website (sections INDICIT documents and Tools; versions September 2018). The protocol detailed all the procedures, from the discovery of dead or live sea turtle, most of them being possible on all turtles, except for the leatherback when the individual should be moved or returned.

Translations were done in order to favor local involvement and fill the gaps of data in certain areas:

- Arabic,
- Greek,
- Spanish,
- French.

In addition to the website, the INDICIT protocol was disseminated by email during the 14 training sessions given to stakeholders, and also during specific events such as the workshop on Marine litter impacts organized by INDICIT consortium, RAC/SPA and Archipelagos Institute during the MedTurtle conference in Poreč (Croatia) in October 2018, and the INDICIT Dissemination meeting in December 2018 in Athens (see minutes in INDICIT website).

INDICIT protocol was presented in several conferences and during other projects' meetings (see Dissemination final report in INDICIT website). This offered the opportunity to make synergy with RAC/SPA center of Barcelona RSC, who also produced a standard protocol for monitoring litter impact on sea turtles. The two documents have been compiled in a RAC/SPA-INDICIT protocol and submitted by CNRS with support of other INDICIT partners to CORMON, which recently approved it (RAC/SPA-INDICIT, in press). This synergy should facilitate the harmonization with stakeholders involved in Barcelona RSC, especially with the Southern countries.

A video-tutorial presenting the external and internal exam of a sea turtle is available in French (Darmon et al., 2017b). During INDICIT project, ISPRA managed the elaboration of a video-tutorial showing all procedures from the discovery of a dead specimens to the opening of a loggerhead turtle, with extraction of the digestive tract, the differentiation of the digestive sections and the extraction and quantification of the litter ingested. This video was published in a peer-review journal (Matiddi et al., 2019). The publication in a journal should facilitate the dissemination for a common procedure.

2.3.3. Costs and recommendations

Only authorized people can manipulate protected species and after contacting local authorities. CITES permits are also to be considered in case that samples have to be sent from/to countries having ratified the Washington convention.

Moreover, manipulations absolutely require sanitary precautions in order to avoid zoonosis risk. This should be highlighted during training sessions with participation certificates. These sessions can be the opportunity to provide a material kit to stakeholders newly involved in the monitoring (list of materials provided in the INDICIT protocol).

To better assess the impact of litter on individual's health, "optional" parameters related to body condition are encouraged to be collected, as well as measuring systematically the dry mass of natural food remains (category FOO, Table 5; see §2.4.3.2).

The time required to collect samples and extract marine litter depends on the body condition level (no manipulation recommended if the autolysis is too advanced). An average of 5 hours with two manipulators should be considered for the collection of data from necropsied individuals, including the external and internal exam of the body. The cost of material can be evaluated from the list provided in INDICIT protocol.

The cost of implementation should consider the equipment and training of each stakeholder by covering the widest possible area. Fees could also target dedicated specialized teams who could support the laboratory analyses. Among stakeholders having been contacted but not yet involved in litter monitoring, some are constrained by financial and human means. In certain areas, the number of turtles found dead is too important to conduct a systematic monitoring of litter impacts, e.g., as reported by the Israel sea turtle rescue centre during the workshop proposed by INDICIT during the MedTurtle conference in Croatia in October 2018. Further studies are needed to evaluate if the monitoring could be performed in a part of the area considered as representative for the country.

In case of material and time/human constraints, stakeholders could be supported by specialized teams for manipulations in laboratory and data analyses. These stakeholders should be equipped with a minimum material in order to conduct the first measures on the field, perform the necropsies and extract the digestive tract,

including a freezer. The laboratory analyses could then be performed by specialized teams coming locally e.g. on a 6-months period. The travel and accommodation costs of such teams should be considered.

2.4. Data banking

2.4.1. Procedures used during INDICIT project for data gathering and cleaning

Each partner was in charge of gathering and making a first cleaning of data collected locally. Then, the data was either sent by email to CNRS/EPHE, ULPGC and ISPRA as leaders of Activities 2, 3 (Atlantic area of the project) and 4 (Mediterranean area) who compiled the entire dataset, or in a private area. CNRS/EPHE indeed proposed a Sharepoint platform accessible via login and password, in order to share documents such as INDICIT databases. As signed in the Consortium agreement (§1.2), only partners were authorized to access to data and only the results from this data can be shared in INDICIT reports and presentations but not the raw data. Stakeholders participating in data collection must be cited. CNRS completed data cleaning during data analysis. This task was highly time-consuming. To this purpose, the use of a dedicated platform with drop-menus, as managed by IFREMER in the framework of the CleanAtlantic project could greatly save time.

2.4.2. Summary of data collected

A standard database (Excel file) for data on litter ingested by sea turtles was built (deliverables D2.8 and D2.9, Appendix 1), differentiating:

(i) two tabs for necropsies and for faeces,
(ii) basic and optional parameters.

At the end of the INDICIT project, a total dataset of **1406 lines** has been gathered (1 line per individual) (1014 data was recorded in January 2018): 948 for necropsies and 458 for faeces. In the "Necropsy" table, 3 data concerning the green turtle *Chelonia mydas* were removed. This species would be possibly monitored since observations were regular, both in Atlantic and Mediterranean. Nevertheless, this should be clearly asked to stakeholders for a systematic monitoring and power analyses. Stakeholders were asked to give priority to the collection of the loggerhead turtle and, if possible, on the leatherback turtle, and other species could be considered in the future. All data from faeces concerned only loggerhead individuals, who were hosted in rescue centers.

In the remaining data from necropsies, 838 were extracted from loggerheads and 107 from leatherbacks. For the leatherback database, only one data was collected in Tunisia and all other in France Atlantic from the stakeholder CESTM-Aquarium La Rochelle. The possibility to integrate this species in future monitoring should be better evaluated. This would require specific training sessions for conducting a necropsy on this species, with specific manipulations and conditions of work due to the size of specimens. Due to the restricted number and distribution of data acquired at this stage, analyses could not be performed to assess the indicator's constraints and GES for this species and only exploratory analyses were done (see §2.4.1).

Table 5 and Table 6 show the number of data collected from live and dead loggerheads respectively:

(i) data collected before the INDICIT project (not always standard and maybe not systematic) were gathered and formatted to INDICIT standard Excel file (total number of data);

(ii) the number of data collected on a 6-year period according to MSFD cycle, from 2013, year of the MSFD guidance (MSFD Technical Subgroup on Marine Litter, 2013), from which a standard methodology was started to be disseminated and known to stakeholders;

(iii) the number of data acquired during the two years of the INDICIT project in order to evaluate the minimum sampling capacity per partners' country. Nevertheless, some stakeholders' data was not available during the INDICIT project duration or the duration was not sufficient to process all the samples. This information thus represented a minimum sampling capacity at the Area level.

Table 5. Number of data collected from excretions of live loggerhead turtles in each area/country.

Area	Country	Total number of turtles	Number of turtles from 2013	Min number of turtles during INDICIT project (2017-2018)*
Atlantic	France	128	5	0
	Portugal	0	0	0
	Spain	12	2	1
	Total	***140***	***7***	***1***
Mediterranean	France	68	68	25
	Greece	3	3	3
	Italy	183	93	33
	Spain	39	21	14
	Tunisia	17	17	17
	Turkey	8	8	8
	Total	***318***	***210***	***100***

Table 6. Number of data collected from necropsied loggerheads in each area/country

Area	Country	Total number of turtles	Number of turtles from 2013	Min number of turtles during INDICIT project (2017-2018)
Atlantic	France	95	21	1
	Portugal	66	33	6
	Spain	13	9	6
	Total	***174***	***45***	***13***

Area	Country	Total number of turtles	Number of turtles from 2013	Min number of turtles during INDICIT project (2017-2018)
Mediterranean	France	81	77	37
	Greece	28	28	28
	Italy	198	129	54
	Spain	188	85	17
	Tunisia	75	46	28
	Turkey	94	94	92
	Total	***664***	***457***	***256***

2.4.3. Synergy with CleanAtlantic project for data banking

A dedicated platform should help win time for data gathering and cleaning. Such platform is being developed by IFREMER in the framework of CleanAtlantic project with the support of INDICIT. his part was extracted from CEMP document written by CNRS, MNHN and IFREMER for OSPAR (April 2019).

Data entry software is under development in the framework of Interreg project CleanAtlantic, in order to store data on litter impacts on sea turtles. The data management process provided a standardized and structured data with common referential, with QA/QC procedures. Data from each partner could be hidden to other partners and a moratorium can be applied on dataset, thus respecting stakeholders' possible condition for using the data they collected. Data could also be made widely available, also compatible to existing mechanisms (e.g. MSFD, OSPAR/ICES databases and EMODNET).

Two options were proposed for the administrative architecture of the central database, depending on organism IT abilities (i.e. operating team, database server, Oracle License). The first was a transfer of the central architecture under use at IFREMER. In that case, each institution would need a trained staff, a database server and an Oracle License. Minor software developments should be required under this option. The second option should offer an adaptation of the existing architecture to use a PostgreSQL central Database. In that case, each institution would need a trained staff, an operating team and a strong IT structure using an open source software. Advanced software development would be necessary under this option.

2.5. Analysis of the indicator's constraints

In its objective of developing the indicator "Litter ingested by sea turtles", INDICIT consortium analysed data gathered by each partner in order to:

(i) assess the prevalence of litter ingestion in sea turtles and the quantity of ingested litter;
(ii) identify the possible biological constraints of the indicator (e.g., individual size);
(iii) evaluate the sample size and temporal and spatial unit.

The data was explored in order to evaluate which factors may influence the prevalence and the quantity of ingested litter. In addition to a better understanding on the impact of litter by ingestion, these influencing factors may indeed be considered to stratify the data when applying the indicator (e.g., using only small individuals).

Given that data collection, gathering and cleaning took much more time than expected while planning the INDICIT project time table for submission, the expected time allocated to data analysis was too small to deeply explore data and test indicator's constraints. Data cleaning was completed during data analyses which highlighted some needs of, for instance, combining factors together or adjusting methodologies. Various explorations were done, especially to select the factors to be considered, in particular concerning the relationships between individual's body condition or health status and litter ingestion. Data analyses brought to light the need to further discuss and standardize the methodologies used by stakeholders (partners included), either by completing the database from stored samples, or by better classifying the parameters used for the qualitative variables (see below). Some analyses are presented here but will be further explored thereafter, especially in order to publish the results and highlight towards experts' community the great harmonized work made by the INDICIT wide network.

The prevalence of litter ingestion in sea turtles was calculated as the percentage of turtles found with ingested litter, based on the occurrence (or incidence) reported as presence or absence. The quantity of ingested litter was measured as a dry mass (grams), a total volume (mL) and a number of items or fragments (see below). The precision of the measurements was 2 decimals (up to 0.01). For really small but non-zero quantities, the value of 0.001 g (or 0.001 mL) was noted. Calculations were then based on arithmetic averages with standard errors (hereafter noted "SE", calculated as the ratio of the population's standard deviation on the squared root of the sample size). Averages of quantities are calculated over all available individuals, including those that contained no litter ("population averages"), and compared with quantities assessed on only those found with ingested litter. When sample sizes are small, arithmetic means may be influenced by local variations and outliers. An alternative could be to apply logarithmic (adding a small value to avoid zeros) and normal transformation of data, which can reduce the role of the higher values (ln(x)). The factors influencing the prevalence of litter ingestion and the quantity of ingested litter were first explored with simple analyses (e.g., Student t test for comparison the means, analysis of variances (ANOVA)) and then a global approach evaluating the possible interaction among factors (e.g., linear generalized models and multivariate analyses).

The analysis of data was done with Excel and R (version 3.4.1; (R Core Team, 2017) software.

2.5.1. Data on the leatherback turtle

From the 106 individuals sampled in the Atlantic France and the only individual from Tunisia (standard straight carapace length ranged from 96 to 203 cm (**143.4 ±1.53 cm**; N = 94)), the occurrence of litter ingestion was really high: 52.83% from 1988 to present (52.38% in Atlantic France) and **53.84%** when considering only the last 6 years (data from 2013, N = 13 individuals all from France). The dry mass of ingested litter was noted for 63 individuals in France Atlantic: On average, 7.67 ±2.82 g of plastics were found ingested among turtles having ingested debris (N=13), which represents **1.7 ±0.73 g** on average at the population level (i.e., when considering all sampled individuals for which the information was filled; N=63)).

The data on litter ingested by the leatherback turtle (data only from necropsies) were insufficient at this stage to enable the development of the indicator from this species. An evaluation of the sampling capacity from this species should be done with a greater number of stakeholders.

2.5.2. Possible discrepancies in the methodologies used to collect samples from live individuals

Only the data collected from living loggerheads who remained at least 1 month in the rescue center was considered. For these turtles, the data were gathered from all faeces collected daily from their arrival until 2 months later.

A total of 458 living loggerheads was sampled, from which 445 were found with ingested litter (Table 7). The individuals' size ranged from 16 to 77 cm (Standard curved carapace length, mean **51.8 ± 0.05 cm**, N = 254). The occurrence of litter in the faeces was recorded in 445 of them, the average being 31.68% (all data from 1988 to present) and **57.94 %** (N = 217 for the last 6 years). The dry mass of ingested plastics, noted for 396 individuals since 1988, was 0.59 ±0.21 g on average **at the population level**, and of **1.06 ± 0.44 g on a 6 year-cycle** (N = 185 from 2013; Table 7). Among those who had ingested litter, the mean dry mass of plastics was 1.93 ±0.69 g since 1988 (N = 120) and 1.84 ±0.76 g since 2013 (N = 107).

Considering the discrepancies among stakeholders in the methodology of collecting samples, INDICIT consortium decided not to include data coming from living turtles for the elaboration of the indicator at this stage (minutes of INDICIT 4th meeting in Azores available in INDICIT website, Summer 2018). Some partners used a dip net to collect floating faeces on the surface and in the water column, scraping the possible remaining materials at the bottom when emptying the water and cleaning the tank. Others used filters on both incoming waters (thus avoiding a possible contamination with micro-plastics) and the water discharge system, then collecting the excreted elements directly on this last filter. This second methodology was probably more appropriate for collecting samples in a standardized way. This difference underlined the need for specific training sessions to stakeholders working with living turtles in rescue centres.

In the light of the high occurrence found on living loggerheads (57,94%), the collection of samples from living individuals is highly recommended. On the one hand, this will allow a better understanding of the factors influencing litter ingestion and the consequences on individual's health, and to assess the possible under-evaluation of litter ingestion from the examination of faeces compared to the examination of the entire digestive tract. On the other hand, thanks to the great number of rescue centres involved in the area (§2.1.3), the analysis of a dataset should enable the comparison with the results from necropsies, and either combine data from the two protocols, or propose a specific GES for living turtles.

Table 7: Mean prevalence (%) and dry mass (grams) evaluated from excretions of living loggerhead turtles in rescue centres per area and country (table "Faeces" of the INDICIT standard database)

Area	Country	All data		Data from 2013	
		Prevalence (N)	Dry mass mean±se (N)	Prevalence (N)	Dry mass mean±se (N)
Atlantic	France	2.34 (128)	0 (125)	20 (5)	0 (4)
	Portugal	-	-	-	-
	Spain	18.18 (11)	(0)	50 (2)	0
	Global	*3.6 (139)*	*0 (N=125)*	***28.57 (7)***	***0 (4)***
Mediterranean	France	48.53 (68)	0.21 ± 0.06 (60)	48.53 (68)	0.21 ± 0.06 (60)
	Greece	100 (3)	0.03 ± 0.02(3)	100 (3)	0.03 ± 0.02 (3)
	Italy	39.9 (183)	1.06 ± 0.45 (183)	64.52 (93)	1.71 ± 0.87 (93)
	Spain	62.96 (11)	2.36 ± 1.31(10)	88.89 (18)	2.36 ± 1.31 (10)
	Tunisia	47.06 (27)	0.2 ± 0.07 (8)	47.06 (17)	0.2 ± 0.07 (8)
	Turkey	25 (8)	(1.4 x 10^{-4}; 7)	25 (8)	(1.4x10^{-4}; 7)
	Global	*44.44 (306)*	*0.86 ±0.31 (271)*	***58.94 (207)***	***1.09 ± 0.38 (181)***

2.5.3. Results on litter ingestion observed in necropsied individuals

Partners gathered data collected prior to INDICIT project. We suppose that loggerhead and leatherback turtles found dead were systematically necropsied at least after the publication of the MSFD guidance in 2013. Before this date, we could not verify if all turtles were systematically analyzed, or more specifically if individuals in poor body condition, or who presented an injury or a sign of presence of litter, were more prone to be examined. However, all data collected before INDICIT project were standardized according to INDICIT database and were thus comparable to the data collected during the INDICIT project. At the end of the project, 838 turtles were recorded, ranging from 1990 to 2018 (last turtle found the 26th November 2018).

Each partner cleaned the data and a second cleaning was made by CNRS with the great support of all partners during data analysis. Data was classified and standardized, e.g. to minimize the number of factors for qualitative parameters (e.g., circumstances of discovery or causes of death, see below). Missing data was either filled by partners or when possible, deduced by CNRS. The phase of cleaning dataset was highly time-consuming and probably under-estimated at the beginning of the project. The use of a platform with drop menus and data control would certainly enable to gain time.

