

# INDICIT-II FINAL REPORT

IMPLEMENTATION OF THE INDICATOR  
“IMPACTS OF MARINE LITTER  
ON SEA TURTLES AND BIOTA”  
IN RSC AND MSFD AREAS  
(INDICATOR IMPACT TAXA)



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## List of acronyms

**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 Affairs 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

**MTS:** French Ministry of Ecological Transition

**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

**UNIVPM:** Università Politecnica delle Marche (Italy)

**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 of the project INDICIT-II

Due to the anthropogenic pressures on the marine environment, the Marine Strategy Framework Directive (MSFD, 2008/56/EC) aims to restore clean, healthy and productive marine ecosystems of the European waters by 2020. To this end, the MSFD strives for the development of indicators aiming to monitor each EU Member States' distance to the Good Environmental Status (GES) defined by 11 Descriptors (Art 1.1). Each MSFD Member State (MS) must set up a "Marine Strategy" in order to achieve the GES and report the state of play every 6 years. According to their Action plan for the marine environment, this should be revised every 6 years i) the initial assessments of environmental status, ii) the determination of GES based on each qualitative Descriptor and iii) the determination of environmental targets with the associated indicators. And develop iv) a monitoring programme for assessing their distance to GES, a target which can be achieved due to iv) a set of concrete and operational actions (programme of measures, PoMs) along with operational targets.

Among these pressures, the pollution caused by marine litter constitutes one of the major socio-economic and ecological threats worldwide and is recognised as a major challenge, in particular in the European seas due to their high touristic and urbanisation pressures, and especially in the Mediterranean because of its spatial configuration which encloses the litter. Marine litter poses a serious threat to ecosystems. Several hundreds of taxa (invertebrates, cetaceans, fish, sea birds, sea turtles) are impacted, especially by entanglement and ingestion, which induce mortality, or more likely a reduced health condition, capacity to feed and to escape from predators and disturbances, and a reduced fitness. These sub-lethal effects on individuals and the contamination of pollutants leached by ingested plastics might have cascading negative impacts on ecosystems and thereby on human activities over a short or long term.

The MSFD dedicates the Descriptor 10 to this, defining the GES as "the properties and quantities of marine litter which do not cause harm to the coastal and marine environment" with two primary and two secondary criteria, the first (D10C1 and D10C2) concerning the composition, amount and spatial distribution of macro (particles > 5mm) and micro litter (particles < 5mm) in the environment, and the second (D10C3 and D10C4) the impacts cause by ingestion and entanglement or other injuries to marine fauna. Marine organisms can be essential bio-indicators for the monitoring of marine litter impacts and would reflect the spatial and temporal presence of litter in the marine environment. However, as the occurrence and quantity of marine litter varies in the environment with several environmental and anthropogenic factors, the spatio-temporal variability of fauna's behaviour has also to be considered in order to establish the GES and standard procedures.

Historically, the sea bird *Fulmarus glacialis* was used as a focal species to monitor litter impacts through ingestion for the north-eastern countries in the Atlantic area. The litter (>1 mm) found in the digestive tracks of stranded individuals (quantities and types) was correlated with the "quantity" of marine litter in the environment. While an EcoQo was proposed in the framework of the OSPAR RSC, no official GES has yet been produced for MSFD. The TG ML group validated a standard protocol for long term monitoring of stranded individuals (Fulmar) in their geographical range. This protocol served as a basis for establishing a protocol for collecting standard data on litter ingested by sea turtles in other geographic region such as Med/Macaronesia areas (Matiddi et al., 2011; Galgani et al., 2012).

Sea turtles have now been selected as an indicator of litter impact caused by ingestion, due to their propensity to ingest debris, their widespread distribution and the large range of habitats used during their life cycle. Specifically, the loggerhead turtle *Caretta caretta* have been used in monitoring protocols for application both in some parts of the Southern Atlantic (OSPAR IV area, OSPAR common indicator) and in the Mediterranean (ECAP pilot indicator 18 of the Barcelona convention). Individuals, either dead or alive, can easily be collected by stranding networks and rescue centres distributed over all these areas, allowing for the standard evaluation of the pressures caused by "litter ingestion" and "entanglement in floating litter" on this species. After a state-of-play realised during INDICIT, with the report (Darmon et al., 2018), and the development of standardised protocol (Matiddi et al., 2019) and harmonisation of network for standard data collection, the indicator's constraints and threshold to reach the GES still need to be defined.

Micro-litter (< 5 mm) is widespread in the marine environment, making them inevitably more readily accessible to marine fauna by ingestion than macro-litter (> 5mm). The ingestion of micro-plastics by fish presents a strong potential for developing monitoring programmes on ingestion of litter by marine organisms and may allow the ability to cover wide spatial scales. OSPAR, HELCOM, and Barcelona Conventions are currently scoping the use of fish for this indicator, underlying that a rigorous monitoring protocol needs to be developed. Furthermore, in 2017, the UNEP/MAP updated the Report “Defining the most representative species for IMA candidate indicator 24 (UNEP, 2017)” underlining the potential of fish as bio-indicators for monitoring ingestion of litter. A literature review and analysis of data collected during INDICIT project are already available, the report “State of the art: Indicator micro-plastic debris ingested by marine turtle and fish” (Silvestri et al., 2018) gives several guidelines for the testing of this indicator with several fish species as well as the sea turtles. In sea turtles, the data collection was already harmonised for data collection for the ingestion of litter indicator, and stakeholders were encouraged to differentiate the micro-plastics from 1 to 5 mm, the sea turtles were also integrated as possible indicator species for micro-litter ingestion. For fish, a standard protocol needs to be harmonised for collecting standard data. Then a first state-of-play need to be established and indicator’s constraints (fish species, biological factors influencing the observed pressure) and the GES threshold should be defined.

