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INDICIT-II Technical Summary of the Monitoring Procedure for the Marine Litter Impact Indicators

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Technical summary of the monitoring procedure for the 3 indicators

SUMMARY

This document provides the technical summary of three protocols developed and tested during the INDICIT and INDICIT-II DG ENV projects, related to the MSFD criteria (D10C3 and D10C4) and corresponding RSCs indicators (IMAP Candidate Common indicator 24). Each protocol is presented with its objective, the monitoring procedure, the data banking used as well as the monitoring assessments, for which the collected data can be used. The document also refers the networks involved (types and numbers of stakeholders).

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GENERAL CONTEXT

The DG ENV INDICIT-II project strives to harmonize the Regional Sea Conventions (RSCs) and Marine Strategy Framework Directive (MSFD) indicators to evaluate the marine water good environmental status regarding impacts of marine litter on fauna. As a strong need of common protocols has been identified, the INDICIT-II project aims to propose, test and support the implementation of 3 protocols related to MSFD criteria (D10C3 and D10C4) and corresponding RSCs (Barcelona and OSPAR, also including HELCOM specifically for micro-litter impact) indicators. The INDICIT II project also search for i) assisting in the harmonization of networks for standard data collection, ii) establishing common databases, and iii) providing proposals for baselines and thresholds assessments.

The present document provides a summary of technical information for monitoring the impacts related to indicators "Litter ingestion by sea turtles" (§I), "Entanglement of sea turtles and biota in floating marine litter" (§II) and "micro-litter ingested by fish" (§III). For Barcelona RSC, the corresponding IMAP Common Indicators belong to "Candidate Common indicators 24: *Trends in the amount of litter ingested by or entangling marine organisms, especially mammals, marine birds and turtles*", related to the Ecological Objective (EO 10) "*Marine and coastal litter do not adversely affect the coastal and marine environment*".

The INDICIT protocols for collecting standard data propose to distinguish the so-called "basic" parameters from "optional" parameters. The firsts are required as fundamental information for evaluating the current situation and the distances to thresholds. While collecting the whole list of parameters can be time-consuming, it is recommended to also collect the optional parameters, which intend to better identify the risky biological, environmental and anthropogenic factors favouring the interactions with litter.

The INDICIT II consortium interacts with stakeholders in charge of data collection for considering experience feedbacks when applying the proposed protocols. Slight changes may be proposed in the next future for optimizing the monitoring procedures, also by considering the required parameters for evaluating the thresholds.

I. Litter ingestion by sea turtles

OBJECTIVES

In 2018, INDICIT project (2017-2019) has developed a standard protocol for monitoring litter ingestion by sea turtles in collaboration with stakeholders in charge of data collection, who provided feedbacks for optimizing the measurements in the field and in laboratory (INDICIT, 2018). In addition to English, the INDICIT protocol has been translated in Arabic, French, Greek and Spanish. The English and French versions of the protocol have been harmonized in a common document with SPA/RAC protocol (SPA/RAC-INDICIT, in press). The monitoring procedures concern the loggerhead turtle *Caretta caretta*, but are all applicable to other sea turtle species, except for the leatherback turtle *Dermochelys coriacea* for some biometric measures requiring to handle the individual.

The objectives of the monitoring are to i) determine the presence as well as the type, quantity and characteristics of litter items superior to 1 mm found either in the digestive tract of dead necropsied individuals or the faeces of living individuals recovered in rescue centres, and ii) evaluate the impacts of litter ingestion on individuals' health. INDICIT II project aims at optimizing the procedures regarding the results obtained from more than 1,100 necropsied individuals and (INDICIT Final report, 2019) and 300 living individuals (impacts on health, threshold assessments, feasibility and other constraints…).

PROCEDURE

A complete necropsy of dead individuals is recommended in order to well assess the possible causes of death and the direct and indirect impact of litter ingestion on individual's health. It is also advised to practice the necropsy with a veterinarian and in a veterinary laboratory for complementary expertise and for respecting sanitary precautions. Only authorized people can handle live or dead individuals after signalling the discovery to local authorities. The complete procedure is feasible only when autolysis status is not too advanced for allowing it. The protocol provides the procedure for collecting data from excretions of living individuals in rescue centre. In this case, it is highly suggested to affix filters <1 mm on the incoming and outgoing water system in order to avoid contamination from outside.