2.5.3.1. Evaluation of metrics of measurements

- Description of the prevalence of litter ingestion and the quantities of ingested litter

Among the 838 necropsied loggerhead turtles from 1988 to the end of INDICIT project (standard curved carapace length ranging from 9.4 to 89 cm with a mean of 53.45 ±0.03 cm), 57.62 % of the turtles have been found with ingested litter in their digestive tract (N = 833 for which the occurrence was clearly notified). Considering data from 2013 (data from Portugal was attributed to the year 2016), the prevalence of litter ingestion was really high with **63.03% of 522 turtles** found with ingested litter (standard curved carapace length ranging from 10 to 89 cm with a mean of 56.32 ±0.04 cm).

The total dry mass of ingested plastics was 0.75 ± 0.08 g (N = 768). This represented 0.12±0.01 g of plastics / 10 cm of turtle carapace (Standard curved carapace length). After 2013, the dry mass was evaluated to **0.78 ±0.11 g** (**N= 480**) at the population level (all sampled turtles considered), representing **0.12±0.02 g/10 cm**. When considering only the individuals for which a presence of litter was found (occurrence= 1), the dry mass was 1.35 ±0.13 g (N = 425) on average since 1990 (0.23 ±0.03 g plastics/10 cm carapace lengths), and 1.27 ±0.17 g since 2013 (N = 294; that is 0.21 ±0.03 g/10 cm). The mean occurrence of litter ingestion and quantities of ingested plastics in necropsied turtles per area (Atlantic/Mediterranean) and country found both on all data and data after 2013 are presented in Table 8.

Table 8: Mean prevalence of litter ingestion (%) and dry mass of ingested litter (plastics, in grams) evaluated from the necropsies of loggerhead turtles per area and country (table "Necropsies" of the INDICIT standard database)

Area	Country	All turtles		Turtles from 2013	
		Prevalence (N)	Dry mass mean±se (N)	Prevalence (N)	Dry mass mean±se (N)
Atlantic	France	26.32 (95)	0.07 ± 0.04 (79)	45 (20)	0.28 ± 0.16 (18)
	Portugal	87.88 (66)	1.31 ± 0.23 (66)	81.82 (33)	1.13 ± 0.35 (33)
	Spain	84.61 (13)	0.16 ± 0.07 (8)	88.89 (9)	0.16 ± 0.07 (8)
	Global	*54.02 (174)*	*0.61 ±0.11 (153)*	***70.97 (62)***	***0.74 ±0.21 (59)***
Mediterranean	France	79.75 (81)	1.15 ± 0.26 (79)	82.43 (76)	1.23 ± 0.27 (70)
	Greece	64.28 (28)	0.13 ± 0.06 (28)	64.28 (28)	0.13 ± 0.06 (28)
	Italy	53.53 (198)	0.74 ± 0.13 (198)	62.01 (129)	0.92 ± 0.19 (129)
	Spain	72.43 (188)	1.05 ± 0.17 (168)	80.49 (85)	0.89 ± 0.27 (70)
	Tunisia	45.33 (75)	0.63 ± 0.41 (52)	52.17 (46)	0.84 ± 0.71 (29)
	Turkey	32.98 (94)	0.36 ± 0.28 (94)	33.33 (93)	0.37 ± 0.29 (93)
	Global	*58.57 (664)*	*0.78 ±0.09 (615)*	***61.95 (457)***	***0.78 ±0.12 (419)***

- Assessing the quantity of ingested litter as a volume: complexity for providing accurate assessments

The mean volume of plastics ingested by sea turtles was evaluated to 0.33 ± 0.09 ml on average (N = 276), 0.05 ± 0.03 ml in Atlantic area of the project (N = 78 available records) and 0.44 ± 0.03 ml in the Mediterranean area of the project (N = 198), respectively when considering all data. On the last 6-year cycle, from 2013 onward, the mean volume was **0.48 ± 0.01 (N=191)**, 0.23 ± 0.15 ml (N=16) in the Atlantic and 0.51 ± 0.14 ml (N=173) in the Mediterranean, respectively. The volume was regularly reported by partners and collaborators to be difficult to measure, especially for the very light plastics which are floating on the surface. Therefore, this metric was not retained for proposing a GES. However, the volume may inform on the impact of plastic ingestion on health, for example by comparing it to the digestive capacity, measured by some partners as the volume of each digestive full and emptied section (oesophagus, stomach and intestines: optional parameters, N = 58 individuals from France and Atlantic Spain).

- Assessing the quantity of ingested litter as a number of litter items: can be confusing

During the 4th INDICIT meeting in September 2018 (minutes available in INDICIT website), while sharing experience on the methodologies to evaluate the quantity of ingested litter, the consortium recognized that partners and collaborators did not use the same approach to evaluate the number: Some evaluated the number of fragments by counting all pieces of litter found (as done on the Fulmar indicator), other evaluated the number of items by gathering all fragments of a same item and counting all supposed items. Partners and collaborators have then specified the methodology they used in order to differentiate the total number of plastic fragments and the total number of plastic items. We found a mean number of 12.11 ± 1.12 fragments and 4.71 ± 0.71 items on average from the total database, and a mean number of **11.46 ± 1.37 fragments** and **5.83 ± 0.98 items per individual** on average from 2013.

The information provided by these metrics could probably be more significant and eloquent for the general public. The two metrics did not provide the same information, the number of fragments being probably more related to the impact on health, the number of items informing more on the type of litter in the environment being possibly more related to the specific measurements associated to them. Whereas the INDICIT protocol proposed to record the number per litter category (Table 4) and each digestive section (oesophagus, stomach and intestines), this information could not be used to develop the indicator since we could not certify that the measurement was assessed in a harmonized way on the whole area. However, as most of samples were stored, data could be completed to distinguish both items and fragments, but this would require time to complete the database. The number of fragments and items were still asked as “optional parameters” in the INDICIT protocol (an update has to be done for specifying the definition and the methodology). A decision to keep or remove these parameters should be taken.

2.5.3.2. Factors influencing litter ingestion (indicator’s units and constraints)

Some factors may influence the sampling strategy and the probability to find turtles having ingested litter. To represent as best as possible the litter abundance and impacts, sampling ideally should be performed randomly, with a sufficient number of specimens to detect changes in occurrence and quantity of ingested litter. However, certain factors might expose turtles to litter more than others, e.g. smaller individuals may be less experienced

and less selective or may use different marine habitats compared to larger ones. If sampling principally target these individuals more/differently prone to ingest litter than the others, there would be a risk to over/under-estimate litter abundance and impacts. Nevertheless, if all individuals show same spatial and temporal variations in litter ingestion, all individuals could be combined in a same dataset for assessing an overall impact of litter at the MS or regional scale. In this way, even if young fulmars have been found to eat more plastics than older fulmars, as the mean mass of ingested plastics shows the same temporal fluctuations for adult and non-adult individuals, the two groups are combined to employ the OSPAR indicator by considering age as co-factor (van Franeker et al., 2011). This verification should be done for the Turtle indicator. The need for stratifying data according to environmental and biological factors was thus be tested. The objective was to specify the possible biological constraints, especially related to the circumstances of discovery and the causes of death, which may influence the sampling strategy, and the individual's size that may influence turtles' exposure to litter.

The INDICIT standard dataset contained a large number of basic and optional parameters, in particular to describe individual circumstances of discovery and body condition (26 parameters), some of them having been combined for analyses. The analyzed database contained 122 columns. A high number of combinations could thus be tested to assess which factors may influence litter ingestion. This part thus shows preliminary analyses which should be deepen in the future.

- Description of sampled specimens

Circumstances of discovery. Specimens were mostly found bycaught, supposedly recovered by fishermen, or stranded (Table 9). However, turtles found dead stranded, floating at sea or dead later in the rescue centre after recovery, may have been bycaught before (Table 9). The information on the probable cause of death enabled to add 21 turtles as bycaught (total known as 209 bycaught turtles).

For turtles found bycaught, the type of engine involved was reported for 105 cases but the differentiation remained unclear: Generally, they were longliners (22 cases), nets (30), trawls (27) and straight threads (24). The distance to the coast, the depth and the target species were not specified, and it was thus not possible at this stage, to test if litter found in bycaught individuals varied with the type of engine or the marine compartment in which they were captured. This information should be further detailed.

Table 9: Circumstances of discovery of the dead individuals recorded per area and per country (all data pooled)

Area	Country	Found at sea	Bycatch	Dead in rescue center	Stranding
Total		**22**	**188**	**20**	**519**
Atlantic	France	2	12	0	80
	Portugal	3	30	0	18
	Spain	2	0	4	7
	Global	***7***	***42***	***4***	***105***
Mediterranean	France	2	40	1	16
	Greece	0	0	0	28
	Italy	5	16	3	174
	Spain	2	87	0	95
	Tunisia	0	3	0	25
	Turkey	6	0	12	76
	Global	***15***	***146***	***16***	***414***

Probable causes of death. The external and internal exams of sea turtles aimed at identifying the probable cause of death. The information was recorded for 189 individuals on the entire dataset. Bycatch was the main cause of death (114 cases) followed by anthropogenic trauma, e.g. collision with boats (32 cases), entanglement, which in fact regularly appeared to be active fishing (bycatch) in set nets (20 cases) or in ghost fishing gear, and natural causes, e.g. disease (14 cases). Litter ingestion was identified as a direct cause of the death with certainty for only 4 individuals. Bycatch is supposed to sample both healthy individuals and individuals in bad condition, as found in our samples: For the individuals for which body condition was recorded, 13 loggerhead turtles were considered as in fair condition, 10 in good and 14 considered in poor condition or with injuries. All body (recorded) conditions were equitably found among stranded turtles (28 fair, 31 good, 31 injured, 24 poor). Bycatch and stranded seem thus a good way to sample specimen randomly according to body condition.

Individual's size. Several measures were taken to describe individuals' stage and body condition. Generally, stakeholders collected the standard curved carapace length (here after StCCL) and/or the minimum curved carapace length (hereafter MinCCL), thus not necessarily the two measures on the same individuals (Table 10). Moreover, it was regularly uncertain to know which measure was taken, as a standard or a minimum curved carapace length (see below). Generally, individuals were found larger in the Mediterranean compared to individuals sampled in the Atlantic area of the project (significant differences with Student t test, p-value<<0.001 for both StCCL and Min CCL), with differences among countries in the Mediterranean area (Figure 3).

Table 10: Stages of dead individuals sampled (all data). [Range and mean of standard curved carapace length (StCCL) and range and mean of minimum curved carapace length (Min CCL) in cm. Number of individuals per stage (Stage 1: ≤20 cm; Stage 2:]20-40 cm]; Stage 3:]40-60 cm]; Stage 4: ≥60 cm)].

Area	Country	StCCL	Min CCL	1	2	3	4
Total		**9.4-89; 53.45 ±0.03**	**12-81; 51.2 ±0.03**	**35**	**184**	**313**	**284**
Atlantic	France	14.4-68; 28.56 ± 1.69	21.2-66.1; 36.46 ± 8.16	21	57	11	3
	Portugal	9.4-71; 41.95 ± 2.22	12-69.5; 46.15 ± 2.17	12	16	29	9
	Spain	34.4-72.5; 47.86 ± 4.6	30-71; 48.62 ± 4.38	0	4	6	3
	Global	***9.4-72.5; 36.76 ±1.51***	***12-71; 45.73 ±1.91***	***33***	***77***	***46***	***15***
Mediterranean	France	31.5-82; 50.99 ± 2.69	27-80; 51.09 ± 1.8	0	25	25	22
	Greece	22.5-89; 59.59 ± 2.95	22-67.5; 47.43 ± 6.52	0	4	9	15
	Italy	21-82.7; 57.15 ± 0.93	-	0	22	94	81
	Spain	23-80; 45 ± 8.52	29-81; 57.5 ± 11.22	2	51	81	51
	Tunisia	43-71; 58.06 ± 1.85	21-77; 57.41 ± 1.84	0	4	41	30
	Turkey	26.8-83; 67.19 ± 0.91	-	0	1	17	70
	Global	***21-89; 59.07 ±0.69***	***21-81; 53.66 ±1.32***	***2***	***107***	***267***	***269***

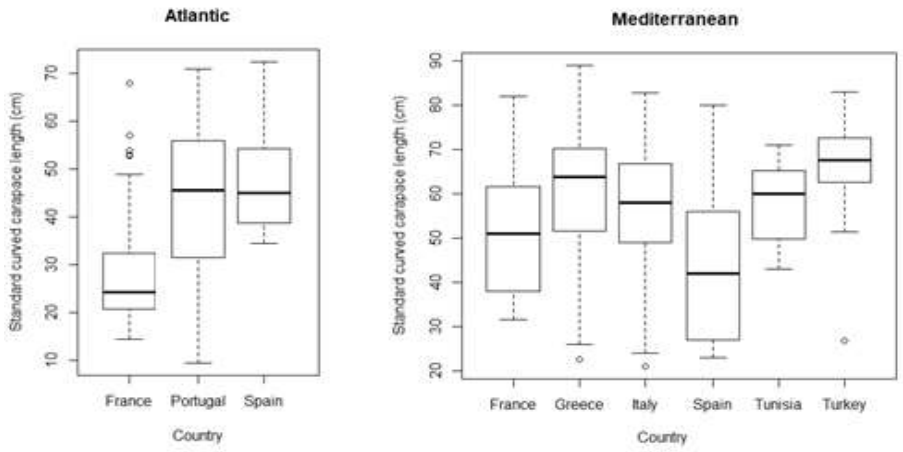

Figure 3: Boxplot (mean 1st and 3rd quartiles) of the standard curved carapace length of turtles sampled per area and country (all data pooled)

- Evaluation of spatial and temporal units

Spatial variations in litter ingestion. We detected no significant differences neither in the frequency of occurrence of litter ingestion in sea turtles nor in the dry mass of ingested litter between the Atlantic and the Mediterranean areas of the project, both when considering data from 1988 to present and data from the last 6 years: Prevalence of 54.02% and 61.95% (Student t test: t = -1.07, df = 268.62, p-value = 0.28) for all data, and **70.91%** and **61.95%** for data after 2013 (t = 1.44, df = 81.077, p-value = 0.15); Mean dry mass of 0.61 ± 0.11 g and 0.78 ± 0.09 g (t = -1.2, df = 368.46, p-value = 0.23) for all data and, 0.74 ± 0.21 g and 0.78 ± 0.12 g after 2013 (t = -0.19, df = 101.1, p-value = 0.85). Nevertheless, there were differences among countries in each of the two areas: Higher prevalence and higher ingested quantities in Portugal compared to France and Spain in the Atlantic area ($p<<0.01$), and e.g. differences between Greece and Turkey compared to other countries in terms of mass (Table 8).

Spatial variations in occurrence and dry mass have been regularly tested during the course of the project with updated database. The results changed regularly, underlying a possible effect of sampling size (see below), especially in areas where sampling just started (e.g. Atlantic Spain, East Mediterranean). The absence of differences found with the final database should be verified later with a larger database and other countries/sub-regions thanks to the involvement of other stakeholders. Meanwhile, the INDICIT consortium voted to differentiate data from Atlantic and Mediterranean and to propose 2 distinct GES (Minutes of INDICIT Intermediate meeting in INDICIT website, see §2.4.4).

Temporal trends in litter ingestion. We found almost no temporal trend (in fact a significant but really slight increase) in the prevalence of litter ingestion over the years from the 1990's till today (predicted slope of occurrence over years with a generalized linear model with family Binomial of 0.009 (±0.003), p-value <<0.01), but a significant slight decrease when considering data after 2013 (slope of -0.06 (±0.01), p-value <<0.01). However, we found no significant temporal trend for dry mass neither by considering all data (slope -0.006 (±0.01, p-value=0.62) nor only data after 2013 (slope -0.13 (±0.07, p-value=0.06). Nevertheless, both,

occurrence and mass varied a lot over time (Figure 4), due to several possible factors as the availability of samples in the different involved country.

Although the data collected before the INDICIT consortium was standardized for the INDICIT database, stakeholders should be contacted to verify that necropsies were indeed performed systematically and in a standardized way. Supposing that the dissemination of the MSFD Guideline in 2013 (MSFD Technical Subgroup on Marine Litter, 2013) encouraged stakeholders to collect data on litter impacts with a standard methodology, the INDICIT consortium decided to use data from 2013 for proposing a GES, which also complies with the MSFD 6-year cycle (Minutes of INDICIT Intermediate meeting available in INDICIT website). Meanwhile, the whole dataset can be considered to better understand the biological factors influencing litter ingestion. Moreover, the temporal trends should be tested by differentiating litter categories (see preliminary analyses below).