The impacts caused by entanglement, for example reduced moving capacity, inability to feed, injuries and lacerations, which can lead to fast or slow mortality, does not only concern sea turtles. Other taxa such as sea birds and marine mammals (cetaceans and seals) need to be considered, in order to increase the probability of observation of these interactions directly at sea. Also based on a literature review and questionnaires to experts produced during INDICIT project, the report “State of the art: Indicator entanglement with marine debris by biota (Claro et al., 2018) analysed the data available in epibenthic invertebrates (corals), fish (Elasmobranches), sea birds, marine mammals and sea turtles and provided guidelines for the test of this indicator with sea birds, marine mammals and sea turtles. For this indicator, the constraints and thresholds also need to be defined.

This report presents the outputs of the two-year INDICIT-II project (2<sup>nd</sup> February 2019 – 31<sup>st</sup> January 2021), 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 belonging to Criteria D10C3 and D10C4 (New Commission Decision 2017/848/EC). It corresponds to the deliverable n° D1.6 (deliverables listed in Appendix 1). Note that due to the COVID19 pandemic and their consequences such as lock-down in the respective countries, a 6 months extension was requested and granted. This final report is thus delivered the 30<sup>th</sup> September 2021.

The INDICIT-II project is a follow-up of the INDICIT project (1<sup>st</sup> February 2017 – 31<sup>st</sup> January 2019).

The INDICIT II project involves 12 partners from the public sector (7 EU countries and 2 non-EU countries), all contracting parties to the OSPAR (Atlantic) and/or Barcelona (Mediterranean) conventions.

The general objectives are:

- (1) to capitalise the practical outcomes related to networking, development of standardised tools, gathering of standard data and constraints and GES assessments for the Indicator “Litter ingested by sea turtles”,
- (2) to support the implementation of the indicators “Entanglement of sea turtles, sea birds, and marine mammals in floating debris” and “Micro-plastic ingestion by fish and sea turtles”,
- (3) to support the next 6-year cycle of MSFD implementation by testing the indicators (especially the more advanced “Litter ingested by sea turtles”) in response to National Programs of Measures (PoMs) in several pilot areas.

By developing standardised knowledge for several fauna taxa at an extended spatial level, the project aims to develop integrative tools to assess anthropogenic risks and distance to GES especially in the framework of the Descriptor 10. The knowledge acquired on species’ biology and anthropogenic impacts can also be shared to contribute to Descriptors 1 and 4. The project also aims to foster synergies with other EU programs and the transfer to competent authorities in 3 of the regional priorities targeted by the call, i.e. the North-East Atlantic Ocean, the Macaronesia and the Mediterranean. Finally, INDICIT-II also aims at communicating its main results to the large audience.



# I. INDICIT-II project: Structure and approaches

## I.1. General presentation of the project

INDICIT-II project started the 2<sup>nd</sup> February 2019 and finished 31<sup>st</sup> July 2021. The total budget represented 1 243 670,09 euros, considering an allocation of DG Environment of 921 399.31 Euros added to the 20% of partners' co-funding.”

The INDICIT II consortium was composed of 12 partners from 6 EU countries (France, Greece, Italy, Portugal, Spain, United Kingdom; UK) and 2 non-EU countries (Turkey, Tunisia) in addition to 2 authority representatives from France and Spain. 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 (Fig. I.1 and table I.1).



**Figure I. 1:** Map of INDICIT-II partners. EPHE = Ecole Pratique des Hautes Etudes (France) ; ISPRa= Istituto Superiore per la Protezione e la Ricerca Ambientale (Italy); CNR= Consiglio Nazionale Delle Ricerche - Istituto Per L'ambiente Marino Costiero (Italy) ; HCMR= Hellenic Centre For Marine Research (Greece) ; UVEG= Universitat De València-Estudi General (Spain) ; ULPGC= Universidad de Las Palmas de Gran Canaria (Spain); PAU-DEKAMER= Pamukkale University - Sea Turtle Research And Application Center (Turkey); FRCT= Fundo Regional para a Ciência e Tecnologia (Regional Fund for Science and Technology) (Portugal); INSTM= Institut National des Sciences et Technologies de la Mer (Tunisia), UNEXE; University of Exeter, MTES; Ministère de la transition écologique et solidaire (France), DGSCM-SGPM- Dirección General de Sostenibilidad da la Costa y da Mar-Subdirección- General para la Protección del Mar (Spain).