An average of 5 hours with 2 manipulators should be considered for the collection of data (classified ingested litter) from necropsied individuals, including the external and internal exam of the individual. For living individuals, besides the time required for the external exam, the manipulation time required to collect the faeces every day (fifteen minutes at least) in the basin until 2 months maximum. The SPA/RAC-INDICIT protocol lists the material required for the monitoring procedure.

The parameters to measure are i) the occurrence of litter ingestion, evaluated as the frequency of necropsied individuals having ingested litter among all individuals, and ii) the quantity of ingested litter expressed both in mean dry mass per category (0.01 g precision) and mean number of pieces per category (0.01 precision). All pieces of size superior to 1 mm are considered by affixing a filter on the water washing the digestive tract (separated in oesophagus, stomach and intestines sections) or the faeces contents. It is recommended to affix a second filter of mesh size 5 mm on the filter of mesh size of 1 mm in order to differentiate the fraction micro from macro-litter (>5 mm). Meso-items (5 mm – 2.5 cm) can also be differentiated from macro-items (>5mm). The ingested litter can also be differentiated per main class of colour ("white-transparent", "light coloured", "dark coloured").

Litter items are characterized from a list adapted from MSFD guideline (MSFD TG Marine Litter, 2013), detailing especially the plastic items, being mostly found ingested by sea turtles (INDICIT Final report, 2019): Industrial plastics (IND PLA), User plastics (sheet (USE SHE), threadlike (USE THR), foams (USE FOA), fragments (USE FRAG) other plastics (USE POTH)), litter other than plastics. For further evaluating the faculty of the indicator to detect significant changes related to specific programs of measures, e.g., related to single use plastics, it is recommended to further characterize the items found ingested, especially for the fragment category (USE FRAG) which can be described in a column "Notes". In addition to anthropogenic materials, it is suggested to measure the quantity of natural material, natural no food items (NFO) and especially the natural food for sea turtles (FOO), which can possibly inform on individual's health status outside the reproductive period.

Several parameters can be collected for acquiring a better knowledge on the biological factors which may influence litter ingestion and the harms caused by litter (sub-lethal effects and lethal doses) on individuals' health status (plastron shape, fat reserves, injuries and description of the external and internal exam).

DATA BANKING

INDICIT II project has provided an Excel file template for gathering data in a standard form. The file contains 2 separate tabs, for data from necropsies and data from faeces (Data_Ingestion_ sea turtle_INDICIT II_2020.xlsx). A template of observation sheet extracted from SPA/RAC-INDICIT protocol is provided (INDICIT _ RAC SPA _ OBSERVATION SHEET _ Modif 09102020.pdf). The project supports the INTERREG project CLEANATLANTIC for developing a data entry software aiming to store the standard data on litter ingested by sea turtles.

BASELINE AND THRESHOLD ASSESSMENT

The threshold and baselines are being analysed from assessments at the population level, comparing evaluations at the country, entire basin or sub-basin spatial scales, of: i) the occurrence of litter or specifically plastic ingestion (percentage of individuals having ingested litter according to the sample size), ii) the mean dry mass with standard error of ingested litter per individual, ii) the mean number of pieces and standard error of ingested litter per individual. Assessments can be reported once a year and analysed over a 6-years cycle for harmonization with MSFD. Each contracting party can assess his own current situation and distance to target. Averages can concern all litter debris or specifically plastic and sub-categories in order to evaluate the efficiency of National specific programs of measures. Several methodological options are being evaluated for proposing a threshold and baseline, based on minimum, mean or quantiles of the different recorded parameters (occurrence, abundance, mass) of ingested litter.

MONITORING NETWORK

More than 100 institutions (stranding networks, rescue centres, laboratories, authorities) are collecting data on sea turtles possibly with other taxa too, in the areas covers by INDICIT II project (France, Spain, Tunisia, Turkey, Cyprus, Greece, Portugal; see the restricted sharing list constituted by INDICIT I and II project: INDICIT II stakeholder contact 2020_INDICIT D2.8 - D3.7.xlsx). Southern countries have been contacted for establishing their current capabilities to collect sea turtle specimens and data on litter ingestion. Some stakeholders have requested conditions for being involved in data collection (e.g., training session, support in material or human means…, see INDICIT Final report, 2019 (INDICIT Final report 06_09_2019.pdf)).

