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**
Mediterr-anean	France	77	82.43	1.23 ± 0.27	12.12
	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]

---

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

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.