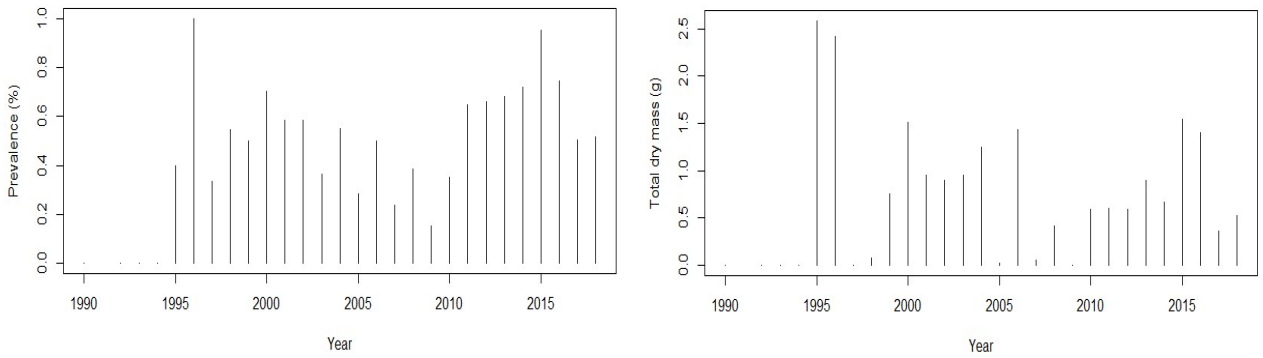

Figure 4. Mean dry mass of ingested litter (plastics; g) (left) and prevalence of litter ingestion (%) (right) in necropsied loggerhead turtles over years from 1988 to present

- Evaluation of the biological constraints

Evaluating the relationship between litter ingestion and individual's health status. Testing the possible relationship between litter ingestion and turtle's body condition is important both to evaluate if data should be stratified (in case of different probability to ingest litter depending on individual's body condition, size or health status) and to better understand the impact of litter ingestion on health. INDICIT partners and collaborators collected a large number of parameters to describe individuals' body condition (see INDICIT protocol for more details). There were:

(i) The size (proxy of development stage), measured by 12 carapace lengths (standard, minimum and maximum curved and straight carapace length, plastron curved and straight carapace length) as well as the body mass. Individuals were also attributed to a class of stage, according to 4 classes (Stage 1: ≤20 cm; Stage 2:]20-40 cm]; Stage 3:]40-60 cm]; Stage 4: ≥60 cm; Table 10). The stage was assessed first with the standard CCL if recorded, and if not, with other available carapace length measures.

(ii) The circumstances of discovery,

(iii) The body condition evaluated by the possible cause of death, the health status, the description of injuries (types and affected body parts) and the fat reserves,

(iv) The digestive capacity evaluated by the volume and wet mass of each digestive section (esophagus, stomach, intestines) both full and emptied.

Defining the impact of litter ingestion on health was quite hard at this stage. The mean digestive capacity (evaluated from the volumes of the 3 digestive full sections) was evaluated to 1554.86 $\pm$ 59.65 ml. On average, plastics represented 0.06 $\pm$ 0.006% of the entire digestive tract, but this did not necessarily feature impact on health. Identifying the causes of death was recognized by most partners and collaborators to be highly difficult. Death was attributed to litter ingestion for sure in only 4 individuals due to perforation or obstruction of the digestive tract (out of 189 cases identified). The mean length (StCCL) of individuals having ingested litter was 54.03 cm, almost equivalent to those with no litter (52.19 cm; t-value=-0.79; p-value = 0.42).

Identifying a parameter for better evaluating body condition. A first work was made to assess which parameter could be considered to evaluate health. Various combinations were evaluated to assess individual's body condition. New parameters were calculated for exploring the data such as a Body Condition Index (BCI) evaluated thanks to the ratio [weight (kg)/straight carapace length (cm^3)] X 10.000 (Barrios-Garrido et al., 2015; Bjorndal et al., 2000). The BCI calculated from INDICIT data was evaluated to 0.21 $\pm$ 0.04 for individuals considered as fat, 0.36 $\pm$ 0.04 for individuals in good condition and 0.76 $\pm$ 0.12 for thin individuals, with no significant difference among the 3 conditions (F=1.23; p-value=0.3). The BCI formula was evaluated from the green turtle *Chelonia mydas* (Bjorndal et al., 2000). This formula is maybe not adapted for the loggerhead and should be adjusted for this species.

It was not possible to compare all parameters in a single analysis because, the individuals have been described by some but not all the parameters proposed in INDICIT protocol, a complete database would have 0 line. Using library ade4 in R (Dray et al., 2018), we performed a multivariate analysis with mixed quantitative variables and factors (Hill and Smith method) (Hill and Smith, 1976) on Area (Atlantic/Mediterranean), Country, Month, Year, a variable "CircumstancesDeath" combined both the Circumstances of discovery and the Cause of death (Anthrop. trauma/At Sea/Bycatch/Entanglement/Euthanasy/Litter ingestion/Natural/Several/Stranding), Condition (Fair/Good/ Injured/Poor), StCCL, Stage3 (1/2/3/4), Occurrence, Total dry mass (TotDMass), logarithm of total dry mass (logMass). As revealed in Figure 5, the Hill and Smith analysis showed only one axis (see histogram of eigenvalues), which explained only 17.92% of the total variance. Although in 2 dimensions, the graph in Figure 5 should thus be interpreted only on the first axis. This first axis is led by Portugal (score 2.09), Atlantic area (1.72) and Stage 1 (1.72), thus possibly interpreting the differences of turtles' stages between the Atlantic (smaller) and the Mediterranean area of the project. However, this analysis could not allow the use of the scores on this first axis as a parameter to interpret a combination of variables.

Figure 5: Scatter diagrams of the projection of each parameter scores of the Hill and Smith method on a vector basis

These preliminary analyses underlined the need to standardize the information on individual body condition in order to better evaluate the impact of litter on health and better assess if inversely the body condition may affect the probability to ingest litter. The numerous optional parameters collected by several stakeholders should enable further studies to better define and assess litter impacts on individual's health.

The percentage of turtles with more litter than food remains as a proxy of individual's health. The use of the comparison of ingested litter (total dry mass) to food remains (FOO, Table 4) was discussed by INDICIT partners as a proxy of individual health. Possible bias due to specific life stage conditions, has been solved with the assumption that sea turtles who do not feed (e.g., adult individual during reproduction period), should also not ingest litter. Considering the entire INDICIT dataset, 14.51 % of sea turtles had ingested more litter than food, which represents **16.76 %** on the 6-year cycle from 2013. Data on food remains was not always collected, but some could be post-evaluated on stored samples, in order to provide more accurate assessments at the country level (Table 11).

Table 11: Percentage of necropsied individuals with more litter than food remains (dry mass)

Area	Country	All data	Data ≥2013
Total		**14.51**	**16.76**
Atlantic	France	21.43	11.11
	Portugal	0	-
	Spain	-	0
	Global	***18.75***	***9.09***
Mediterranean	France	11.59	12.12
	Greece	3.7	3.7
	Italy	26.26	30.23
	Spain	6.52	16
	Tunisia	9.09	9.09
	Turkey	0	0
	Global	***14.37***	***17.01***

Evaluating the influence of intrinsic factors on litter ingestion. We evaluated the factors that may influence occurrence and dry mass using generalized linear models with a Binomial and Normal distributions respectively. The distribution of data on litter mass was skewed towards zero. A logarithmic transformation of mass data was better although it did not significantly help to normalize the distribution. A transformation would be necessary to analyse correctly the factors affecting the dry mass of litter and should be further explored. The following exploratory analyses were done with a log transformation of the mass, which gave better results.

Effects of Area and Country have been evaluated above. The detection of a possible influence of body condition or size varied with the data collected during the project and in particular with the parameter chosen. We choose here the standard curved carapace length (StCCL) since was recorded most often. StCCL had no significant influence on occurrence neither when considering all data (t-value =0.8 and p-value = 0.42) or only data after 2013 (t-value = -1.19 and p-value = 0.23), and no on the total ingested dry mass (log-transformed +1) (respectively t-value= 1.72 and p-value = 0.08 with all data, and t-value = 0.519 and p-value = 0.6).

We studied models with one, two or all of the 3 additive or interactive parameters Area, Country and StCCL (the measure mainly recorded to describe individuals' body condition). We selected the model based on parsimony and considering a difference in Akaike's Information Criterion (ΔAIC) of ≥ 2 as equivalent models. The model selected for explaining the occurrence of litter ingestion contained the additive effects of the 3 parameters (AIC= 583.81). The same model was selected for the dry mass (AIC = 701.32), but the model Country x StCCL was almost equivalent (AIC = 702.57; Table 11).

Table 12: Models predicting the occurrence of litter ingestion and the dry mass of ingested litter (with log transformation) according to Area (Atlantic/Mediterranean), Country and StCCL (Standard Curved Carapace length) for necropsied loggerheads. Models are selected using Akaike's Information Criterion (AIC).

Model	Occurrence	Log(Dry Mass)+1
	AIC	AIC
1	1137.34	1304.52
Area	1138.18	1306.08
Country	1066.5	1263.62
StCCL	689.14	765.09
Area + Country	1033.32	1236.66
Area x Country	1017.41	1238.2
Area + StCCL	682.13	754.77
Area x StCCL	663.87	741.35
Country x StCCL	598.82	702.57
Country + StCCL	600.61	704.75
Area + Country + StCCL	583.81	701.32
Area x Country + StCCL	585.81	701.32
Area + Country x StCCL	590.02	702.77
Area x StCCL + Country	583.79	700.45
Area x StCCL x Country	596.02	702.99

According to available data and these first exploratory results, data were not stratified (all individuals pooled) but GES will be differentiated for the 2 areas (see above).

- Categories of litter

Ingested litter was differentiated in 7 categories in addition to food remain and natural no food remain (see Table 4).

Table 13: Mean mass of ingested litter (±standard error). Litter categories represent industrial plastics (IND), sheet like plastics (SHE), threadlike plastics (THR), foam (FOA), fragments (FRA) and other types of plastics (POTH), as well as non-plastic items, food remains (FOO) and natural items not considered as natural food (NFO)

Litter category	All data	≥2013
IND	0.001 ±0.0005	0.001 ±0.0007
SHE	0.23 ±0.03	0.2 ±0.03
THR	0.15 ±0.05	0.19 ±0.07
FOA	0.18 ±0.13	0.24 ±0.2
FRA	0.26 ±0.04	0.21 ±0.04
POTH	0.05 ±0.01	0.03 ±0.01
Non plastic	0.58 ±0.46	0.78 ±0.68
FOO	78.35 ±11.8	96.87 ±15.21
NFO	2.75 ±0.78	3.68 ±1.16

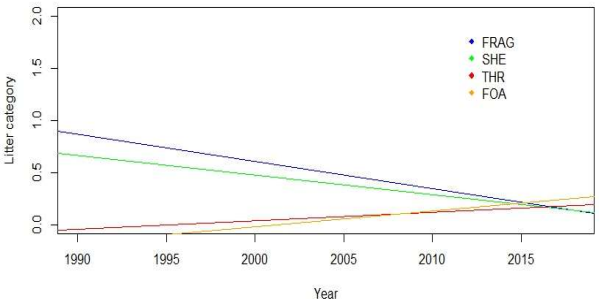

Figure 6. Temporal variations of the main plastic categories found in loggerhead turtles

All categories of "USE" plastic litter (see Table 4) was found in necropsied loggerheads' digestive tract, especially fragments (FRA), sheet like (SHE), threadlike (THR), with different variations over time (slope for FRAG: -0.03 ± 0.006 (p-value <<0.01); SHE: -0.02 ± 0.005 (p<<0.01); THR: 0.008 ± 0.009 (p=0.36); FOA: 0.01 ± 0.02 (p=0.54); Table 13, Figure 6). Inter-annual variations should be further studied.

- Sample size

The evaluation of occurrence and dry mass of ingested litter varied with the number of data considered. Figure 7 shows an example of result (dry mass in this case) according to a random selection of data, from 1 to the entire dataset. Sample size should correspond to the number of data from which adding new data should not change the results.

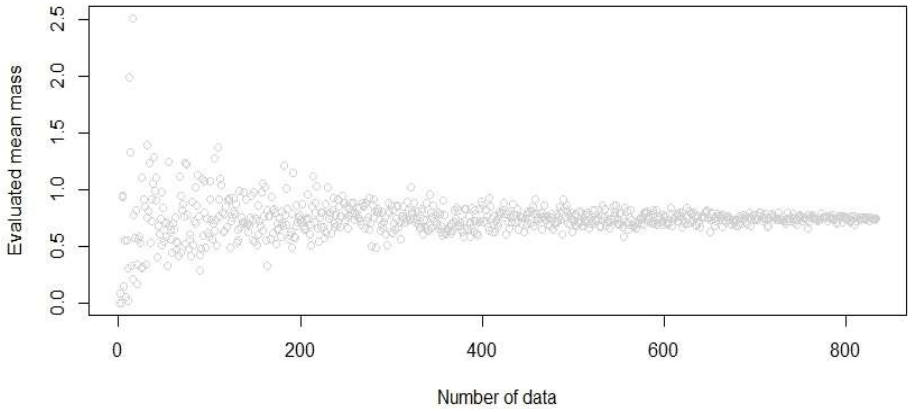

Figure 7: Mean dry mass (g) of ingested litter evaluated in the population according to number of data selected randomly

A power test was done in R with library pwr (Champely et al., 2018). It aims to determine the sample size required to detect an effect of a given size with a given degree of confidence. Four factors were evaluated: (i) size of the response to detect, (ii) the variance of the response, (iii) the significance level and (iv) the power. A significance of 0.05 (Type I error, that is the probability to reject the null hypothesis when it is true) and a power of 0.90 (1 – Type II error, that is the probability to not reject the null hypothesis when it is true, in other words to not detect an effect when it is present) were considered. The analysis took into consideration the effect size that combines the minimal relevant difference and the variability into one measurement Δ/σ, based on current evaluations. Our results showed that, for detecting **a change of 10 %** in dry mass, **a sample of 236** (N = 235.8) would be required for the whole INDICIT area.

Further analyses should be conducted to better assess sampling size, and by separating data for the Atlantic and the Mediterranean areas in order to provide accurate evaluations.

2.5.3.3. Synergies and perspectives

More statistical analyses will be done while collecting more standard data. This will aim specially to perform more powerful tests to assess the indicator's possible biological constraints. The analyses should also differentiate the litter categories in order to better evaluate specific Programs of Measures, as well as to litter size in order to plan how supporting the stakeholders to collect data separately on micro (1-5 mm) and macro-litter (>5 mm) according to the GES definition.

Moreover, the knowledge learnt from INDICIT database could be used for other projects and MSFD Descriptors, such as related for example to biodiversity (Descriptor 1), food webs (Descriptor 4), reduction of bycatch, or more generally for conservation of sea turtles. The INDICIT consortium agreement does not allow partners to share raw data, some of which belonging to stakeholders who gathered the data only for INDICIT project's purpose. Only results (analyzed data) could be shared. A set of biological questions could be defined with other experts involved in such projects/Descriptors in order to provide the results of these specific analyses.

INDICIT partners collaborated with other projects. For example, a small number of data was shared with CleanAtlantic project (partner IFREMER) to start building a platform for data banking (see §2.3.3). This platform should facilitate and possibly accelerate the standardization of data by avoiding the phase of data cleaning. INDICIT also collaborated with MedSeaLitter, an Interreg project which, targeting the Mediterranean level, aims establish standardized approaches for monitoring litter abundance and impacts and support networking, cooperation and harmonized approaches among Marine Protected Areas. The two projects interacted in order to share protocols (INDICIT protocol) and data on ingested litter in necropsied loggerheads.

As almost no empirical standard data on litter was available at the Mediterranean level, an evaluation thanks to simulations would help assessing indicator's units. Therefore, maps of floating litter distribution across the Mediterranean was set up in the framework of MedSeaLitter in collaboration with INDICIT in order to 1) assess high risk areas where sea turtles may be exposed to litter and 2) test the correlation between litter distribution and litter ingestion in sea turtles. These maps represented the mean monthly distribution of litter calculated from 10 years of simulations (Mansui et al., in revision (MedSeaLitter project); Figure 8). While a diffusion of litter at the whole basin scale was shown at the year level, thus with no clear pattern of distribution, some accumulation areas appeared at the trimester level with seasonal variations (Figure 8). An analysis of the mean monthly litter distribution (study in review) showed fluxes of litter, with litter spread over all the Mediterranean on Winter, while litter concentrate in the Western basin in Summer. These maps may help interpreting INDICIT

results. A perspective would be to test the spatial constraints of the indicator "Litter ingested by sea turtles" and if 2 (or more) thresholds for GES corresponding to different hydro-ecological sub-regions, should be proposed for the Mediterranean area.

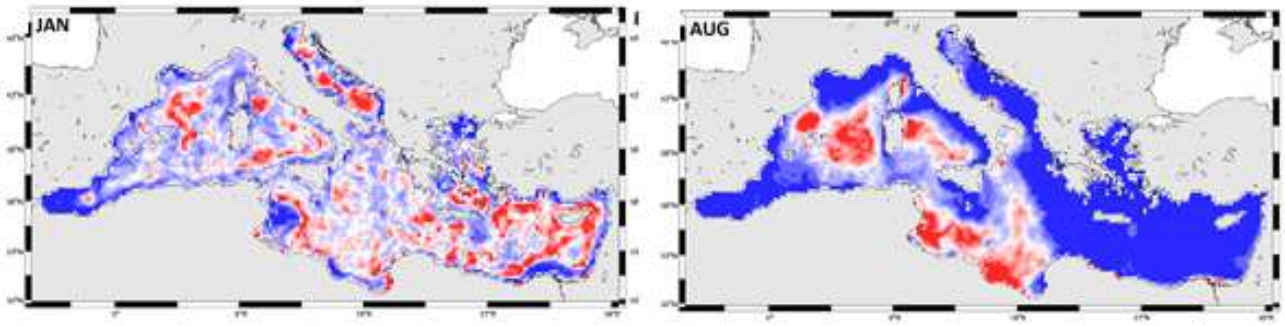

Figure 8: Simulations of monthly distribution of floating litter across the Mediterranean Sea, examples in January and August. Red tones are used for particle accumulation. Grey and blue tones show emptying areas (from Mansui et al., article in review; Collaboration with MedSeaLitter project).