II. Entanglement of sea turtles and biota in marine litter

OBJECTIVES

INDICIT II project proposed to define Entanglement as “the process of being wrapped, trapped or stuck in marine litter”. The project has developed a standard protocol for monitoring “*Entanglement of sea turtles and biota in marine litter*”. This protocol has been shared for standardization to the MSFD Target Group Marine Litter (TG ML) and is under revision. The main objectives are to identify the types and characteristics of litter items in cause of entanglement, and assessing the impacts on individuals’ health.

The proposed procedure aims to characterize the individual’s circumstances of discovery and describe his general body condition, including with biometric measurements. The next steps consist in characterizing the litter items following a detailed list intending to i) better differentiate bycatch and entanglement; ii) target possible associated Programs of Measures, in particular related to litter from marine origin (for example related to fishing) and litter originating from land (for example related to agriculture), and iii) collect knowledge on health impacts caused by entanglement.

One of the main challenges registered by researchers and policy makers to monitor entanglement focus on the great difficulty to differentiate entanglement in marine litter (i.e., in items deliberately discarded or unintentionally lost), from bycatch on active fishing gears (i.e., corresponding to an inadvertent catch). For this reason, an insert with the main concepts and a list of criteria to distinguish both types of cases, has been included in the protocol. Moreover, a specific parameter called “severity” has been added for better classify the impacts of entanglement on individual’s health.

The INDICIT II proposed tool aims to be implemented by the organizational structures managing stranded or injured live or dead animals found at sea or on the shores (stranding networks). Generally, alive animals are carried to adequate facilities for their rehabilitation (rescue centres). For animals found dead or who die in rescue centres, a complete necropsy is highly recommended. INDICIT II

project aims to involve these key stakeholders (stranding networks and rescue centres) to monitor entanglement through standardized data collection proposed on this protocol.

SPECIFICATIONS

1. Taxonomic groups and species: sea turtles (loggerhead, leatherback and green turtle), marine mammals, seabirds and seals. A preliminary analysis is undergoing for the last 3 taxa.
2. Technical requirements: The finding of sea turtle individuals must be signalled to local authorities and an official permit is required for handling this protected species. CITES permits are required to move the samples from or to countries having ratified the Washington convention. Sanitary precautions have to be considered to avoid any risk of zoonosis.
3. Preliminary recommendations:

- Taking pictures is highly recommended: An image is an important source of data that could be used to improve the description of the stranding event, or to collect relevant information that was not registered upon the specimen discovery.
- Review of stakeholders' historical databases: The review of historical databases and pictures from key stakeholders is highly recommended to try identifying the impacts of entanglement in each specific region in the precedent years.

4. Visual guide: a guide with images has been developed for dissemination towards stakeholders in order to facilitate the standard data collection (litter classification, individual size, biometric measures…).

PROCEDURE

The protocol aims to characterize the types of litter, which categories have been proposed by consulting the lists used in various programs (beach clean-ups, floating debris, etc.) or conventions (OSPAR, BARCELONA, HELCOM), in order to homogenize terminologies. A proposal of litter causing entanglement has been developed by INDICIT II Partners based on TG ML protocols, and reference codes from TG-ML, OSPAR and UNEP-MAP have been included. The proposed list intends to differentiate litter according to their origin (land based sources, related to packaging or other land base sources, and fisheries, aquaculture and maritime uses). A photo-guide is being developed for differentiating the types of fishing litter (net mesh size, diameter of ropes and lines, etc.). The guide will include reference schemes of range sizes in order to facilitate the evaluation of individuals and litter items relative size, especially when the interaction is observed at sea.

The protocol also provides indications to support the differentiation of entanglement from bycatch. In addition to the description of injuries caused by litter, the severity of impact can be classified from minor to severe. Biometric measurements and description of individual's body condition will enable further evaluations of the possible biological constraints promoting entanglement risks.

ASSESSMENTS

The assessments are being evaluated from averages calculated at the population level and at the country and basin scales. They consist in evaluating the frequency of occurrence of entanglement (percentage of individuals found entangled according to the sample size, per species). Averages can target all litter debris or explicit categories for evaluating specific programs of measures.

DATA BANKING

Two templates of common database developed for compiling and gathering data at the population and individual levels, can be disseminated to stakeholders for facilitating data acquisition and analyses (INDICIT II _ Data_Entanglement Standard protocol GENERAL FO.xlsx and INDICIT I _ Data _ Entanglement Standard Protocol INDIVIDUAL DATA.xlsx). This template may evolve according to the protocol currently under review by TG ML.