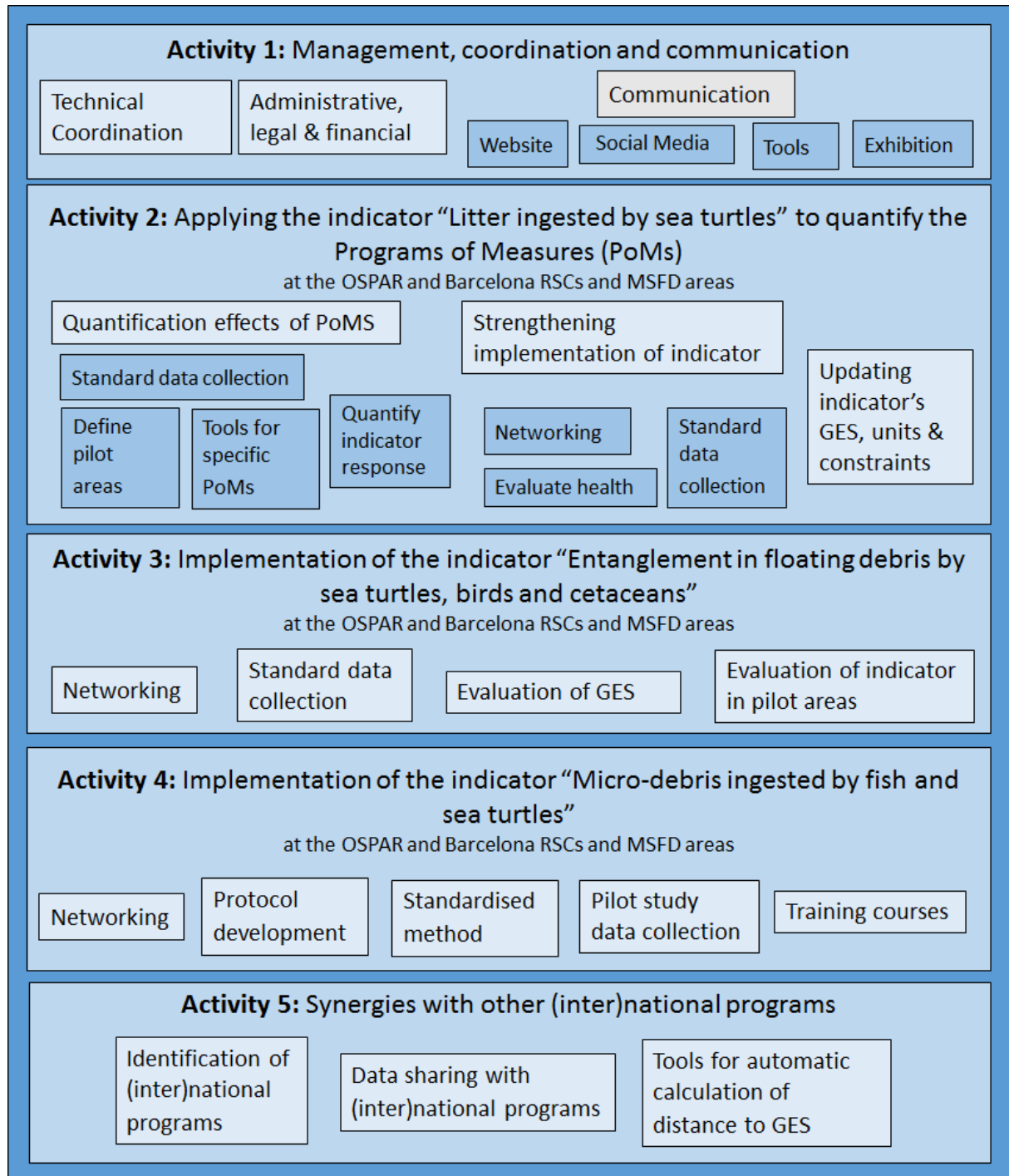
**Table I.1:** List of partners, scientific and administrative representatives

<b>Acronym</b>	<b>Identity</b>	<b>Partners' name(s)</b>	<b>Legal representative</b>
EPHE	ECOLE PRATIQUE DES HAUTES ETUDES	Claude MIAUD	Jean-Pierre VERDIER
HCMR	HELLENIC CENTRE FOR MARINE RESEARCH	Eleni KABERI	Spyridon MAVRAKOS
ISPRA	ISTITUTO SUPERIORE PER LA PROTEZIONE E LA RICERCA AMBIENTALE	Marco MATIDDI	Alessandro BRATTI
UNEXE	UNIVERSITY OF EXETER	Brendan GODLEY	Enda CLARKE
PAU-DEKAMER	PAMUKKALE UNIVERSITY SEA TURTLE RESEARCH AND APPLICATION CENTER	Yakup KASKA	Hüseyin BAĞ
FRCT	FUNDO REGIONAL PARA A CIÊNCIA E TECNOLOGIA (REGIONAL FUND FOR SCIENCE AND TECHNOLOGY)	Maria Luís ADRIÃO DO VALE	Bruno Miguel CORREIA PACHECO
UVEG	UNIVERSITAT DE VALÈNCIA (ESTUDI GENERAL)	Jesús TOMÁS	M. Dolores REAL GARCÍA
IAS-CNR	CONSIGLIO NAZIONALE DELLE RICERCHE	Giuseppe Andrea DE LUCIA	Crisafi ERMANNO
INSTM	INSTITUT NATIONAL DES SCIENCES ET TECHNOLOGIES DE LA MER	Olfa CHAIEB	Hechmi MISSAOUI
ULPGC	UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA	María M. GÓMEZ CABRERA	José Pablo SUÁREZ RIVERO
MTES	MINISTÈRE DE LA TRANSITION ECOLOGIQUE ET SOLIDAIRE	Anastasia WOLFF	Thierry VATIN
DGCM-SGPM	DIRECCION GENERAL DE SOSTENIBILIDAD DE LA COSTA Y DEL MAR- SUBDIRECCION GENERAL PARA LA PROTECCION DEL MAR	Marta MARTINEZ-GIL PARDO DE VERA	Itziar MARTÍN PARTIDA

University of Siena, Department of Environmental, Earth and Physical Sciences (Italy) has been selected as sub-contractor for the ecotoxicological analysis of this project.

## I.2. Organisation of the project in “Activities”

The INDICIT II project consists in 5 interconnected Activities (Figure I.2), each led by two partners (Table I.2). The organisation and objectives of each activity activities are described below.



**Figure I.2:** Inter-relationships among the five Activities.

**Table I.2:** Activity leaders and co-leaders

Activity	Leader	Co-leader
Activity 1	Claude MIAUD – Gaëlle DARMON (EPHE)	Brendan GODLEY - Emily DUNCAN (UNEXE)
Activity 2	Gaëlle DARMON – Claude MIAUD (EPHE), Jesús TOMÁS (UVEG)	Andrea Giuseppe DE LUCIA – Andrea CAMEDDA (IAS-CNR)
Activity 3	Ana LIRIA LOZA - Maria M. GOMEZ CABRERA (ULPGC)	Olfa CHAIEB (INSTM)
Activity 4	Marco MATIDDI – Cecilia SILVESTRI (ISPRA)	Maria Luz PARAMIO MARTIN - Maria Luís ADRIÃO DO VALE (FRCT)
Activity 5	Eleni KABERI (HCMR)	Claude MIAUD – Gaëlle DARMON (EPHE)

**I.2.1. Activity 1 – Management, coordination and communication**, led by Claude MIAUD and Gaëlle DARMON (EPHE), and co-led by Brendan GODLEY and Emily DUNCAN (UNEXE).