2.5.4. GES scenarios

GES is expected to determine a threshold value (TV) and the extent to which this TV has to be achieved (Art. 9(1) of MSFD 2008/56/EC, see Appendix 2). TV should represent agreed level(s) of pollution above which GES is approved to have been achieved. TV should be evaluated thanks to a value, a range of values or a trend reduction. TV should reflect the significance of an adverse effect and should be compared against a reference condition (MSFD Commission Decision 2017/848/EU). It should be set on the precautionary principle, reflecting the potential risks of pollution to the marine environment consider the dynamic nature of marine ecosystems and their elements and possible future risks. The definition of GES for D10C3 according to MSFD 2017/848/EU corresponds to the amount of litter and micro-litter ingested by marine animals (i.e. sea turtles) that is at a level that does not adversely affect the health of the species concerned.

Various approaches have been proposed to establish TV, e.g. based on the study of the dose-effect relationship, defined as "the dose of the toxicant below which no adverse effects will occur" (Rosenthal and et al., 1997). However, information collected until now, does not allow determining the relationship between litter ingestion and death for loggerhead turtles. At this stage, INDICIT data did not enable to assess individual's health status with certainty. From data on necropsies in Australia, Wilcox et al. (2018) estimated that 14 pieces of plastics in turtles' digestive tract should lead to a 50% probability of mortality. However, INDICIT consortium agreed that this approach could not be employable as baseline for a GES, since 1) at this stage, the number of fragments is a confusing parameter and would need further analyses to be used as a parameter, and 2) only 4 deaths caused by plastic ingestion were identified with certainty among the 189 individuals for who the cause of death was possible to evaluate (but with degree of certainty not asked to experts). Wilcox et al. (2018)'s study was discussed within INDICIT consortium, as well as with EAB and external experts such as Jan Van Franeker who shared his experience in the development of the Fulmar indicator for the OSPAR RSC (van Franeker et al., 2011). With some questionings regarding Wilcox et al. (2018)'s methodology, it was also recognized that evaluating the impact of litter ingestion on the cause of death would require an objective statement. Moreover, this would need a larger number of data associated to quantities of plastics to make power models. All parameters

describing body condition collected within INDICIT project would not be usable for testing such an approach for now, but this information, in partnership with all experts identified during the project, would make possible to propose an objective judgment of impact of litter ingestion on body condition and health. Nevertheless, TV should be based on more ambitious thresholds, based on impacts on health before reaching death. An approach comparing litter ingestion and body condition assessed either from external exam or evaluation of toxins in blood or tissues should be tested.

Considering MSFD definitions and requirements (see Appendix 2), INDICIT data and current results (main results reported in Table 14), INDICIT consortium took several decisions:

- Reference: It was not possible to (i) find an existing control area (without pressures) neither in the current environmental situation nor (ii) by defining it *a posteriori*, due to the lack of past data to reconstruct past events. In the future, INDICIT data could be used with modelling on litter distribution, coupled with expert judgement in order to defined a reference area (Borja et al., 2013). For now, the INDICIT consortium decided to define the reference condition to which TV should be compared, as the minimum of litter impact observed in the studied area. Further studies could test other approaches, e.g., use of the median.

- Spatial unit: As underlined by the New Commission Decision 2017/848/EC, TV needs to be set at appropriate geographical scales, taking into account the biotic and abiotic characteristics of regions, sub-regions and subdivisions. INDICIT consortium decided to propose two different GES scenarios, for the Atlantic, and for the Mediterranean areas of the project, in order to consider their specificities although the INDICIT results with data collected until January 2019 showed no significant difference in prevalence of litter ingestion and ingested dry mass (§2.4.3.2). Due to the differences between the Eastern and Western basins of the Mediterranean, with a high prevalence of litter ingestion in the Western compared to the Eastern basins, suggest that 1 separate GES should be proposed for each of the two basins, as confirmed by the simulations of litter distribution (Figure 8).

- Temporal unit: An evaluation and revision on a 6-year cycle appeared relevant regarding INDICIT data (§2.4.3.2).

- Stratification of data: No biological constraint was considered, such as individual's size or circumstances of discovery (i.e., capture mode) which was either not significant or further standard data should be collected for power analyses (§2.4.3.2).

- Sample size. Thirty individuals per country was considered as a minimum for allowing parametric tests. A first evaluation of 236 turtles was found for assessing GES for the area. This is reached for the Mediterranean but not yet in the Atlantic. The INDICIT consortium suggested a minimum of 50-100 samples per country. A minimum of 30, as generally required for parametric statistical analyses, could be tested, since it could allow reaching 236 turtles for a 6-year period. This is not yet reached for all countries (Table 14), but this would be possible now that a network has been implemented.

Table 14: Main results on necropsied loggerhead turtles from data from 2013 included to January 2019. [Means per area and country of: prevalence (mean percentage of turtles found with ingested litter), dry mass (mean dry mass of ingested plastic ± standard error), dry mass per StCCL (x10), and percentage of turtles having more plastics than food remains, calculated at the population level. Minima are highlighted in light red. In grey, the results of the countries where the 30 turtles proposed by INDICIT consortium as a minimum sample size are not achieved.

Area	Country	Number of necropsies	Prevalence (%)	Dry mass mean (g)	Dry mass x 10 per StCCL	% turtles with Litter>FOO
Atlantic	France	21	45	0.28 ± 0.16	0.07 ± 0.05	11.11
	Portugal	33	81.82	1.13 ± 0.35	0.23 ± 0.07	-
	Spain	9	88.89	0.16 ± 0.07	0.04 ± 0.02	0
	Global	***45***	***70.97***	***0.74 ± 0.21***	0.16 ± 0.04	***9.09***
Mediterranean	France	77	82.43	1.23 ± 0.27	0.21 ± 0.06	12.12
	Greece	28	64.28	0.13 ± 0.06	0.02 ± 0.01	3.7
	Italy	129	62.01	0.92 ± 0.19	0.16 ± 0.03	30.23
	Spain	85	80.49	0.89 ± 0.27	-	16
	Tunisia	46	52.17	0.84 ± 0.71	0.03 ± 0.03	9.09
	Turkey	94	33.33	0.37 ± 0.29	0.05 ± 0.04	0
	Global	***457***	***61.95***	***0.78 ± 0.12***	0.11 ± 0.02	***17.01***

Several scenarios have been discussed by INDICIT consortium considering that GES should aim to appreciate the environmental situation and support Member States to easily refer to it. Two scenarios were retained for further analyses but are still being discussed:

- The **first scenario** was based on baselines (see Appendix 2), as for the Fulmar approach. For this, three approaches are possible: 1) based on the minimum prevalence, 2) based on the minimum dry mass of ingested litter, which could be reported to the individual's carapace length (see Table 14), or 3) on both the minimum prevalence and the minimum dry mass, this third option being retained by INDICIT consortium (Table 15).
- The **second scenario** aimed to consider the impact on health, as required by the new Commission Decision (Definition of MSFD 2017/848/EU considering "a level that does not adversely affect the health of the species concerned"), and which is not considered in the previous scenario. Nonetheless, the notion of "Health of the species" should be further specified. Health could indeed be evaluated at the population level, which would make sense for establishing species conservation measures or measures aiming to restore/maintain ecosystem equilibrium. However, this may be difficult to evaluate because this would require a long term spatial and temporal monitoring of population structure (age and sex distribution) and an evaluation of survival and reproduction rates. Although some evaluations have been made thanks to analyses of bycatch (Casale, 2011), this is highly challenging for long-lived and late reproductive species. Assessing the impact of litter ingestion on health at the level of individual appears more relevant, supposing that a decrease in survival or reproduction would have consequences on population dynamics. However, the health of sea turtle individuals was difficult to evaluate, especially on necropsied individuals

(§2.4.3.2). The INDICIT consortium hence proposed to evaluate the percentage of turtles with more plastics than food remains as a proxy of individual's health (§2.4.3.2; Table 15).

The approach of these two scenarios was presented to EAB especially during the INDICIT Final meeting (Minutes of the Dissemination meeting available in INDICIT website) and generated debates. Other approaches were envisaged, such as adding trends to highlight efforts, e.g. a target of 10% to 30% reduction in prevalence, dry mass or percentage of turtles having ingested more plastics than natural food. Finally, the GES should be evaluated for the plastic categories mostly observed in sea turtles (FRAG, SHE, THR, see §2.4.3.2).

Table 15: Pro and cons of the main GES scenarios discussed by INDICIT consortium (3.7 % the following minimum percentage). In blue, the two scenarios retained by INDICIT consortium for further discussion.*

Scenario			Atlantic*	Mediterranean	Pro	Cons
1	**Baseline**	**Prevalence**	"There should be less than 45 % of turtles with ingested plastics"	"There should be less than 33 % of turtles with ingested plastics"	Easy and useful for assessing the environmental situation	The impact on health is not assessed
		Dry mass	"There should be less than 0.13 g of plastics ingested by sea turtles on average"	"There should be less than 0.37 g (0.13 g?*) of plastics ingested by sea turtles on average"		
		Prevalence and dry mass	"There should be less than 45 % of turtles with more 0.13 g of ingested plastics on average"	"There should be less than 33 % of turtles with more 0.37 g of ingested plastics on average"		
2	**Target towards a threshold (impact on health)**		"There should be less than 11.11 % of turtles with more plastics than food remains"	"There should be 0 % (be less than 3.7 %*) of turtles with more plastics than food remains"	Best proxy of health possible currently	Might be constrained by environmental availability or biological constraints (to be further studied); FOO not considered in official definition.

The second scenario proposed by INDICIT consortium, raised questions both because the "quantity of food remains" does not clearly appear in the D10C3 definition, and because it may depend on individual's location (i.e., availability of natural resources) and stage (feeding capacity and selectivity). The notion of "health" remains highly discussed within INDICIT consortium and the definition of Criteria D10C3 referring to the "health of species concerned" has led to debates within the consortium. We considered that the health should be

measured at the individual level, while we should refer to "viability" for the population or the species. Therefore, INDICIT consortium focused on the evaluation of health at the individual level, and tried to assess the contribution of the individual to the population ideally thanks to parameters related to reproduction and survival (i.e., fitness). INDICIT consortium considered that as long as a turtle has ingested litter, this could have an impact on its health. Litter ingestion should be confronted to morbidity or mortality, which was not possible with the data collected during the project. Some EAB members suggested considering the relationship between the number of ingested litter items and the direct mortality caused by litter ingestion (a dose effect). It would indeed enable to propose a simple scenario corresponding to the D10C3 definition. However, INDICIT consortium has discussed this approach which was proposed in Wilcox et al. (2018)'s study (the probability of death due to ingested plastic increases with the amount of plastic in their gut). This approach allows proposing threshold and one result was that a load of 14 items corresponds to a probability of mortality of 50% (Wilcox et al. 2018). The INDICIT consortium was very skeptical about this work, especially on the way to evaluate the cause of mortality during necropsies. According to experts' own experiences, the number of items cannot be the only cause of death, but the type and shape of plastic items would have to be taken into account. During the INDICIT project, a death caused by litter ingestion was indeed found in only 4 among the 189 cases where the information was filled out. Moreover, the methodology to assess the number of items/fragments appeared to be confusing (see §2.3). The relation between the probability of death and the amount of ingested plastic cannot be use with the currently available data.

Various proxies of body condition and health, and the relationship with the dry mass of ingested litter, have been tested and no clear and simple link appeared with the quantity of (optional) data acquired at this stage (§2.5.3). The necessity to prove an impact on the health of the individual was highly debated, some of the partners considering that the impact of litter is proved by the high percentage of turtles found with ingested litter (population level effect). Proposing a threshold based on the effective mortality (individual effect) would be not enough ambitious, as it would be better not observing turtle mortality caused by marine litter.

INDICIT consortium discussed if the sea turtle's feeding behavior could give information on its health status. A turtle in good health should be able to feed on natural items, and be more selective when exposed to both natural items and marine litter. Stage is important to take into account, as adults during the reproductive period (summer months) may no feed, but in this period, they should have ingested neither natural food nor litter. This hypothesis which leads to the second scenario, was also highly debated. While considered as a good proxy of individual's health, the partners recognized that the threshold-ratio Litter/Food remains should be analyzed more accurately, and recommended to acquire more data on the FOO category.

For the two scenarios and whatever the proxy of health (mortality, body condition index, ratio FOO/litter…) and the statistical approach considered, the evaluation of the threshold raised the question of what could be judged "acceptable" and "enough ambitious". A reference to 0 (e.g. pristine and un-impacted area) had been judged unrealistic. Searching for a compromise (e.g. a reachable target and a positive effect on the species) will be more realistic. INDICIT consortium concluded that further research is needed to collect more data, especially to acquire knowledge on the evaluation of health in Sea turtle and the impact of litter ingestion on health. Research is also needed to better assess the ratio food remains/litter, considering spatial and inter-individual variations and digestibility of items.

Thresholds are commonly evaluated in human health (e.g., exposure to lead poisoning), for example through physical symptoms and cognitive capacities to a more or less long term (Needleman, 2004). Other approaches are based on experimentations or inter-species comparisons, not possible on sea turtles due to their protection

status. Other studies rest on the study of the correlations between symptoms and concentrations. Such toxicological study have been initiated for example on marine mammals (Desforges et al., 2016) and are suggested for a better assessment of GES (Fossi et al., 2018). Using such methodology for sea turtles, considering gradient of pollution and ideally a control area or control individuals, are also further discussed.

2.6. Summary of recommendations

2.6.1. Networking

The organization of networks in charge of collecting and manipulating specimens in the field is highly variable among countries, and sometimes the local networks may not be reached easily. Elaborating a diagram of existing local organizations, considering the role of each stakeholders and the contact of possible referent(s), for each country or region would facilitate the speed of procedures locally. Moreover, it could inspire the organization of new networks in other countries where monitoring is being set up.

The perpetuation of the networks should be ensured for a sustainable standard monitoring and data reporting. For this, institutions reported needs and conditions of implication, reported by INDICIT consortium (Table provided to the EU and EAB). Institutions generally asked for more human and material needs that have to be addressed, considering that the protocol for data collection, from the discovery and handling of the specimen to the extraction and classification of ingested litter, would require a minimum of 5 hours for one individual, without considering data reporting and analyses. It is also recommended to consider providing and maintaining the skills for collecting specimens and extracting/classifying the litter ingested, thanks to regular workshops and training sessions, which should help promoting the collaborations. Other tools like mobile or web applications and blogs should facilitate discussions and mutual support in case of misunderstanding of the protocol or difficulties for identifying items found ingested by the turtle.

The lack of data in some regions prevents to assess the situation in a global and consistent way. Developing the networks in these areas appears as a priority. This is especially the case in the OSPAR zone as well as in the South Mediterranean Sea. Our results showed that the impacts of litter were found to be minimum in the Eastern Mediterranean basin (minimum occurrence of ingestion and minimum ingested litter quantities). However, data collection is just beginning in this area and a more global view (in time and space) could strongly change the evaluation. It is therefore necessary to reinforce the networks and collect more data in this area in order to better understand the processes and provide results that are more accurate. This is particularly important as the region where the impact (occurrence and quantity of ingested litter) is evaluated as minimum can be used as reference for the GES.

2.6.2. Harmonisation of data collection

The INDICIT protocol with a video-tutorial has been highly shared. Pursuing the dissemination of the INDICIT protocol would enable collecting more information and data, and engaging more stakeholders. The INDICIT protocol has been merged with the RAC/SPA protocol and is being disseminated to Southern Med Countries.

The protocol proposes basic and optional parameters. “Optional” parameters, especially those related to body condition are encouraged to be collected, as well as measuring the dry mass of natural food remains. This was highly difficult to assess accurately the impacts on individual’s health with the data collected at this stage, while it is required in the definition of the criteria D10C3. More data on the size of the items found ingested, also

asked as an optional parameter, should help differentiating micro-items (1-5 mm) to macro-items (>5 mm) and further progress on the feasibility of the implementation of the indicator "Micro-plastics ingested by sea turtles" (see §3).

The collection of more data will enable refining some parts of the protocol, such as the method to count the items, which seems to vary among stakeholders. The GES scenarios have been proposed from data on dead turtles (necropsies). However, thanks to the number of data that can be collected from living individuals (faeces), and the high occurrence of litter found in faeces, pursuing the collection of these samples from living individuals is highly recommended. Nevertheless, there are discrepancies in the methodologies used among stakeholders to collect samples and training sessions and workshops could be proposed specifically for rescue centres on this topic. This should allow a better understanding of the consequences of litter ingestion on individual's health, and to assess the possibility of comparing results from the two protocols on dead and on living turtles.

An average of 5 hours with two manipulators should be considered for the collection of data from necropsied individuals, although the time required to collect data depends on the body condition level and the quantity and variety of ingested litter. The cost of implementation should consider the equipment and training of each stakeholder. Training sessions should consider providing a tool kit to support the stakeholders who will be authorized to manipulate the specimens to handle the turtle, perform the necropsies and extract and analyse the litter.

The intervention of professional teams of 2 or 3 persons specialized to extract/classify litter from digestive tracts and faeces in laboratory and competent to report and analyse the data may also be considered. These teams could support stakeholders, especially when they are too busy with other tasks, especially during the nesting period or after stranding events for examples. In this case, stakeholders would be asked to perform the first measures on the field, to do the necropsies, to collect the digestive tracts, and store them in a freezer as recommended in the standard protocol.

2.6.3. Data banking and cleaning

Raw data cleaning required a very long time and had led to lose time for data analyses. A dedicated platform should help for this task, from data gathering and cleaning, with a specific pipeline in order to homogenize data. Such platform is being developed by IFREMER in the framework of CleanAtlantic project.