MONITORING NETWORK

More than 120 institutions (stranding networks, rescue centres, laboratories, authorities) are being involved for collecting data on entanglement in sea turtles, birds and mammals including seals (see the restricted sharing list constituted by INDICIT I and II project: INDICIT II stakeholder contact 2020_INDICIT D2.8 - D3.7.xlsx

III. Indicator "micro-litter ingested by fish"

OBJECTIVES

The objective is to consolidate a standard protocol for MSFD D10C3 criteria which Good Environmental Status goal is that "*the amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned*". This protocol is dedicated to micro-litter ingestion by fish. Micro-items are defined as inferior to 5 mm. The protocol is currently under review by the MSFD TG ML.

METHODOLOGY

1. *Fish species selected*: 3 species differing in their trophic ecology (diet and position in the ecosystem i.e. benthic, demersal and pelagic) with commercial value, common in the MSFD and RSCs areas, and potentially already used for evaluating other Ecological Objectives (e.g., related to contaminants), were selected: *Merluccius merluccius, Scomber scombrus* and *Mullus barbatus*.
2. *Sampling in the field:* Long hauls should be avoided as the net is scraped on the seabed and re-suspends micro-plastic particles. Gill nets are chosen for sampling in shallow waters and hot spot (harbor, river mouth, etc.). Other methods are also permitted while ensuring that all sources of biases are reduced to the minimum. Fish should be collected directly on board, checking for evidence of any disease, ensuring that all fish showing signs of net feeding are rejected (control of the mouth). Fish with everted stomach are rejected. All specimens are rinsed with ultrapure water, stored in aluminium foil and frozen upon collection.
3. *Dissection in the laboratory:* Each fish is weighed (up to 0.1 g) and the total length measured (up to the nearest mm). The entire gastro intestinal (GI) tract from the mouth to the cloacae is extracted. Fish with completely empty stomach should be excluded. Entire GI tracts are weighed (up to 0.1 g).
4. *Digestion and filtering:* The GI is immersed in hydrogen peroxide (H_2O_2 15%) or potassium hydroxide (KOH 10%) for digestion. Both reagents could affect polymers structures and colors, so temperature bath should be maintained at no more than 40 °C and digestion bath for no more than 5 days. If the resulting solution in the beaker is not completely clean, pre-filter the solution through 100 µm sieve, under laminar flow cabinet, collecting all the material by washing the sieve with ultrapure Milli-Q water. Filter the samples on a fiber glass membrane or anodisc or other membrane, with a mesh size less than 100 µm, using vacuum pump. Rinse glass funnel above the membrane with ultrapure water. Insert the membrane on a Petri dish, covered by glass top. Place the Petri dish in a clean cupboard for drying membrane at room temperature.
5. *Micro-litter detection and identification:* All the micro-litter items are detected under stereo-microscope. Uncertain micro-plastic items can be recognized using optical microscope or hot needle. The collected items are categorized according to both size classes and shape.
6. *Micro-spectrophotometry FT-IR or Raman micro-litter identification:* At least 10% of the collected items should be analyzed if this analysis is available in the facility.
7. *Micro-litter classification:* Total number, size class and shape are noted. The litter are classified as Fibers (from textile), Filament (threadlike artificial polymer element, flexible, elongated, thin), Granule (sphere), Pellet (only industrial), Film (Layer, foil, flexible), Fragment (rigid) and Foam (soft).

The number of fragments per colors is differentiated into White (include yellow), Black (include brown), Green (all the tonalities), Blue (from sky blue to light blue), Red (including orange and pink), Other (including multi-colors), each one as transparent or opaque.

It is recommended to collect a minimum of 30 specimens per species (within a defined standard length) from the same location and at the same time (ideally from the same haul). However, there is some flexibility to collect the samples, during the same week or month.

ASSESSMENTS

The analyses consist in evaluating the frequency of occurrence of micro-litter among the different species and the frequency of occurrence according to characteristics (size, shape, colour classes), including origin (e.g. industrial, fishing).

DATA BANKING

A template (excel file) of common database developed for compiling and gathering data has been disseminated to stakeholders for facilitating data acquisition and analyses (INDICIT II _ Data FISH monitoring_2020.xlsx). This template may evolve according to the protocol currently under review by TG ML.

NETWORK

Twenty stakeholders have been contacted for data collection. Among them, 9 were able to apply the protocol and collect data during the year 2020.

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