The objective was to ensure the proper implementation and management of the project with respect to its objectives, time frame and budget constraints, allowing a smooth communication between the consortium members. The communication effort, based on a communication plan, is a specific task of this activity. This activity, involved all the members of the INDICIT Consortium, was divided in three tasks:

*Task 1.1. Technical coordination of the project*

This sub-task aims at ensuring an efficient technical coordination of the project thanks to the organisation of meetings and workshops for the smooth and effective communication among partners and ensure the production of deliverables on time. EPHE coordinated the project, and was responsible of the official reporting (e.g. discussion with the Policy officer (PO) and submission of deliverables). The deliverables were sent to EPHE by each Activity leader two weeks before the deliverable deadline (see each specific activity time table).

The planning of meetings and workshops was:

- 20<sup>th</sup> February 2019: Kick of meeting in Brussels (Belgium)
- 10-12<sup>th</sup> July 2019: Intermediate meeting # 1 and workshop # 1 in Monastir (Tunisia)
- 14-16 January 2020: Intermediate meeting # 2 and workshop # 2 in Paris (France)
- 6-10 October 2020: Intermediate meeting # 3 and workshop #3, video conferences
- 14<sup>th</sup> June 2021: Dissemination meeting, video conference
- 8<sup>th</sup> January 2021: Final Meeting, video conference

[Deliverables D1.1; D1.2; D1.3; D1.4; D1.5; D1.6; D1.8; D1.9; D1.10; D1.11; D1.12]. The description of the deliverables is available in Appendix 1.

*Task 1.2. Administrative, legal and financial management of the project*

This task aimed at coordinating 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. The consortium 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 I.3), 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 and all kind of important decisions needing voting, validated and completed the EAB composition during the project (Table I.4).

**Table I.3: Members of INDICIT-II External Advisory Board (EAB)**

<b>Organization</b>	<b>EAB members</b>
OSPAR Commission	Philip STAMP (Deputy secretary)
HELCOM/OSPAR Commission	Stefanie WERNER (UBA, German Environment Ministry)
Barcelona convention	Tatjana HEMA
Barcelona convention (RAC/SPA)	Lobna BEN NAKHLA
AFB French Biodiversity Agency	Benjamin GUICHARD
IEO (Spanish Institute of oceanography)	Jesus GAGO
IFREMER	François GALGANI
DRAM, Azores government	Marco A.R. SANTOS
European Environment Agency	Ana TEJEDOR ARCEREDILLO
UN Environment/MAP	Christos IOKEIMIDIS
Italian Ministry of Environment	Roberto GIANGRECO
Museum National d'Histoire Naturelle	Françoise CLARO
DRGM, Portuguese Ministry	Sandra MOUTINHO
DRAM, Azores government	Gilberto CARREIRA
DFMR, Cyprus	Konstantinos ANTONIADIS

**Table I.4: Members of INDICIT Steering Committee**

<b>Institution</b>	<b>StC representative</b>	<b>Possible substitute</b>
EPHE	Claude MIAUD	Gaëlle DARMON
UNEXE	Brendan GODLEY	Emily DUNCAN
ISPRA	Marco MATIDDI	Cecilia SILVESTRI
CNR	Giuseppe DE LUCIA	Andrea CAMEDDA
HCMR	Eleni KABERI	Catherine TSANGARIS
UVEG	Jesús TOMÁS	Natalia FRAIJA
ULPGC	Ana LIRIA LOZA	Maria M. GOMEZ CABRERA
PAU-DEKAMER	Yakup KASKA	Dogan SOZBILEN
FRCT	Maria Luz PARAMIO MARTIN	Maria VALE
INSTM	Oifa CHAIEB	Ahmed AFLI
MTES	Bénédicte JENOT	Laure DUCOMMUN
DGCM-SGPM	Marta MARTINEZ-GIL PARDO DE VERA	

[Deliverable D1.13]

### *Task 1.3. Communication*

This task aimed at communicating about the project and its outcomes by managing the technical tools developed in INDICIT-II (e.g. website, private area), which will be used by partners to share data and disseminate the standardized tools to EAB and stakeholders within and outside the INDICIT-II project area. This task also includes the internal communication especially internal meetings for partners (listed above), and communicating about the project context, objectives and results towards a general audience in order to raise public awareness, thanks to e.g., Facebook (tools developed during INDICIT), Twitter or specific events.

The Project Management Team (CNRS/EPHE) regularly communicated with the PO and DG ENV. All draft deliverables were submitted to the PO for a continuous information on the project progress.

[Deliverables D1.14; D1.15; D1.16]

**I.2.2. Activity 2 – Applying the indicator “Litter ingested by sea turtles” to quantify the Programs of Measures at the OSPAR and Barcelona RSCs and MSFD areas**, led by Jesús TOMÁS (UVEG) and Gaëlle DARMON – Claude MIAUD (EPHE) co-led by Andrea Giuseppe DE LUCIA and Andrea CAMEDDA (IAS-CNR).

This activity was organised in three tasks:

#### *Task 2.1. Support the quantification of the effects of programs of measures (PoMs)*

This task aims at assessing the capacity of the indicator to evaluate the effect of PoMs (e.g., related to the ban of single use plastics) in specific pilot areas selected in various partners’ countries. The knowledge of amount and categories of ingested litter by sea turtles is related to different scenarios of litter reduction (e.g., targeted items) induced by specific PoMs. Pilot areas were selected according to the information (capacity to collect samples, characteristics of the marine environment, pollution pressures etc.) collected during the first 6 months of the project. Each partner aimed at evaluating for his country, the capacity of evaluate the PoMs implemented at the national level thanks to this indicator.

[Deliverables D2.7; D2.11]

#### *Task 2.2. Strengthening the implementation of the indicator*

The mobilisation of more stakeholders was identified as an important objective in the first INDICIT project. Efforts have been made in the OSPAR-Macaronesia area where networking established within the INDICIT project faced some difficulties due both, to the configuration of the zone and the specific conditions and needs required by stakeholders to collect and gather their data (e.g., financial assistance, training, involvement in meetings with partners, requirements related to data access and privacy, etc.). Data was needed in particular in the continental Spain and Portugal areas. An effort on promoting harmonisation (e.g. the use of standard protocols for data collection and banking) was made in Eastern and Southern countries in the Mediterranean area and in mainland Portugal and in the Atlantic area of Spain. The current constituted Mediterranean and Atlantic networks pursued the collection of standard data needed to refine the spatial precision (sub-regions) of the GES and indicator’s characteristics.