2.6.4. Need of further analyses to evaluate GES scenarios and indicator's constraints more accurately

Evaluating individual's health. The INDICIT consortium selected two major GES scenarios, the first being based on the Fulmar approach and the second proposing an approximate of impact on individuals' health. The notion of health was difficult to assess with the data collected during the INDICIT project. The INDICIT consortium had difficulty to agree on the way to respond to the definition of Criteria D10C3 as currently written, since the "heath of the species concerned" may be confusing. Discussing the health of individuals in regards to the viability of populations and species, the consortium decided to further work on the "health of the individual" by collecting "optional" data on injuries and body condition. Further studies should be performed to better assess the impact on health, especially considering parameters related to individuals' chances of reproduction and survival, which may be related to population dynamics and viability.

Acquiring more data on natural food remains. The second scenario considers the quantity of natural food remains in relation to the quantity of ingested litter. The ratio food/litter should be further studied, considering possible differences in individuals' characteristics related to stages or reproductive period for example, and comparing the transit digestibility between natural and plastics items. Considering that natural food can consist in large and hard shells, the transit digestibility could be comparable. For the moment, the second scenario was therefore retained as the best approach with the current state of knowledge. For this reason, and to further acquire more knowledge to verify the relevance of the scenario, INDICIT consortium recommends collecting the dry mass of natural food retained in filters of 1 mm and 5 mm.

Testing the biological constraints more accurately. The constraints related to intrinsic and methodological factors which may influence the observed occurrence and quantity of ingested litter should be further analysed. None of the factors that we tested had a clear influence on litter ingestion. Different parameters were used to test the influence of individual's size, and the results vary according to the parameter considered. Measures should be standardized among stakeholders. We recommend the measurement of the standard curved carapace length, as it is the most commonly body size measurement used in sea turtle, in order to get enough data for more powerful statistical tests. Body condition indices, currently developed for the green turtle, should be tested and evaluated for the loggerhead species. In addition, the circumstances of discovery should be specified to test e.g., if stranding and bycaught turtles would have the same risk of litter ingestion. Nevertheless, the typology should be revised, since stranded individuals may have been bycaught before.

Verifying the units. More data is necessary to better assess the spatial and temporal units of GES reference and target. Our results and the collaboration with the programme MedSeaLitter suggested no difference in litter ingestion (occurrence and quantities) between the Atlantic and the Mediterranean. Further data will enable for more powerful tests and more accurate assessments.

Perspectives for further studies. The results obtained by the INDICIT consortium and collaborators enabled defining the modalities of use of the indicator (the spatial unit i.e. Atlantic versus Mediterranean; the temporal unit, i.e., a 6 years-cycle), the biological constraints; i.e., none at this stage) and a proposal of GES scenarios. At this stage, it appeared necessary to collect more data, and start evaluating the indicator in pilot areas, where the response to relevant programs of measures could be evaluated, e.g. related to the ban of plastic bags, which corresponds to the category USE SHE, a category most often found ingested by sea turtles.

3. Feasibility studies of other marine litter impact indicators

The INDICIT consortium discussed the relevance and feasibility of two indicators for monitoring other impacts of marine litter on marine wildlife, related to entanglement and to the ingestion of micro-plastics (< 5 mm). Two feasibility studies were carried out during the first 6 months of the project. The reports on the Entanglement indicator (Claro and INDICIT consortium, 2018) and on the Micro-plastic ingestion in fish and sea turtles (Silvestri and INDICIT consortium, 2018) with detailed results, are available in a compiled document ("Pilot and feasibility studies for the implementation of litter impacts indicators in the MSFD and RSCs OSPAR-Macaronesia, HELCOM and Barcelona", INDICIT consortium, 2018) on the project website (https://indicit-europa.eu/indicit-documents/). The summaries are provided in the two following paragraphs.

3.1. Indicator "Entanglement by biota with marine litter"

This indicator belongs to the Criteria D10C4 "the number of individuals of each species which are adversely affected due to litter, such as by entanglement, other types of injury or mortality, or health effects" with a unit of measurement being "the number of individuals affected (lethal; sub-lethal) per species". Our feasibility report focused on a review of available grey and published literature and the responses of a questionnaire disseminated to experts in sea turtles, marine mammals, marine birds, fish or marine litter. The objectives were to:

i) assess the state of knowledge on entanglement in these taxa (prevalence per taxa/species, types of litter, type of injuries, etc.),

ii) evaluate the constraints (methodologies, species biology, etc.),

iii) identify and analyse the available data.

It was also to identify the networks and skills necessary to implement the monitoring of entanglements. All taxa were assessed in order to identify which of them and which species could be relevant as indicators especially in the OSPAR-Macaronesia areas, HELCOM and Barcelona RSCs and of MSFD. All marine compartments were considered, from the surface to the bottom. The analysis of the literature and of the 21 responses received to the questionnaire was carried out by taxon and region.

Few data on entanglement reported in literature. The literature review showed that marine mammals, birds, fish and reptiles have been recorded to suffer from entanglement. Individuals found entangled were mainly Megafauna observed at sea or stranded and invertebrates on the sea floor. For all taxa, the observations of entanglement seemed to have increased over time. Intrinsic factors such as behaviour or age, for example in mammals, played a significant role in the probability to be entangled in litter, and some debris may be more attractive than others. However, the partial or opportunistic data found did not allow to make statistical analyses. We did not find any standard protocol and no standardized typology of the types and sizes of marine litter that cause entanglement.

Types of litter. The litter causing entanglement came from various sources, but the fragments of abandoned fishing gears and human product packages are the most frequently type of litter found. For litter from fishing activities, the distinction between passive (e.g., ghost nets) and active (e.g., animals escaped with a part of the gear after an accidental capture) entanglement was highly difficult to differentiate with certainty, although ghost gears may cause more entanglement than active bycatch. Few data were already available to better describe the entanglement, and its consequences on individuals' body condition, health or behaviour were rarely reported.

Methods. The methods used for collecting individuals or acquiring information on entanglement varied according to taxa. The observations were generally made by NGOs, rescue centres, fishermen or scientists. Entangled individuals were generally observed stranded, either opportunistically or during regular surveys, and more occasionally during observation campaigns, on the sea surface or in the water columns. The involvement of a larger audience (e.g. yachting activities) can contribute to this data collection. On the sea bed, photographic sampling performed during sea campaigns and diving explorations with Remotely Operated Vehicles or submarines could be a good way to collect information on entanglement.

Taxa and species. In mammals, despite some constraints, e.g. related to their migration, harbour or grey seals appeared as relevant indicator species due to their number, the prevalence of entanglement (Figure 9) and the existence of a network for the observation and the rescue of these species. These characteristics were also true for sea turtles. The presence of large network trained to collectively collect data on litter ingested by the loggerhead turtle (§2.2) and the standard protocol established and disseminated for the indicator "Litter ingested by sea turtles" (§2) should confer a direct benefit for the development of this new indicator on entanglement. In birds, the European shag *Phalacrocorax aristotelis*), the Scopoli's Shearwater *Calonectris diomedea* and the northern Gannet *Morus bassanus* appeared as suitable indicator species. Observations appeared most often during nest surveys or fisheries observer campaigns. In fish, the large distribution of the blue shark *Prionace glauca* was considered as a good species, regularly observed during fishery observer programs. In invertebrates, corals and sponges were the more affected, and gorgonians, black corals, scleractinians.

Figure 9. Entanglement of a seal in fishing litter. Picture from Picardie Nature

Figure 10. Injuries caused by entanglement on a sea turtle. From HCMR

Networks. Entanglement was observed in a wide range of depths. The structures producing data in the project area were heterogeneous in their target and their governance, and generally, they did not benefit from stable human/financial resources, which may ensure a constant data flow on entanglement for each taxon. Skills, human and logistical resources, exist at some level but the majority of the existing data was collected by volunteers (NGOs, rescue centres) and thanks to public and private fund raising, the other part by professional scientific oceanography/fishery monitoring networks, or by national entities responsible for the protection of the environment. Our results in listing all the stakeholders who collect/may collect data of interest were significant but incomplete. More time would be requested to identify and contact stakeholders, and especially generate confidence and sign agreements for sharing information.

Recommendations. A better knowledge on entanglement impacts on individual's health and the type of litter causing entanglement is necessary. Pursuing data collection is thus necessary, e.g., thanks to the INDICIT protocol, which proposes to gather data on entanglement on sea turtles as optional parameters. Therefore, the feasibility report recommended to carry out: i) a survey of available databanks owned by oceanographic institutions/projects; ii) a study aiming to design a protocol proposal for monitoring entanglement/smothering of epibenthic invertebrate using ROV; and iii) to collect data and perform pilot tests.

3.2. Indicator "Micro-plastic ingestion in fish and sea turtle"

This indicator belongs to the criteria D10C3 of MSFD Descriptor 10. The aim is to assess the feasibility of an indicator distinguishing ingestion of plastic litter < 5 mm in size (diameter), considered as primary microplastics, entering the environment as particles already < 5mm, or secondary microplastics, i.e. coming from the fragmentation of larger items. They may have been ingested directly in the environment, or by trophic transfer (first ingested by the preys) or both. This feasibility study focused on sea turtles and fish. Indeed, this fraction of litter size is not differentiated in the monitoring of indicator "Litter ingested by sea turtles". As the network was constituted for sea turtles, it seemed interesting to evaluate the feasibility of this indicator for this taxon. Nevertheless, the occurrence of ingestion of micro-particles of plastics in fish is important, and their use could cover a wider area than indicator "Litter ingested by sea turtles", notably by being used in the HELCOM zone. A

review of the literature on micro-debris ingested by these two taxa has been conducted, in order to assess the prevalence, sampling techniques and laboratory procedures according to species, their functional group and their geographical location.

3.2.1. In fish

The feasibility study consisted in a literature review. The aim was to discuss the choice of a good indicator species, considering its habitat, trophic level, sensitivity to litter, abundance, spatial distribution and commercial importance. The sampling techniques were also evaluated. A wide distribution may enable to include the monitoring of litter impacts in the HELCOM RSCs, which is not possible with the Turtle indicator. Moreover, working on fish would enable targeting items with size below 1 mm.

A total of 159 fish species has been evaluated. The ingestion of micro-plastics appeared very variable with the geographical location, the species' functional groups, size and feeding behaviour, as well as the capture methods.

Differentiation of the micro-plastics from natural items. Micro-plastics were generally differentiated by visual inspection, density separation by buoyancy tests or in hyper-saturated saline solution, or thanks to a previous desiccation in oven. Several authors proceeded to the digestion of the biotic material thanks to different methods, mostly using potassium hydroxide (KOH, 10%), sodium hydroxide (NaOH), oxidizing agents such as hydrogen peroxide (H2O2), at different temperatures depending on the studies. Each digestion method would have its advantages and inconvenient depending on the objective.

Characterization of plastic items. The authors verified rarely the nature of items. Applying a hot metal tip on the particle would enable to differentiate plastics and natural item in case of doubt. Some authors proposed to identify the polymers thanks to micro-spectroscopy, such as the Fourier transform infrared (FT-IR) or the Raman spectroscopy.

Number and size of items. The minimum value of ingested litter found in literature was 0.03 ± 0.18 and the maximum 3.8 items. More than 80 % of the fish with plastic contained only a single particle. However, the number of items found ingested highly depends on the mesh size. The high variability of lower sizes tested by authors prevented comparing the studies.

Types of plastics. Fibres were the prevalent type of plastic found ingested. This can be related to contamination during sampling in the field and analyses in the laboratory, or to waste management strategies on land which vary among countries. The nylon fragments from fishery activities were also highly represented, and might also be due to contamination. "Fragments" was also an important category.

Avoidance of the contamination. Micro-fibres are ubiquitous. Easily transported by the air, they can contaminate the samples during all the stages of the procedure, from field sampling to laboratory manipulations, e.g. originating from the examiner's clothing and/or already present in the laboratory room. The MSFD TG ML with the "Guidance on Monitoring of Marine Litter in European Seas" suggested that the contamination should be less than 10% of the average values of micro-plastic items determined in the samples. To limit such contamination, Petri dishes should be rinsed, dried with compressed air and checked under a microscope for any plastic contaminants. The use of a pre-filtered deionized water and a hypersaline solution for the material should be done, under a clean airflow cabinet for example. The use of blank samples during all the stages of the procedure, from the dissection of the animal until the litter detection under the microscope. Some authors

performed the filtration under a pyramid glove box, with incorporated gloves permitted sample handling with no interference from the external environment. The contamination in the field cannot be controlled, especially when buying fish from markets. The caught should be done using polymer nets and all the equipment should be cleaned before sampling. Trawling net with long hauls should be avoided, since when scraping the bottom, the net creates and re-suspends micro-plastic particles. Lastly, it is recommended to minimize the stress during handling, because fish can regurgitate their stomach content due to stress, stomach compression or stomach inversion.

Selection of relevant indicator species. Several species have been proposed according to habitat and with the selection criteria (habitat, trophic level, sensitivity to litter, abundance, spatial distribution and commercial importance): *Sardina pilchardus* and *Scomber sp*. (pelagic), *Boops boops*, *Mullus sp*. and *Mugil sp*. (demersal), *Scyliorhinus canicula* (elasmobranchs), *Raia clavata*.

3.2.2. In sea turtles

Given their propensity to ingest litter, their wide distribution, the large range of habitats used during their life and the networks trained to collect litter impacts (>1 mm; §2), the loggerhead species may be proposed as a possible indicator.

The literature review showed few papers on the topic, since most studies concerned macro-litter (> 5 mm) or were based on the indicator “Litter ingested by sea turtles” who combined both micro and macro-litter.

Differentiation of plastic items. The differentiation of plastics from natural food items were generally made thanks to visual identification and more exceptionally using chemical or enzymatic digestion of the digestive tract content or of the faeces (KOH or H2O2). The micro-plastics found ingested were generally hard fragments, fragments of sheets and industrial pellets.

To collect the specific fraction of 1 to 5 mm, a filter of 1 mm mesh could be easily affixed on the 5 mm mesh filter, which may allow working from data collected for the indicator “Litter ingested by sea turtles”. The same networks of stakeholders could be involved in this monitoring, considering the additional time this would require.

Avoidance of contamination. The possibility of contamination depends on the manipulations in the laboratory or in the rescue centre, where filters should be affixed in incoming water in the tank. The digestive tract of dead individuals should be washed intensively before opening to extract gut content, as well as all working instruments (scissors, sieves, etc.) before use. Working on an airflow cabinet or laminar flow hood may help reducing the contamination risks when identifying and classifying the micro-debris. For living turtles, intensive lavages of the individual and filtering systems in the water circuit before entering into the tank is recommended. Fish used as food would also be eviscerated before being given to the turtles. Lastly, water samples in empty tanks should be taken in order to evaluate possible air contamination.

4. From INDICIT to INDICIT-II

The INDICIT-II project (2019-2021) is a follow-up of the INDICIT project (2017-19). The project aims to:

1) Capitalize the INDICIT results concerning the networking, the elaboration of standard guidelines, the collection of large standard data set and the evaluation of GES scenario for the indicator "Litter ingested by sea turtles". Specifically, the new project will evaluate the indicator's response in regards to the effect of the Programmes of Measures (PoMs) in pilot areas (e.g. the quantity of USE SHE category ingested by sea turtles where the ban of plastic bags or of single use plastics have be implemented), to fill the gaps of data collection identified in the areas (e.g. Macaronesia and continental Portugal), and promote harmonization with Eastern and Southern countries in the Mediterranean area.

2) Identify key elements for the implementation of two other indicators for the monitoring of litter impacts "Entanglement of sea turtles, birds, cetaceans in floating debris" and "Micro-debris ingestion by fish and sea turtles".

For all these indicators, GES scenarios will be tested and propose according to different spatial and temporal scales. Following the New Decision Commission 2017/848, these indicators will be developed considering the potential effect on health. For this task, analysis in ecotoxicology for evaluating the pollutant exposure will be tested on sea turtle biological samples. A specific attention will be devoted on the use of these 3 indicators in pilot areas identified thanks to a set of criteria (availability of means for sampling, pollutant sources, etc.) and especially the implementation of PoMs.

5. References

Andrady, A.L., 2011a. Microplastics in the marine environment. Marine Pollution Bulletin 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Andrady, A.L., 2011b. Microplastics in the marine environment. Marine Pollution Bulletin 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Araújo, M.C.B., Silva-Cavalcanti, J.S., Costa, M.F., 2018. Anthropogenic Litter on Beaches With Different Levels of Development and Use: A Snapshot of a Coast in Pernambuco (Brazil). Frontiers in Marine Science, Sci. 5:233. doi: 10.3389/fmars.2018.00233.

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Phil. Trans. R. Soc. B 364, 1985–1998. https://doi.org/10.1098/rstb.2008.0205

Barrios-Garrido, H., Espinoza, N., Shimada, T., Wildermann, N., 2015. Body condition index in rescued Green turtles (Chelonia mydas) in the Gulf of Venezuela: a seven years assessment.

Bjorndal, K.A., Bolten, A.B., Chaloupka, M.Y., 2000. Green turtle somatic growth model: evidence for density dependence. Ecological Applications 10, 14.

Borja, Á., Dauer, D.M., Grémare, A., 2012. The importance of setting targets and reference conditions in assessing marine ecosystem quality. Ecological Indicators, Marine Benthic Indicators 12, 1–7. https://doi.org/10.1016/j.ecolind.2011.06.018

Borja, A., Elliott, M., Andersen, J.H., Cardoso, A.C., Carstensen, J., Ferreira, J.G., Heiskanen, A.-S., Marques, J.C., Neto, J.M., Teixeira, H., Uusitalo, L., Uyarra, M.C., Zampoukas, N., 2013. Good Environmental Status of marine ecosystems: What is it and how do we know when we have attained it? Marine Pollution Bulletin 76, 16–27. https://doi.org/10.1016/j.marpolbul.2013.08.042

Botterell, Z.L.R., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R.C., Lindeque, P.K., 2019. Bioavailability and effects of microplastics on marine zooplankton: A review. Environmental Pollution 245, 98–110. https://doi.org/10.1016/j.envpol.2018.10.065

Camedda, A., Marra, S., Matiddi, M., Massaro, G., Coppa, S., Perilli, A., Ruiu, A., Briguglio, P., de Lucia, G.A., 2014. Interaction between loggerhead sea turtles (Caretta caretta) and marine litter in Sardinia (Western Mediterranean Sea). Marine Environmental Research, Large marine vertebrates as sentinels of GES in the European MSFD 100, 25–32. https://doi.org/10.1016/j.marenvres.2013.12.004

Casale, P., Abbate, G., Freggi, D., Conte, N., Oliverio, M., Argano, R., 2008. Foraging ecology of loggerhead sea turtles Caretta caretta in the central Mediterranean Sea: evidence for a relaxed life history model. Mar Ecol Prog Ser 372, 265–276. https://doi.org/10.3354/meps07702

Casale P. 2011. Sea turtle by-catch in the Mediterranean. Fish and Fisheries 12: 299-316.

Champely, S., Ekstrom, C., Dalgaard, P., Gill, J., Weibelzahl, S., Anandkumar, A., Ford, C., Volcic, R., Rosario, H.D., 2018. pwr: Basic Functions for Power Analysis.

Claro, F., Darmon, G., Miaud, C., Galgani, F., 2014. Project of EcoQO/GES for Marine Litter Ingested by Sea Turtles (MSFD D10.2.1.) (Minutes of the european workshop, October 13th, 2014.). Marseille (France).

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: A review. Marine Pollution Bulletin 62, 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025

Commission Decision (EU) 2017/848 of 17 May 2017 laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardised methods for monitoring and assessment, and repealing Decision 2010/477/EU (Text with EEA relevance.), 2017. , OJ L.

Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, Á.T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernández-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. Proc Natl Acad Sci U S A 111, 10239–10244. https://doi.org/10.1073/pnas.1314705111

Darmon, G., Miaud, C., 2016. Darmon, G., Miaud, C., 2016. Elaboration d'un indicateur de dechets ingérés par les tortues marines (D10-2-1) et d'un bon etat écologique (BEE) pour la Directive Cadre Stratégie pour le Milieu Marin (DCSMM), et d'un objectif de qualité écologique (EcoQO) pour la convention internationale pour la protection du milieu marin de l'Atlantique nord-est (OSPAR). (Rapport final de contrat d'étude CNRS-IFREMER). Montpellier (France).

Darmon, G., Miaud, C., Claro, F., Dell'Amico, F., Gambaiani, D., Galgani, F., 2014. Pertinence des tortues caouannes comme indicateur de densité de déchets en Méditerranée dans le cadre de la Directive Cadre Stratégie pour Ie Milieu Marin (indicateur 2.1 du descripteur n°10) (Rapport technique pour l'IFREMER).

Darmon, G., Miaud, C., Claro, F., Doremus, G., Galgani, F., 2017a. Risk assessment reveals high exposure of sea turtles to marine debris in French Mediterranean and metropolitan Atlantic waters. Deep Sea Research Part II:

Topical Studies in Oceanography, Abundance, distribution and habitats of Atlantic and Mediterranean marine megafauna 141, 319–328. https://doi.org/10.1016/j.dsr2.2016.07.005

Darmon, G., Raymond, Q., Miaud, L., 2017b. Examen externe et interne d-une tortue caouanne Caretta caretta.

Derraik, J.G.B., 2002. The pollution of the marine environment by plastic debris: a review. Marine Pollution Bulletin 44, 842–852. https://doi.org/10.1016/S0025-326X(02)00220-5

Desforges, J.-P.W., Sonne, C., Levin, M., Siebert, U., De Guise, S., Dietz, R., 2016. Immunotoxic effects of environmental pollutants in marine mammals. Environment International 86, 126–139. https://doi.org/10.1016/j.envint.2015.10.007

Domènech F, Aznar FJ, Raga JA, **Tomás J**. 2019. Two decades of monitoring in marine debris ingestion in loggerhead sea turtle, *Caretta caretta*, from the western Mediterranean. *Environmental Pollution* 244: 367-378.

Dray, S., Dufour, A.-B., Thioulouse, and J., Jombart, with contributions from T., Pavoine, S., Lobry, J.R., Ollier, S., Borcard, D., Legendre, P., Chessel, S.B. and A.S.B. on earlier work by D., 2018. ade4: Analysis of Ecological Data: Exploratory and Euclidean Methods in Environmental Sciences.

Duncan, E.M., Botterell, Z.L.R., Broderick, A.C., Galloway, T.S., Lindeque, P.K., Nuno, A., Godley, B.J., 2017. A global review of marine turtle entanglement in anthropogenic debris: a baseline for further action. Endangered Species Research 34, 431–448. https://doi.org/10.3354/esr00865

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLoS ONE 9, e111913. https://doi.org/10.1371/journal.pone.0111913

Fossi, M.C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M., 2018. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environmental Pollution 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019

Galgani, F., Hanke, G., Maes, T., 2015. Global Distribution, Composition and Abundance of Marine Litter, in: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 29–56. https://doi.org/10.1007/978-3-319-16510-3_2

Gall, S.C., Thompson, R.C., 2015. The impact of debris on marine life. Marine Pollution Bulletin 92, 170–179. https://doi.org/10.1016/j.marpolbul.2014.12.041

Gall, S.C., Thompson, R.C., n.d. The impact of debris on marine life. Marine Pollution Bulletin. https://doi.org/10.1016/j.marpolbul.2014.12.041

Groffman, P.M., Baron, J.S., Blett, T., Gold, A.J., Goodman, I., Gunderson, L.H., Levinson, B.M., Palmer, M.A., Paerl, H.W., Peterson, G.D., Poff, N.L., Rejeski, D.W., Reynolds, J.F., Turner, M.G., Weathers, K.C., Wiens, J., 2006. Ecological thresholds: The key to successful enviromental management or an important concept with no practical application? Ecosystems 9, 113. https://doi.org/10.1007/s10021-003-0142-z

Hardesty, B.D., Good, T.P., Wilcox, C., 2015. Novel methods, new results and science-based solutions to tackle marine debris impacts on wildlife. Ocean & Coastal Management, Making Marine Science Matter: Issues and

Solutions from the 3rd International Marine Conservation Congress 115, 4–9. https://doi.org/10.1016/j.ocecoaman.2015.04.004

Hill, M.O., Smith, A.J.E., 1976. Principal Component Analysis of Taxonomic Data with Multi-State Discrete Characters. Taxon 25, 249–255. https://doi.org/10.2307/1219449

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771. https://doi.org/10.1126/science.1260352

Laist, D.W., 1997. Impacts of Marine Debris: Entanglement of Marine Life in Marine Debris Including a Comprehensive List of Species with Entanglement and Ingestion Records, in: Coe, J.M., Rogers, D.B. (Eds.), Marine Debris: Sources, Impacts, and Solutions, Springer Series on Environmental Management. Springer New York, New York, NY, pp. 99–139. https://doi.org/10.1007/978-1-4613-8486-1_10

Law, K.L., Thompson, R.C., 2014. Microplastics in the seas. Science 345, 144–145. https://doi.org/10.1126/science.1254065

Matiddi, M., van Franeker, J.A., Sammarini, V., Travaglini, A., Alcaro, L., 2011. Monitoring litter by sea turtles: an experimental protocol in the Mediterranean. Proceedings of the 4th Mediterranean Conference on Sea Turtles.

Matiddi, M., Hochsheid, S., Camedda, A., Baini, M., Cocumelli, C., Serena, F., Tomassetti, P., Travaglini, A., Marra, S., Campani, T., Scholl, F., Mancusi, C., Amato, E., Briguglio, P., Maffucci, F., Fossi, M.C., Bentivegna, F., de Lucia, G.A., 2017. Loggerhead sea turtles (Caretta caretta): A target species for monitoring litter ingested by marine organisms in the Mediterranean Sea. Environmental Pollution 230, 199–209. https://doi.org/10.1016/j.envpol.2017.06.054

Matiddi, M., deLucia, G. A., Silvestri, C., Darmon, G., Tomás, J., Pham, C. K., Camedda, A., Vandeperre, F., Claro, F., Kaska, Y., Kaberi, H., Revuelta, O., Piermarini, R., Daffina, R., Pisapia, M., Genta, D., Sözbilen, D., Bradai, M. N., Rodríguez, Y., Gambaiani, D., Tsangaris, C., Chaieb, O., Moussier, J., Loza, A. L., Miaud, C. Data Collection on Marine Litter Ingestion in Sea Turtles and Thresholds for Good Environmental Status. J. Vis. Exp. (147), e59466, doi:10.3791/59466 (2019).

MSFD Technical Subgroup on Marine Litter. Hanke, G., Galgani, F., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R., Palatinus, A., Van Franeker, J.A., Vlachogianni, T., Scoullos, M., Veiga, J.M., Matiddi, M., Alcaro, L., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J., Liebezeit, G., 2013. Guidance on Monitoring of Marine Litter in European Seas (EUR - Scientific and Technical Research Reports). Publications Office of the European Union. https://doi.org/10.2788/99475 (pdf)

MSFD GES TG ML. Werner, S., Budziak, A., van Franeker, J., Galgani, F., Hanke, G., Maes, T., Matiddi, M., Nilsson, P., Oosterbaan, L., Priestland, E., Thompson, R., Veiga, J., Vlachogianni, T., 2017. Harm Caused by Marine Litter. MSFD GES TG Marine Litter - Thematic Report. http://dx.doi.org/10.2788/690366. JRC Technical report; EUR 28317 EN.

Needleman, H., 2004. Lead Poisoning | Annual Review of Medicine. Annual Review of Medicine 55, 209–222.

Nelms, S.E., Duncan, E.M., Broderick, A.C., Galloway, T.S., Godfrey, M.H., Hamann, M., Lindeque, P.K., Godley, B.J., 2015. Plastic and marine turtles: a review and call for research. ICES Journal of Marine Science: Journal du Conseil fsv165. https://doi.org/10.1093/icesjms/fsv165

OSPAR, 2009. EcoQO Handbook - Handbook for the application of Ecological Quality Objectives in the North Sea, OSPAR Commission. ed, OSPAR Biodiversity Series Publication 307/2009. London.

OSPAR- EIHA 16/5/13, 2016. Marine Litter in sea turtles: A risk assessment as a scientific background for including ingestion of debris by sea turtles as a candidate indicator for impact of marine litter on biota in southern OSPAR area (region IV)., in: Agenda Item 5 Document EIHA 16/5/13 Presented by France. OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic. Presented at the Meeting of the Environmental Impact of Human Activities Committee (EIHA), Berlin (Germany).

Pham, C.K., Rodríguez, Y., Dauphin, A., Carriço, R., Frias, J.P.G.L., Vandeperre, F., Otero, V., Santos, M.R., Martins, H.R., Bolten, A.B., Bjorndal, K.A., 2017. Plastic ingestion in oceanic-stage loggerhead sea turtles (Caretta caretta) off the North Atlantic subtropical gyre. Marine Pollution Bulletin 121, 222–229. https://doi.org/10.1016/j.marpolbul.2017.06.008

R Core Team, 2017. R: A Language and Environment for Statistical Computing.

Rochman, C.M., 2015. The Complex Mixture, Fate and Toxicity of Chemicals Associated with Plastic Debris in the Marine Environment, in: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, pp. 117–140. https://doi.org/10.1007/978-3-319-16510-3_5

Rochman, C.M., Hoh, E., Hentschel, B.T., Kaye, S., 2013a. Long-Term Field Measurement of Sorption of Organic Contaminants to Five Types of Plastic Pellets: Implications for Plastic Marine Debris. Environ. Sci. Technol. 47, 1646–1654. https://doi.org/10.1021/es303700s

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013b. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific Reports 3, 3263. https://doi.org/10.1038/srep03263

Rosenthal, A., et al., 1997. Legislating Acceptable Cancer Risk from Exposure to Toxic Chemicals, in: Oundations of Environmental Law and Policy. Revesz, Richard L., New York, N.Y., pp. 52–58.

Schuyler, Q., Hardesty, B.D., Wilcox, C., Townsend, K., 2014. Global Analysis of Anthropogenic Debris Ingestion by Sea Turtles. Conservation Biology 28, 129–139. https://doi.org/10.1111/cobi.12126

Schuyler, Q.A., Wilcox, C., Townsend, K., Hardesty, B.D., Marshall, N.J., 2014. Mistaken identity? Visual similarities of marine debris to natural prey items of sea turtles. BMC Ecology 14, 14. https://doi.org/10.1186/1472-6785-14-14

Suaria, G., Aliani, S., 2014. Floating debris in the Mediterranean Sea. Mar. Pollut. Bull. 86, 494–504. https://doi.org/10.1016/j.marpolbul.2014.06.025

Tanaka, K., Takada, H., Yamashita, R., Mizukawa, K., Fukuwaka, M., Watanuki, Y., 2013. Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. Marine Pollution Bulletin 69, 219–222. https://doi.org/10.1016/j.marpolbul.2012.12.010

Thompson, R.C., Gall, S.C., Secretariat of the Convention on Biological Diversity, United Nations Environment Programme, Global Environment Facility, Scientific and Technical Advisory Panel, 2014. Impacts of marine debris on biodiversity: current status and potential solutions.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at Sea: Where Is All the Plastic? Science 304, 838–838. https://doi.org/10.1126/science.1094559

Valente, A.L., Marco, I., Parga, M.L., Lavin, S., Alegre, F., Cuenca, R., 2008. Ingesta passage and gastric emptying times in loggerhead sea turtles (Caretta caretta). Research in Veterinary Science 84, 132–139. https://doi.org/10.1016/j.rvsc.2007.03.013

van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P.-L., Heubeck, M., Jensen, J.-K., Le Guillou, G., Olsen, B., Olsen, K.-O., Pedersen, J., Stienen, E.W.M., Turner, D.M., 2011. Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea. Environmental Pollution, Nitrogen Deposition, Critical Loads and Biodiversity 159, 2609–2615. https://doi.org/10.1016/j.envpol.2011.06.008

Vegter, A.C., Barletta, M., Beck, C., Borrero, J., Burton, H., Campbell, M.L., Costa, M.F., Eriksen, M., Eriksson, C., Estrades, A., Gilardi, K.V.K., Hardesty, B.D., Sul, J.A.I. do, Lavers, J.L., Lazar, B., Lebreton, L., Nichols, W.J., Ribic, C.A., Ryan, P.G., Schuyler, Q.A., Smith, S.D.A., Takada, H., Townsend, K.A., Wabnitz, C.C.C., Wilcox, C., Young, L.C., Hamann, M., 2014. Global research priorities to mitigate plastic pollution impacts on marine wildlife. Endang Species Res 25, 225–247. https://doi.org/10.3354/esr00623

Vroom, R.J.E., Koelmans, A.A., Besseling, E., Halsband, C., 2017. Aging of microplastics promotes their ingestion by marine zooplankton. Environmental Pollution 231, 987–996. https://doi.org/10.1016/j.envpol.2017.08.088

Wilcox, C., Puckridge, M., Schuyler, Q.A., Townsend, K.A., Hardesty, B.D., 2018. A quantitative analysis linking sea turtle mortality and plastic debris ingestion | Scientific Reports. Scientific Reports 8.

Xanthos, D., Walker, T.R., 2017. International policies to reduce plastic marine pollution from single-use plastics (plastic bags and microbeads): A review. Marine Pollution Bulletin 118, 17–26. https://doi.org/10.1016/j.marpolbul.2017.02.048

Appendix 1 – INDICIT deliverables time table

This table presents the percentage of execution for each expected deliverable. Colours represent the degree of execution, with red, orange or green from bad to full satisfaction. All tasks have been completed. Nevertheless, the standard "basic" data (see §2.2. and §2.3.2) collected throughout the INDICIT project in collaboration with stakeholders (see §2.1.3), did not enable to fully assess the impact of litter ingestion on health, as defined by the New Commission Decision 2017/848/EC (see Appendix 2). While GES scenarios are proposed regarding the results obtained from analyses of available data (see §2.4.4), further studies are needed to better assess individual health and the relationship with ingested litter, a perspective of INDICIT-II project.