[Deliverables D2.6; D2.8; D2.9]

#### *Task 2.3. Updating indicator’s GES, units and constraints at the RSCs scale*

All the data collected and corrected were gathered in a common database, allowing the consolidation of the indicator’s constraints and GES evaluation, improving the evaluation made during the INDICIT project. The increasing size of this data set allowed to further test e.g. the relationships between occurrence of litter ingestion and quantities of ingested litter with body condition. Body condition has been approximated by biometric measures and other parameters considered as “optional” (considering stakeholders’ time

constraints), especially those describing the individuals' health status, which were encouraged to be measured on both, live and dead individuals. Stakeholders were also encouraged to standardise the collection of some parameters (e.g., carapace length) and to collect more specific requests, such as e.g. the ingested food remains, in order to further evaluate one scenario proposed during INDICIT project (the number individuals with more ingested litter than food remains), as a proxy of litter impact on individual's health.

In this task, a specific action was devoted to "individual health" in respect of the New Decision Commission 2017/848 (where the "health" of species is considered). To this purpose, samples (from dead and live individuals) were collected for ecotoxicological analysis (pollutant dose, biomarkers) in synergy with the project PlasticBuster MPAs.

[Deliverables D2.10; D2.12]

### **I.2.3. Activity 3 – Implementation of the indicator "Entanglement in floating debris by sea turtles, birds and cetaceans" at the OSPAR and Barcelona RSCs and MSFD areas, led by Ana LIRIA LOZA and Maria M. GOMEZ CABRERA (ULPGC) and co-led by Olfa CHAIEB (INSTM).**

This activity aims at mobilising and improving the networks of stakeholders identified in the INDICIT feasibility study report, defining standard procedures and collecting standard data in order to provide a first state-of-play at the country level as well as a first assessment of GES and characteristics for this indicator (entanglement of sea turtles, birds and cetaceans in floating debris). This Activity benefits (data available and expert knowledge) of synergies (Activity 5) and also provide expertise and original data for MSFD Descriptors 1 ("Biological diversity") and 4 ("Food webs").

The activity was divided in 4 tasks:

#### *Task 3.1. Networking and standardization of monitoring*

This task aims at establishing the networks notably identified and contacted during INDICIT, and train stakeholders to the collect of standard data.

[Deliverables D3.6; D3.7; D3.8; D3.9; D3.10]

#### *Task 3.2. Collection of standard data*

This task aims at constituting a common standard database in which to gather standard data.

[Deliverable D3.11]

#### *Task 3.3. Evaluation of GES and indicator's characteristics at the RSC scale*

This task aims at statistically analysing the data from Task 3.2. in order to propose a first assessment of GES (baseline/threshold) and evaluate the indicator's characteristics: species, biological constraints and units.

[Deliverable D3.12]

#### *Task 3.4. Evaluation of the indicator in pilot areas*

This task aims at collecting specific data in the pilot areas selected in Task 2.1 in order to provide a first assessment of the capacity of this indicator to react to the effect of PoMs.

[Deliverable D3.13]

**I.2.4. Activity 4 – Implementation of the indicator “Micro-litter ingested by fish and sea turtles” at the OSPAR and Barcelona RSCs and MSFD areas**, led by Marco MATIDDI and Cecilia SILVESTRI (ISPRA) and co led by Maria Luz PARAMIO MARTIN and Maria Luís ADRIÃO DO VALE (FRCT)

This activity benefits from the INDICIT feasibility study on this topic (INDICIT Deliverable 2.5), which highlighted the strong potential of this Indicator for D10C3 and the need for elaboration and dissemination of a rigorous standardised monitoring protocol. While the network mobilised for the implementation of Indicator “Litter ingested by sea turtles” was mobilised for collecting standard data on micro-plastic ingested by sea turtles in the framework of Activity 2, this Activity was dedicated to the collection of knowledge on fish.

*Task 4.1 Networking and standardization of monitoring for fish*

This task aimed at proposing and disseminating protocols (with a harmonisation of methods used both in the field and in the laboratory), by confronting the expertise in isolating and categorizing micro-litter (size-limit to define) among partners, stakeholders and external experts.

[Deliverables D4.6; D4.7; D4.8: D4.9; D4.10]

*Task 4.2 Collection of standard data for fish*

Following the selection of standard protocols, the data were collected in different pilot areas of several partners’ countries. The collection of these data is used to test the harmonised protocol, and verify its applicability within a monitoring program.

[Deliverable D4.11]

*Task 4.3 Report on GES and indicator’s characteristics at the RSC scale for fish*

Considering the lack of previous standardised data on this topic, it is probably not possible to define a Threshold value for this indicator, but the data analysis provides insights into the most suitable species and the relevant biological constraints for a definition of GES.

[Deliverable D4.12]

*Task 4.4 Evaluation of the indicator “micro-litter ingested by sea turtles” (coordination ULPCG/EPHE)*

As this task concerned sea turtles, the networks of stakeholders involved were globally the same than those of Activity 2 (stranding networks and rescue centres). Laboratories able to record micro-litter (1-5 mm) have to be identified, and the results are presented here within the Activity 2.

[Deliverable D4.13]

**I.2.5. Activity 5 – Synergies with other (inter)national programs**, led by Eleni KABERI (HCMR) and co led by Claude MIAUD and Gaëlle DARMON (EPHE)

INDICIT-II also devoted an activity for fostering the collaboration with other programs for a better harmonisation and effort mutualisation (e.g. sharing expertise, tools, and when agreed with collaborators, the standard data, working hand-in-hand with policy-makers by integrating them directly into the reflexions and the work with stakeholders, disseminating the tools and better allowing their appropriation by other stakeholders on a wider scale). The implementation of coordinated practical monitoring provides a greater coherence within and between marine (sub)regions as at a larger scale (beyond the MSFD Member states) thanks to the dissemination of INDICIT-II’s outcomes.