Number	Title	Leader	Date	% of execution
D1.1	Consortium Agreement	CNRS	Month 3	100%
D1.2	Minutes of project meeting 1	CNRS	Month 1	100%
D1.3	Minutes of project meeting 2	CNRS	Month 6	100%
D1.4	Minutes of project meeting 3	CNRS	Month 13	100%
D1.5	Minutes of project meeting 4	CNRS	Month 20	100%
D1.6	Minutes of final meeting	CNRS	Month 24	100%
D1.7	Inception Report	CNRS	Month 1	100%
D1.8	Progress Report 1	CNRS	15 days after M6	100%
D1.9	Progress Report 2	CNRS	15 days after M13	100%
D1.10	Progress Report 3	CNRS	15 days after M20	100%
D1.11	Final Report	CNRS	60 days after M24	100%
D2.1	Inception Report on Activity 2	CNRS	Month 1	100%
D2.1	Short progress Report 1 on Activity 2	CNRS	Month 6	100%
D2.2	Short progress Report 2 on Activity 2	CNRS	Month 13	100%
D2.3	Short progress Report 3 on Activity 2	CNRS	Month 20	100%
D2.4	Short progress Report 4 on Activity 2	CNRS	Month 24	100%
D2.5	Reports on pilot studies	CNRS	Month 8	100%
D2.6	Report on set of procedures for a standard monitoring using the Indicator "Debris ingestion by sea turtles"	CNRS	Month 15	100%
D2.7	Final Report on set of procedures for a standard monitoring using the Indicator "Debris ingestion by sea turtles"	CNRS	Month 24	90%
D2.8	Establishment of common databases	CNRS	Month 13	100%
D2.9	Final common databases	CNRS	Month 24	100%
D3.1	Inception Report on Activity 3	ISPRA	Month 1	100%
D3.2	Short progress Report 1 on Activity 3	ISPRA	Month 6	100%
D3.3	Short progress Report 2 on Activity 3	ISPRA	Month 13	100%
D3.4	Short progress Report 3 on Activity 3	ISPRA	Month 20	100%
D3.5	Short progress Report 4 on Activity 3	ISPRA	Month 24	100%
D3.6	Report on the validated GES and clarified indicator criteria for the standard monitoring at the RSC scale	ISPRA	Month 15	100%
D3.7	Final report on the validated GES and clarified indicator criteria for the standard monitoring at the RSC scale	ISPRA	Month 24	90%
D3.8	List of identified stakeholders	ISPRA	Month 15	100%
D3.9	Final list of identified stakeholders	ISPRA	Month 24	100%

Number	Title	Leader	Date	% of execution
D3.10	List of evaluation indicators of the implementation of the monitoring program	ISPRA	Month 24	100%
D4.1	Inception Report on Activity 4	ULPGC	Month 1	100%
D4.2	Short progress Report 1 on Activity 4	ULPGC	Month 6	100%
D4.3	Short progress Report 2 on Activity 4	ULPGC	Month 13	100%
D4.4	Short progress Report 3 on Activity 4	ULPGC	Month 20	100%
D4.5	Short progress Report 4 on Activity 4	ULPGC	Month 24	100%
D4.6	Report on the validated GES and clarified indicator criteria for the standard monitoring at the RSC scale	ULPGC	Month 15	100%
D4.7	Final report on the validated GES and clarified indicator criteria for the standard monitoring at the RSC scale	ULPGC	Month 24	90%
D4.8	List of identified stakeholders	ULPGC	Month 15	100%
D4.9	Final list of identified stakeholders	ULPGC	Month 24	100%
D4.10	List of evaluation indicators of the implementation of the monitoring program	ULPGC	Month 24	100%
D5.1	Inception Report on Activity 5	MNHN	Month 1	100%
D5.2	Short progress Report 1 on Activity 5	MNHN	Month 6	100%
D5.3	Short progress Report 2 on Activity 5	MNHN	Month 13	100%
D5.4	Short progress Report 3 on Activity 5	MNHN	Month 20	100%
D5.5	Short progress Report 4 on Activity 5	MNHN	Month 24	100%
D5.6	Plan for dissemination	MNHN	Month 1	100%
D5.7	Final report on dissemination	MNHN	Month 24	100%
D5.8	Plan for communication	MNHN	Month 1	100%
D5.9	Final report on communication	MNHN	Month 24	100%
D5.10	Website + intranet	MNHN	Month 3	100%
D5.11	Tool kit for standardized monitoring of debris impacts on marine fauna	MNHN	Month 22	100%
D5.12	Dissemination meeting in Greece	MNHN	Month 22	100%

Appendix 2 – General definitions

The Marine Strategy Framework Directive

Urgent efforts for reducing litter in the marine environment and stopping the trends in environmental impacts are needed. The European Commission makes the struggle against marine litter one of its priorities to recover the Good Environmental Status (GES) of marine waters, by restoring clean, healthy and productive marine ecosystems and protecting marine biodiversity and resources. Indeed "Marine Litter" constitutes the 10th of the 11 Descriptors of state and pressure for the European Marine Strategy Framework Directive (MSFD, 2008/56/EC). MSFD consists in an adaptive management approach based on the assessment of anthropogenic pressures and impacts on the marine environment, kept up-to-date and reviewed every 6 years.

The European Commission considers a set of detailed criteria and methodological standards to help Member States (MS) to implement the MSFD thanks to Programs of Measures (POMs). For this and for each of the 11 Descriptors, MSFD should be based on:

- an initial assessment of the current environmental status of national marine waters,

- the determination of GES signification,

- the establishment of environmental targets to achieve GES by 2020, associated to indicators,

- the establishment of a monitoring programme,

- the development of a programme of measures aiming to achieve or maintain GES by 2020.

In the 2014 report providing the first stage of MS reporting, the EC revealed a number of weaknesses and requirements in particular for the updates of the determination of GES and environmental targets. For greater benefits to the next cycle of implementation of MSFD, the report concluded that the 2010 "GES Decision" needed to be revised, strengthened and improved. Repealing Decision 2010/477/EU, the New Commission Decision 2017/848/EC (*Commission Decision (EU) 2017/848 of 17 May 2017*) lays down criteria and methodological standards on GES, and gives specifications and standardised methods for monitoring and assessment.

GES implies a condition which has been or can be compared against an anthropogenically altered state (Borja et al., 2012). Therefore, assessing the current environmental status requires the comparison with a reference condition. GES is expected to determine threshold values (TVs) and the extent to which TV have to be achieved (Art. 9(1) of MSFD 2008/56/EC). According to MSFD Commission Decision 2017/848/EU, TV should underline, where appropriate, the quality level that reflects the significance of an adverse effect for a criterion according to the reference condition. TV should correspond to levels of pollutions above which GES is approved to have been achieved. They must be set through union, regional or sub-regional cooperation ideally at EU level, in order to achieve equal protections. Commission Decision 2017/848/EC Art. 2 defines TV as a value or a range of values that allows an assessment of the quality level achieved for a particular criterion, thereby contributing to the assessment of the extent to which the GES has been achieved. Trend reduction can be used as a basis to set a TV, in order to achieve a given operational threshold. Commission Decision 2017/848/EC specifies that TV should be set on the basis of the precautionary principle, reflecting the potential risks to the marine environment.

Moreover, they should accommodate the dynamic nature of marine ecosystems and their elements. TV and baseline have to be proposed/updated for each Criteria of Descriptor.

Borja et al. (2013) proposed four ways to determine the Reference condition by:

(i) finding a control area, similar to the one under study but without the pressures;
(ii) defining it a posteriori considering the time before the pressures had exerted an influence;
(iii) modelling a non-impacted condition numerically;
(iv) by using expert judgement, if none of these are possible.

The notion of Threshold Value is more debated. WG GES reminds the definitions from Oxford dictionary as "the magnitude or intensity that must be exceeded for a certain reaction, phenomenon, result, or condition to occur or be manifested". The one proposed by Groffman et al. (2006) is "the point at which there is an abrupt change in an ecosystem quality, property or phenomenon, or where small changes in an environmental driver produce large responses in the ecosystem". Lastly, there is the definition proposed by Rosenthal et al. (1997) as "the dose of the toxicant below which no adverse effects will occur" (Minutes of the 20th WG GES). TV should be based on baselines where possible.

The New Commission Decision 2017/848/EC underlines that TVs need to be set at appropriate geographical scales, taking into account the biotic and abiotic characteristics of regions, sub-regions and subdivisions. For this, MSFD attempts to work in close partnership with the Regional Sea Conventions (RSC), which also are committed to develop strategies in full synergy with MSFD, including the implementation of an integrated monitoring program based on indicators and reduction measures. In this way, RSCs and MSFD harmonise their approaches, favouring MS to coordinate their actions in order to ensure coherence of approaches and possibility to better analyse the efficiency of restoration measures, thanks to spatial analyses and adaptive management. RSCs deploy a set of criteria and indicators, with similar objectives to those of the MSFD, thus allowing spatial and legal articulations between regional conventions and the European directive. In addition, MS have to determine the mechanisms to evaluate the effectiveness of their PoMs to achieve TV and assess their distance to GES, thanks to the indicators applicable at (sub)regional levels.

Definitions for MSFD Descriptor 10 "Marine Litter".

Under MSFD Descriptor 10 "Marine Litter" (D10), GES is supposed to be achieved when "Properties and quantities of marine litter do not cause harm to the coastal and marine environment". A set of primary and secondary criteria are given:

- Primary Criteria D10C1 and D10C2 (New Commission Decision 2017/848/EC):
  GES for D10C1 represents "the composition, amount and spatial distribution of litter on the coastline, in the surface layer of the water column, and on the seabed, are at levels that do not cause harm to the coastal and marine environment". The element concerned is "Litter", excluding micro-litter, classified as the categories artificial polymer materials, rubber, cloth/textile, paper/cardboard, processed/worked wood, metal, glass/ceramics, chemicals, undefined, and food waste. D10C2 is specific for micro-litter, defined as particles < 5mm, classified in the categories 'artificial polymer materials' and 'other'.
- Secondary Criteria D10C3 and D10C4:

GES regarding D10C3 corresponds to "the amount of litter and micro-litter ingested by marine animals that is at a level that does not adversely affect the health of the species concerned". For D10C4, it is "the number of individuals of each species which are adversely affected due to litter, such as by entanglement, other types of injury or mortality, or health effects". Where appropriate, the outcomes of criterion D10C3 and D10C4 shall also contribute to assessments under Descriptor 1 (Biodiversity).

The New Commission Decision 2017/848/EC specifies that the use of criteria shall be agreed at Union level. The methodological standards are also defined, such as the scales of GES assessment. For D10C1, D10C2 and D10C3, they shall correspond to the subdivisions of the region or sub-region, divided where needed, by national boundaries. For D10C4, they should correspond to those used for assessment of the species group under Descriptor 1 ("Biodiversity").

The units of measurement are:

i) for criteria D10C1 and D10C2: "the amount of litter or micro-litter per category";
ii) for D10C3: "the amount of litter/micro-litter in grams (g) and number of items per individual for each species in relation to size (weight or length, as appropriate) of the individual sampled";
iii) for D10C4: "the number of individuals affected (lethal; sub-lethal) per species".

The standardised methods for monitoring and assessment regarding the secondary criteria should be based on incidental occurrences (e.g. strandings of dead animals, entangled animals in breeding colonies, affected individuals per survey).

In 2014, European Commission reported that the implementation of indicators, associated to Marine Litter in particular, needs to be further advanced, thanks to the improvement of the knowledge for defining the GES baselines and indicators' characteristics, and of the network capacity for a standard monitoring. The Task Group Marine Litter (TG ML), which, co-chaired with the Joint Research Centre (JRC), supports the implementation of MSFD for D10, has developed a Guidance for monitoring marine litter in the European Seas (MSFD Technical Subgroup on Marine Litter, 2013). WG GES discussed the way to propose GES scenarios for D10 Criteria (Minutes of 20th WG GES). The reference situation, according to WG GES, can be based on an expected, a usual, a normal or a pristine area. Set on the basis of precautionary principle, cut-off values could be defined as proportion or percentile in relation to litter baselines, averages or maximum concentrations. The lower concentrations, which already exist in certain areas, could be a target for other areas. This could be supported by the requirement of an equal level of protection in different areas and seas.

Marine taxa used as bio-indicators of marine litter impacts in MSFD and RSCs

Plastic ingested by the fulmar *Fulmarus glacialis* is a bio-indicator for trends in marine litter for OSPAR (EcoQO 3.3; OSPAR, 2009) and an "impact on biota" indicator for MSFD (indicator 10.2.1, EC 2010). Individuals found dead on beaches, especially after storms, are necropsied in order to determine the type and quantity of litter in their stomach (MSFD Technical Subgroup on Marine Litter, 2013). The GES, represented for OSPAR by EcoQO ("Ecological Objective"), relates to almost pristine conditions. Therefore, the target level for EcoQO 3.3 is a baseline level, which currently does not exist in relatively clean artic environments: "*There should be less than 10% of northern fulmars (Fulmarus glacialis) having more than 0.1 g plastic particles in the stomach in samples*

of 50 to 100 beach-washed fulmars from each of 4 to 5 areas of the North Sea over a period of at least five years" (OSPAR, 2009; van Franeker et al., 2011).

Sea turtles are relevant indicator for monitoring litter impact through ingestion in MSFD (Criteria D10C3) and RSCs Barcelona and OSPAR-Macaronesia areas (Matiddi et al., 2011; MSFD Technical Subgroup on Marine Litter, 2013), and possibly for impacts related to entanglement (Criteria D10C4) (Duncan et al., 2017; Nelms et al., 2015). "Litter ingested by sea turtles" was proposed by France in 2015 and 2016 at EIHA meetings, then retained as a candidate indicator for measuring impact on biota for OSPAR in 2016 (OSPAR- EIHA 16/5/13, 2016). INDICIT results reported in CEMP document (2019) participated in changing the status of the indicator from candidate to common for the OSPAR southern zones.

The trends in the amount of litter ingested by or entangling sea turtles is also a candidate indicator for the Barcelona Convention, as part of the 10th Ecological Objectives (EO10) of the Integrated Monitoring Assessment Programme (IMAP, Candidate indicator CI24) belonging to the Regional Plan on Marine Litter Management in the Mediterranean (RPML), adopted in 2013 during the COP18 and came into force in 2014. The sea turtle indicator EI 18 was also proposed within MedPol Marine Litter Action Plan to monitor litter impact at the Mediterranean scale. The loggerhead turtle *Caretta caretta* being the most abundant species of sea turtle in the Mediterranean and commonly seen in the surrounding Atlantic waters, is the target species. Specimens are regularly collected by existing rescue centres and stranding networks, which enables the collection of data useful for acquiring knowledge on the species' biology and on the factors leading to litter ingestion or entanglement. The leatherback species *Dermochelys coriacea* was also recommended especially for the OSPAR zones III, IV and V (Claro et al., 2014; Darmon and Miaud, 2016).

Status of the indicator "Litter ingested by sea turtles" in the Regional Sea Conventions concerned by INDICIT project

OSPAR

The OSPAR Convention for the Protection of the Marine Environment in the North-East Atlantic. Litter ingested by sea turtle is a candidate indicator for OSPAR (10.2.1 of the 10th Ecological Quality Objectives (EcoQO)), which, as other relevant impact indicator (e.g. entanglement of biota in marine litter), should be discussed and validated by the Environmental Impact of Human Activities Committee (EIHA), within the Regional Action Plan for Marine Litter executed over the period 2014-2021 and supervised by the marine litter group (ICG-ML).

Barcelona convention

The Barcelona Convention (UNEP-MAP) for the Protection of Marine Environment and the Coastal Region of the Mediterranean, which Med Action Plan (MAP) is an ecosystem-based approach for the management of human activities (EcAp), established on 11 Mediterranean Ecological Objectives, the 10th concerning the marine litter. Turtles are considered as an indicator for ingestion and entanglement. The Specially Protected Areas Regional Activity Centre (SPA/RAC) assists to the Contracting Parties for their implementation. The loggerhead species is one of the target species for the Integrated Program for Monitoring and Assessment of the Mediterranean Sea and Coasts (IMAP) signed by Contracting Parties under the Regional Plan on Marine Litter Management (RPML) (Candidate Indicator 24 (CI24) of the 10th Ecological Objectives (EO10)).

HELCOM

The Helsinki Convention (HELCOM) for the Protection of the Marine Environment in the Baltic Sea Area, which regional action plan on marine litter plans to establish indicator of litter impacts.

Summary of the outcomes of the European project INDICIT

Implementation of the "Impacts of marine litter on sea turtles and biota" indicator in RSC and MSFD areas (1 February 2017 – 31 January 2019)

Marine litter, a major concern

Anthropogenic marine litter is having an alarming impact on marine fauna: more than 700 species have been shown to be affected by litter, primarily by ingestion and entanglement: among these a number are classified as vulnerable or endangered on the IUCN Red List. Urgent efforts to reduce litter in the marine environment and stop these harmful environmental trends are required. The European Commission's **Marine Strategy Framework Directive** (MSFD, 2008/56/EC) makes the combat against marine litter a priority in recovering the **Good Environmental Status** (GES) of marine waters. The MSFD sets out a list of 11 descriptors of environmental status, of which 'Marine litter' is number 10.

The quantity and characteristics of litter ingested by sentinel species can reflect the spatial and temporal trends of litter in the environment that organisms are exposed to. Sea turtles have been proposed as an indicator of the impact of litter by ingestion in the framework of the MSFD (for Criteria D10C3) and the OSPAR (indicator 10.2.1) and Barcelona (CI 24 IMAP) Regional Sea Conventions (RSCs). Sea turtles may also be relevant for monitoring impacts related to entanglement. The loggerhead turtle *Caretta caretta* is considered a target species due to its wide distribution, its use of various marine habitats and its propensity to ingest litter. The leatherback turtle *Dermochelys coriacea* has also been recommended as an indicator species, especially for OSPAR (Atlantic) zones III, IV and V, where it is regularly observed. For litter impacts related to entanglement or ingestion of micro-particles inferior to 5 mm, target taxa/species remain to be defined.

The INDICIT project

The INDICIT project started on 1 February 2017 and ended on 31 January 2019. The total budget was 1,328,119 euros, of which 999,955 euros was allocated by the EC's Directorate-General for the Environment, and the remaining 20% was co-funded by the other partners.