### *Task 5.1 Identification of international projects*

After listing finished and ongoing international or EU projects related to the studied taxa, marine litter, anthropogenic risks, the aim of this task is to evaluate the synergies (data and methodology sharing, complementary approaches, etc.) to build during the progress of the program.

[Deliverable D5.6]

### *Task 5.2 Data sharing with international programs*

This task aims at sharing data, approaches and knowledge with experts of other projects (concomitant to Task 5.1), especially by writing and signing the respective MoU (Memorandum of Understanding).

[Deliverable D5.7]

### *Task 5.3 Tools for automatic calculation of the distance from GES*

This task aims at supporting the development of a GIS tool to support the implementation of the D10C3 and D10C4 criteria by calculating the distance from GES at the regional to local scales thanks to the data collected by the respective stakeholders.

[Deliverable D5.8]

## 1.3. Achievement of expected deliverables

INDICIT II proposed 62 deliverables (Appendix 1). Most of them are considered as fully achieved.

For Activity 1, all the deliverables are fully achieved. D1.10 and D1.11 (minutes of intermediate meeting and dissemination meeting) were included to the Supplementary progress report provided in November 2020 (as the program was extended by 6 months due to the Covid 19 pandemic).

For Activity 2, the deliverable D2.6 was impacted as it was impossible to organise the training sessions as they had been planned (e.g. in rescue centers and laboratories). These training sessions were managed as video conferences, when possible (the % of progress was thus evaluated at 75 %). A new deliverable (D2.11) has been produced with the detailed results of the ecotoxicological analysis.

For Activity 3, there was same issues as faced by Activity 2 (D3.6 Training sessions, 75 % of progress). For Activity 4, several training sessions have been again impossible to organize, replaced by video conferences. ISPRA has started shooting the laboratory procedure in order to produce a video following the model of what was done in INDICIT for the indicator litter ingested by sea turtle and published in the scientific journal JoVE (Matiddi et al., 2019). There was not a choice between the film and the publication on Jove Journal. JoVe journal is a scientific journal which requires professional quality video for explaining published protocols. This was done for the protocol "litter ingestion in sea turtle" during INDICIT I. This kind of publication was selected for "ingestion by fish" because it is a perfect dissemination tool. Due to the pandemic, instead of the training course, ISPRA proposed to replicate in INDICIT II, the previous video tutorial with the protocol "micro-litter ingestion by fish". The sharp increase in the cost of publication in this journal and the mobilisation of the budget to extend fix-term several contracts (with the 6-month extension) did not allow the payment of these costs. The film shooting of the protocol "ingestion by fish" started by ISPRA was interrupted and was not submitted to this journal. This protocol will be published as "Material & Methods" in the manuscript of a classical scientific journal presenting the data collected in the Mediterranean Sea by the implicated partners.

For Activity 5, D.5.8 (Report on methodology integrative tools) was evaluated at 95% of achievement. While the tool is finalised, not all partners have yet entered their summarised data into the GIS and as for the other Activities, data will be further collected and gathered after INDICIT II. A GIS tool "package" with guidelines has been disseminated among the stakeholders collecting and storing the data, and encouraged to use with their respective data sets.

## II. INDICIT-II Communication

UNEXE leads this task with close interaction with EPHE, with all partners participating in the task (with deliverables D1.20 and D1.21 available through the INDICIT website).

This task is multifaceted, specific objectives include:

1. Dedicated website <https://indicit-europa.eu/>
2. Management of social media accounts (e.g. Facebook <https://www.facebook.com/indicitproject/> and Twitter <https://twitter.com/indicitii?lang=en-gb>)
3. Communication between partners and external advisory board (EAB) and targeted communication with MSFD policy makers in EU MS and the TG ML.
4. Exchange of information and dissemination of INDICIT II Project
5. Exhibition and comic (led by ISPRA)
6. Standardised tools

### II.1 Website

The INDICIT website has continued to provide online the technical tools and outputs of the project, as well providing information on the partners involved latest work, events and results. In addition, latest publications from the partners have also been uploaded. From April 2022, the website will be hosted by the CEFE-CNRS (Montpellier). It will continue to be a tool for cohesion between the former partners of the INDICIT programs and a resource centre on the theme of "plastic ingestion" and "entanglement" of marine organisms.

The UNEXE worked with graphic designers to redesign the logo for INDICIT II (Indicator Impact Taxa).

The website also hosted a dedicated page for the INDICIT II Challenge. This ran between April-June 2020 (during the major lockdown period). This encouraged the general public to send in photos, videos and links that they had found posted on social media relating to the entanglement of marine wildlife in plastic pollution

Dedicated deliverables pages INDICIT I and INDICIT II including reports and standardised protocols.



Figure II.1. INDICIT II new logo & INDICIT II challenge

## II.2. Social media

Dedicated INDICIT II Facebook and Twitter accounts were run throughout the project. One to two posts are put up once a week on both Facebook and Twitter. The posts vary from news related to the INDICIT II project, work by different partners and other related current international studies or news about the issue of marine plastic pollution and biota

Facebook: <https://www.facebook.com/indicitproject/?ref=bookmarks>

- Total numbers of followers (September 2021): 1,345

Twitter: The Twitter page for INDICIT II has been created: <https://twitter.com/indicitii>

- Total number of followers (September 2021): 473
- Connected with other marine litter projects e.g. Clean Atlantic, Plastic Busters MPA and RAGES and Quiet Med 2 (INDICIT II twitter follows all of these projects and they follow the project's twitter back). This aids in Synergies (Activity 5.) as these projects can see update to date work by INDICIT II.

The INDICIT II Challenge engagement was promoted on both social media channels and aided greatly in the participation.

## II.3. Communication with EAB

Communication with the External Advisory Board (EAB) has been delivered in the form of a number of newsletters as well as regular update announcements on the website. These have been written with and sent in conjunction with the coordination team EPHE.