The INDICIT consortium was composed of 10 institutional public-sector partners from five European countries (France, Greece, Italy, Portugal and Spain) and two non-European countries (Tunisia and Turkey). The consortium was advised by a Policy Officer and an External Advisory Board (EAB) composed of experts and representatives from the MSFD, RSCs and the seven member states.

The INDICIT project focused on three litter impact indicators: '**Litter ingested by sea turtles**', '**Entanglement with debris by marine biota**' and '**Micro-plastic ingestion in fish and sea turtles**'. The main goal was to allow litter impact indicators to be implemented in a standardized way in the framework of the MSFD and the OSPAR (Atlantic), Barcelona (Mediterranean) and HELCOM (Baltic) RSCs, using sea turtles as a bioindicator. The specific

objectives were to develop a common approach for monitoring litter ingested by sea turtles by 1) developing a set of standardized tools, 2) creating a network and providing training sessions to stakeholders, 3) collecting and analysing data on living and dead turtles, 3) analysing the indicator's spatial and temporal limits, biological constraints and GES criteria. Contrary to the GES Decision which establishes the 5 mm threshold for distinguishing micro- from macroplastics, litter was defined as items >1mm, including both. This is a provisional solution, taking into account available historic data and the time and resources' limitations of the project. A further objective was to assess the relevance and feasibility of two other litter impact indicators: one related to entanglement (MSFD Criteria D10C4), for which all taxa were evaluated, and one related to the ingestion of micro-plastics (litter items <1mm) (MSFD Criteria D10C3) by fish and sea turtles.

The project consisted of five interrelated activities:

- **Activity 1: Management and coordination** led by the French National Centre for Scientific Research and the École Pratique des Hautes Études (CNRS/EPHE) and involving all the members of the INDICIT consortium, to ensure the proper implementation and management of the project.
- **Activity 2: Acquisition and use of scientific knowledge to develop the litter impact indicators at a sub-regional and global MSFD spatial scale**, led by the CNRS/EPHE and involving all the members of the INDICIT consortium, with the aim of 1) addressing knowledge gaps by collecting and analysing standardized data for defining GES, constraints and units for the indicator 'Litter ingested by sea turtles', and 2) evaluating the relevance of two new impact indicators 'Entanglement with debris by marine biota' and 'Micro-plastic ingestion by marine biota' through feasibility studies based on a literature review and a questionnaire.
- **Activity 3: Implementation of the indicator 'Impacts of marine litter on sea turtle and biota' in RSC OSPAR/Macaronesia regions**, led by the University of Las Palmas Gran Canaria (ULPGC), involving CNRS/EPHE, the French National Museum of Natural History (MNHN) and the Regional Fund for Science and Technology of the Azores (FRCT), with the aim of implementing a perennial and sustainable monitoring programme for Indicator 1 'Litter ingested by sea turtles' in OSPAR-Macaronesia through creating a network, training engaged stakeholders and improving the tools by considering feasibility and relevance in the field.
- **Activity 4: Implementation of the indicator 'Impacts of marine litter on sea turtle and biota' in the Ecosystem Approach (EcAp) of the UNEP/MAP Barcelona convention**, led by the Italian National Institute for Environmental Protection and Research (ISPRA), involving the CNRS/EPHE, MNHN, the Italian Institute for Coastal Marine Environment (CNR-IAMC), Spain's University of Valencia (UVEG), the Hellenic Centre for Marine Research (HCMR), Tunisia's National Institute of Marine Science and Technology (INSTM) and Turkey's Sea Turtle Research and Rehabilitation Centre (PAU-DEKAMER), with the same objectives as Activity 3 but targeting the Barcelona RSC.
- **Activity 5: Communication and knowledge sharing**, led by the MNHN, involving all the members of the INDICIT consortium, with the aim of providing information about the project and its outcomes and disseminating the technical tools.

INDICIT main results

1. 'Litter ingested by sea turtles' indicator

Pilot study A **pilot study** was performed during the first months of the project in order to evaluate the state of knowledge about potential constraints to consider when using the indicator, and to define the means for monitoring in existing networks (Darmon and INDICIT consortium in INDICIT consortium, 2018).

Networking The INDICIT consortium gathered together **106 stakeholders**, principally from rescue centres, stranding networks and veterinarian and research laboratories: 68 work in the Mediterranean, 43 in the Atlantic and 6 in both areas. Fourteen training sessions, including two international sessions, were held to train these stakeholders to collect in a standardized way the litter found in the digestive tracts of necropsied dead individuals and in the faeces excreted by living turtles. The stakeholders' contact information and locations were recorded in a **Google map and an Excel table**, which was kept private to protect potentially sensitive information. The table contains both stakeholders currently involved in monitoring and the **preconditions for involvement** for other stakeholders contacted (e.g. need for material or human resources).

Technical tools Several **tools** were developed in order to assist the collection of **standard data** on litter ingested by sea turtles. A **protocol** with an observation sheet, now available in five languages (English, Arabic, French, Greek and Spanish), and a video-tutorial have been created. The protocol improves on the MSFD guidelines published by the MSFD Marine Litter Task Group in 2013, integrating stakeholder feedback and details on all stages of handling, from the discovery of a specimen to the extraction and analysis of the litter ingested by a living or dead sea turtle. It recommends **'basic' parameters**, i.e. information essential to determining GES, and **'optional' parameters**, which are more time-consuming to collect, but provide valuable information for better understanding the factors that may influence litter ingestion by sea turtles and impact individual health.

Data bank A **standard database** was built to share data, hosted in a private area accessible only to partners who signed the Consortium Agreement specifying the sharing guidelines (some stakeholders asked that their raw data be used only in the framework of the INDICIT project). The information was collected in an Excel file, with data corresponding to each individual turtle recorded on its own line, under either a tab for necropsies or a tab for faeces. INDICIT supports the development of a dedicated **platform** in synergy with the **CleanAtlantic** project.

Data gathered A total of **1406 individuals** were recorded in the INDICIT database, 948 necropsies (841 loggerheads and 107 leatherbacks) and 458 observations of faeces. Past data (from 1988), collected before the INDICIT project and most of them, before the MSFD TG ML guideline of 2013, was insufficient to accurately assess the temporal trend in litter ingestion in sea turtles. In data obtained from the **last 6 years** (from 2013 to December 2018), findings showed that **53.84% of leatherbacks** had ingested litter, with an average of **1.7 ±0.73 g** per individual at the population level (N = 13 individuals, all from France's Atlantic waters); **57.94% of living loggerheads** excreted litter (N=254; **1.06 ± 0.44 g** with N = 185); and **63.03% of dead loggerheads** (N = 522) were found with litter, with a mean of **0.78 ±0.11 g** (N = 480) at the population level.

Main findings

Area	Country	Number of necropsies	Litter prevalence (%)	Dry mass (g)	% turtles litter > food
Atlantic	France	21	45	0.28 ± 0.16	11.11
	Portugal	33	81.82	1.13 ± 0.35	-
	Spain	9	88.89	0.16 ± 0.07	0
	Global	**45**	**70.97**	**0.74 ± 0.21**	**9.09**
	France	77	82.43	1.23 ± 0.27	12.12

Mediterr-anean	Greece	28	64.28	0.13 ± 0.06	3.7
	Italy	129	62.01	0.92 ± 0.19	30.23
	Spain	85	80.49	0.89 ± 0.27	16
	Tunisia	46	52.17	0.84 ± 0.71	9.09
	Turkey	94	33.33	0.37 ± 0.29	0
	Global	**457**	**61.95**	**0.78 ± 0.12**	**17.01**

Main results obtained from necropsies of dead loggerhead turtles collected during a 6-year period (2013 to 2018) per area (Atlantic/Mediterranean) and per country. Litter prevalence: percentage of turtles having ingested litter; Dry mass: mean dry mass of ingested litter; % turtles litter > food: percentage of turtles having ingested more litter than food (measured in terms of food remains).

Indicator assessment criteria and constraints Due to the small amount of data on the leatherback and the possible discrepancies in methodologies used by different stakeholders to collect litter from living turtle faeces, the indicator criteria were tested only on the data from necropsied loggerheads. The GES was evaluated from individuals with conservation status 2 (fresh death) and 3 (relatively fresh, early stage of decomposition). Various measures were considered to assess the quantity of ingested litter, such as the **volume** or the **number** of items or fragments. It was decided to adopt **dry mass** (to 2 decimals) as this appeared to be the most trustworthy parameter in terms of the use of a common methodology between stakeholders. Then all biological factors (collected as 'basic' or 'optional' parameters) were tested individually or in combination in order to assess the possible constraints. We found contrasting results when evaluating the factors influencing litter ingestion according to the parameter chosen, but no significant or major difference in:

i) the circumstances of discovery, especially between the most common way to collect specimens, from **stranding and bycatch**, which may be due to the low amount of data or by confounding factors related to typology or type of gear causing bycatch (e.g. depth, distance to the coast and turtle life stage)
ii) the **individual's size**, evaluated with 12 measurements of carapace length and classified in 4 categories (≤20 cm; 20–40 cm; 40–60 cm; ≥60 cm), and the individual's life stage classified in juvenile or adult.
iii) the **body condition**, which indicated health status, evaluated by body weight, fat reserves, injuries and the possible cause of death.

We also evaluated whether a 'Body condition' index could be used, based on indices used for the green turtle *Chelonia mydas*, but further research is needed to adapt an index for the loggerhead species. Lastly, we considered the dry mass of food remains compared to the total dry mass of litter as a proxy of individual health, with the assumption that individuals that did not consume natural items did not ingest litter and that the digestibility of food and litter items is comparable. From 2013, over the entire area (Atlantic and Mediterranean Sea), an average of **16.76%** of the necropsied loggerheads were found **with more litter than food remains** (9% in the Atlantic and 17% in the Mediterranean). These results (obtained from all the data collected during the INDICIT project) indicate that **more standard data** would be necessary to perform more powerful tests. However, the INDICIT consortium recommended not to stratify the dataset for now and to **consider all data without constraint**.

Indicator units The project also evaluated temporal and spatial units for the indicator. Highly variable between countries, no significant temporal trend was found, but this should be further evaluated with a higher sample size. The INDICIT consortium recommended evaluating GES over a **6-year period** in order to better ensure a standardized methodology for collecting data and to correspond to the MSFD cycle. The data obtained between 2013 and January 2018 showed no significant differences in litter prevalence between the **Atlantic** (**70.91%**) and the **Mediterranean** (**61.95%**) areas of the project, although differences between countries in each of these areas appeared. The INDICIT consortium voted to define **two distinct GES for the Atlantic and the Mediterranean**,

because of the possible differences in ecological processes between the two areas. It also highlighted the need for further studies in the Atlantic area to collect more data. Moreover, simulations of the monthly distribution of floating litter made in partnership with the **MEDSEALITTER** project revealed at least two distinct processes between the Eastern and the Western Mediterranean basins, suggesting that two distinct GES could also be proposed for the two basins.

Sample size An initial evaluation indicated that data from almost 200 individuals would be needed during a 6-year period to detect change in the mean dry mass of litter evaluated at the population level. Based on the available samples, the INDICIT consortium suggested a minimum of 50 individuals per country and per year for evaluating the GES.

Categories of litter Further evaluations could be made considering litter categories based on the MSFD's 'Programmes of Measures', especially for evaluating indicator responses to **USE SHE** (plastic sheets), **USE FRA** (hard plastic fragments), **USE THR** (threadlike plastics) and **USE FOA** (foam), the categories most often found ingested by the loggerheads.

GES scenarios Two GES proposals were retained by the INDICIT consortium. The first is: "**There should be less than X% of turtles with more than Y g of ingested plastics**", based on the 'Fulmar' scenario used in OSPAR, with X and Y being the minimum occurrence and dry mass of ingested litter found in the concerned area (X = 45% in the Atlantic and 33% in the Mediterranean, and Y = 0.13 and 0.37 respectively). However, according to the new Commission Decision 2017/848/EC, the definition of Criteria D10C3 should refer to the "health of species concerned". As the cause of death could not be used to propose a threshold (only four deaths were attributed to litter ingestion of the 189 known cases), mortality could not be used to recommend GES. Thus, the ratio of dry mass of ingested litter to food remains was recommended as the best proxy of 'individual health' with available data on body condition, assuming that healthy individuals with no food remains did not ingest litter either. The second GES proposal is: "**There should be less than Z% of turtles with more plastics than food remains**", Z being the minimum occurrence, which was 11.11% for the Atlantic and 3.7% for the Mediterranean as assessed with the last available data. Collecting further data should help better assess which litter/food remains ratio should be considered and the ecological factors that may influence it. Moreover, further analyses should deal with the fraction of micro-plastics of 1 to 5 mm within the dataset.

Main recommendations In the aim of allowing more accurate assessments and refining GES guidelines, the INDICIT consortium recommends the following actions:

i) Enlarge the network of stakeholders involved in monitoring litter ingestion, especially in the OSPAR and Mediterranean areas where no or little data is available, and consider the preconditions for involvement required by stakeholders who were contacted but are not yet involved (especially material and human means, considering that an average of five hours with two handlers is required per dead turtle from handling in the field to analysing the litter ingested). The professionalization of a specialized team in support of some stakeholders could be envisaged.
ii) Extend the dissemination of the INDICIT protocol and the video-tutorial.
iii) Propose regular training sessions, with a kit offering the essential equipment for turtle handling and litter analysis, envisage the development of tools to allow stakeholders to share their skills, and recommend workshops with expert biologists (rescue centres, stranding networks, veterinarians, researchers, etc.) and MSFD, RSC and member state representatives.

iv) Plan data gathering in a dedicated platform and consider how to integrate stakeholders' private data in order to pursue the statistical analyses.
v) Encourage the collection of the 'optional' parameters in the INDICIT protocol in order to acquire more knowledge about the impacts on individual health and define a simple way to evaluate this.
vi) Continue reflection with experts from multi-disciplinary approaches in order to recommend GES in line with Criteria D10C3 and the notion of "health of the species concerned".
vii) Encourage the collection of data on food remains in order to advance work on the second GES proposal regarding the percentage of turtles with more litter than food remains as a proxy of individual health.

2. 'Entanglement by biota with marine litter' indicator

The feasibility study of this Criteria D10C4 indicator was based on a review of available grey and published literature and the responses to a questionnaire disseminated to experts in sea turtles, marine mammals, marine birds, fish or marine litter. The report, available on the INDICIT website, provides the detailed results per taxa. At this stage, information is often partial or based on opportunistic data, making an accurate assessment of entanglement impossible. The constraints, relative to methodology for the collection of specimens and intrinsic factors such as behaviour or age, are analysed. Several relevant species are proposed, but no standard protocol exists at this time. The INDICIT consortium highly recommends defining a standardized typology of the types of marine litter causing entanglement, particularly to differentiate passive entanglement due to litter and active entanglement due to bycatch. Relevant networks have started to be identified. A survey of available databanks should be conducted, and initial standard data collected in pilot tests. The INDICIT protocol recommends collecting data on sea turtle entanglement as an optional parameter. This information could be further refined using the first collected data.

3. 'Micro-plastic ingestion in fish and sea turtle' indicator

The feasibility of this Criteria D10C3 indicator, which distinguishes micro-plastics < 5 mm in size, was evaluated through a literature review on (i) fish, in order to enlarge the proposed area, including the HELCOM RSC, and (ii) on sea turtles. For the latter, the feasibility was further evaluated in the laboratory with involved stakeholders. The review highlighted the strong discrepancies between studies in terms of extracting the litter (e.g. digestion techniques), differentiating plastic particles from natural items, and avoiding contamination in the field and the laboratory. A selection of relevant species was proposed for fish. For sea turtles, a simple method for collecting data when monitoring the indicator 'Litter ingested by sea turtles' was proposed. A complete protocol should be developed for these two taxa; the collection of further standard data would allow the assessments to be refined.

4. Sharing the knowledge

The INDICIT project targeted a large audience: scientists and laboratories, decisional stakeholders, RSC secretaries, national focal points and fishery management organizations, experts and technical groups, specific networks (e.g. stranding networks or discussion lists), local groups and sea users, non-governmental organizations, as well as the general public. To communicate its findings and recommendations, INDICIT participated in or organized international and national conferences, expert workshops, training sessions and dissemination meetings. Several technical and awareness-raising tools have been built, and INDICIT collaborated

with 11 research projects. A 28' documentary was produced during the project[1]. These actions reached an audience of more than 12,730 people. The video tutorial[2] on sea turtle protocol was disseminated among the scientific communities (researchgate), Unep/Map delegates and MSFD Task Group on ML.

Perspectives: from INDICIT to INDICIT II

The INDICIT II project started in February 2019 and will continue for two years. The new project aims to capitalize on the outcomes of INDICIT in order to 1) collect further data to refine the GES proposed for the indicator 'Litter ingested by sea turtles', and test it in pilot areas where 'Programmes of Measures' are implemented in regards to specific litter categories (e.g. evaluation of a decrease in ingested fragments of plastic sheets where plastic bags have been banned); 2) develop the two other litter impact indicators related to i) entanglement, especially for marine taxa that spend time at the ocean surface (turtles, mammals, birds) and ii) micro-plastic ingestion in fish and sea turtles. For these two new indicators, new standard methodologies will be developed in parallel to networking initiatives and training of new stakeholders. The collected standard data would enable the evaluation of the constraints and inform possible GES proposal. INDICIT II also aims to better evaluate the impacts of litter (ingestion and entanglement) on the health of individuals, especially through eco-toxicological approaches.

[1] https://indicit-europa.eu/documentary/
[2] https://www.jove.com/video/59466/