Figure II.2. INDICIT II EAB newsletter

## II.4. Dissemination of project

A Google Drive was created for internal communication and aid in report writing and exchanges between partners. Partners continued to input their dissemination of the project with a variety of avenues for example events and conferences as well as publications.

Due the COVID-19 pandemic events have often been cancelled or delayed and training sessions unable to be hosted.

Scientific publications relating to and mentioning the INDICIT II project:

Novillo et al. 2020 - Evaluating the presence of microplastics in striped dolphins (*Stenella coeruleoalba*) stranded in the Western Mediterranean Sea. Marine Pollution Bulletin.  
<https://doi.org/10.1016/j.marpolbul.2020.111557>

Antonella et al. 2020 - Floating marine macro litter: Density reference values and monitoring protocol settings from coast to offshore. Results from the MEDSEALITTER project. Marine Pollution Bulletin.  
<https://doi.org/10.1016/j.marpolbul.2020.111647>

Pereira et al 2020 - Microplastic in the stomachs of open-ocean and deep-sea fishes of the North-East Atlantic. Environmental Pollution. <https://doi.org/10.1016/j.envpol.2020.115060>

Scientific publication for exporting INDICIT I and II results to expert communities will be pursued after INDICIT II project (Papers related to debris ingestion in sea turtles, GES evaluation and stakeholder's mobilization are in progress, papers on the other indicators – ingestion of microliter in fish, entanglement in litter – will be started.

## II.5. Exhibition and Comic

The exhibition and comic were managed by ISPRA and involved all partners. The exhibition utilised media gathered during INDICIT I and INDICIT II including high quality images; this includes plastic pollution and images of scientists working in the field. The comic was designed to raise awareness of the impact of plastic pollution on marine biota like sea turtles.

Due to the COVID 19 pandemic, it was not possible to show the exhibition in the partners' countries. Therefore a virtual photo exhibition was organised online <https://artspaces.kunstmatrix.com/node/7219759>.

Additionally, an Itinerary Exhibition catalogue was printed in order to disseminate the Exhibition with or without shown in person. This Exhibition was opened in the Azores on the 17/09/2021 and shown in person in Italy during ecological beach event in Favignana (IT) and European researchers' night in Rome opening date 19/09/2021 and 24/09/2021.

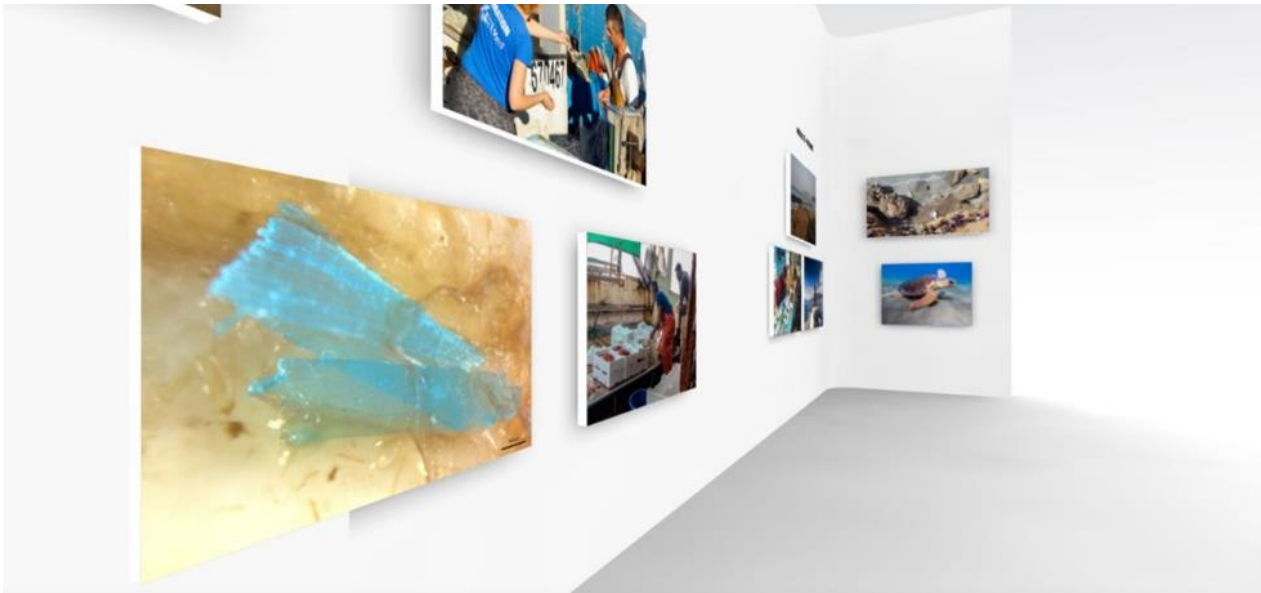


Figure II.3. INDICIT II virtual exhibition

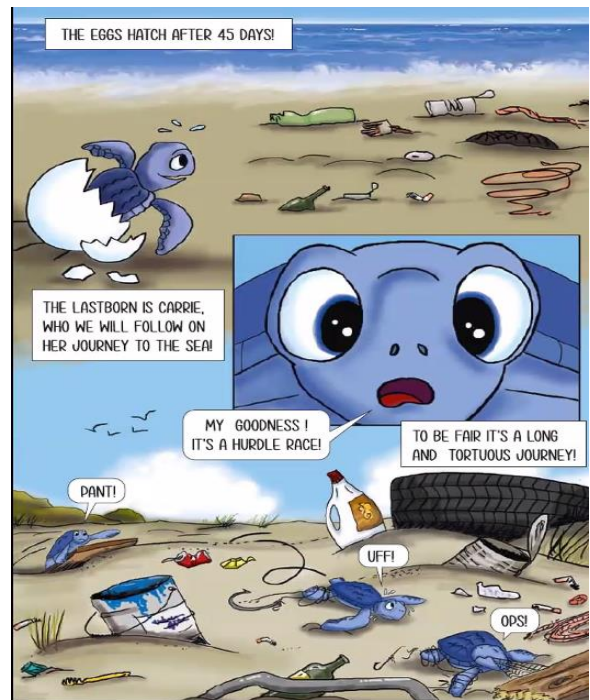


Figure II.4. INDICIT II Comic



Figure II.5. INDICIT II Itinerary Exhibition catalogue

### II.6. Standardised tools

As in the former INDICIT project (protocol for ingestion in sea turtle, [https://indicit-europa.eu/cms/wp-content/uploads/2018/09/Protocole\\_v8.pdf](https://indicit-europa.eu/cms/wp-content/uploads/2018/09/Protocole_v8.pdf)), UNEXE worked with a graphic designer to create a set of standardised tool resources for the website for Activity 3: Entanglement of sea turtles and biota in marine debris, social media review and Activity 4: Micro-litter ingestion in marine fish. These have been shared with the EAB and EU MS authorities in the Mediterranean and NE Atlantic and the respective RSCs for consultation. They are now available on the website for use from wider stakeholders and the international scientific community.

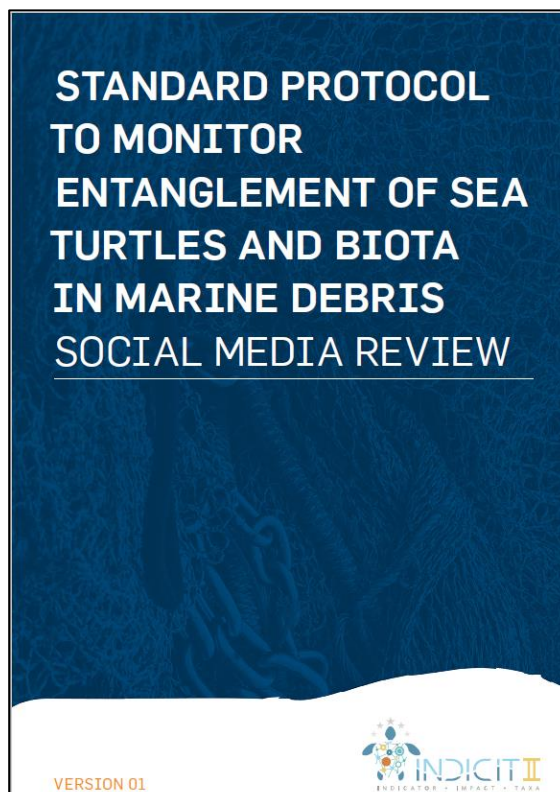


Figure II.6. Title pages of the standardised protocol documents

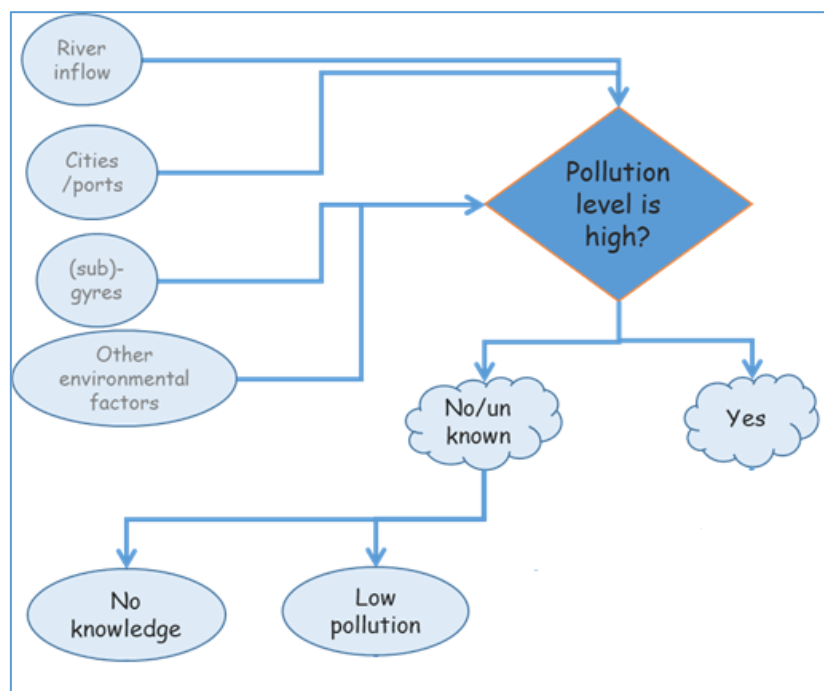
### III. Supporting the quantification of the effects of programs of measures

#### III.1. Validating the pilot areas for indicator “ingestion in sea turtle”

The first step to measure the capacity of the indicators (ingestion and entanglement) to evaluate the effect of Programs of measures (PoMs) was the selection of specific pilot areas (D2.7). These areas should enable to accurately measure litter ingestion and impacts on individuals in relationships with litter abundance and PoMs implemented locally. This was completed using previously collected data (for ingestion), presence, expertise and empirical knowledge of partners and experts, and knowledge on litter and sea turtles in the local environment. At the end of INDICIT-II project, the indicator litter ingestion has been fully evaluated with data recorded during both INDICIT and INDICIT II and new data are now available for the indicator entanglement. We proposed a more standardised methodology (than the way we selected these pilot areas during the first 6 months of the INDICIT-II project) to test for the identification of pilot areas for these two indicators, in connection with PoM which have been implemented in each MS country.

*Method:* A decision tree has been developed in order to identify these pilot areas (e.g. example in fig. III.2). This tree is based on three main entrances (fig III.1a, b, c). The first step is to select the testing area (e.g. where the stakeholders are able to collect information on ingestion/entanglement):

- *Entrance “Pollution levels”:* it concerns the main source of pollution (litter) affecting the area (main rivers and cities, existence of litter gyre/sub-gyre (with can affect the accumulation of litter in the area) and all relevant information on litter source in the area (fig III.1a). This is inferred by literature review, empirical data or experts’ knowledge. For better evaluating the impact of litter and the efficiency of restauration measures, the objective was to consider a gradient of pollution levels in order to test the relationships between litter abundance (per category), ingestion and impacts.

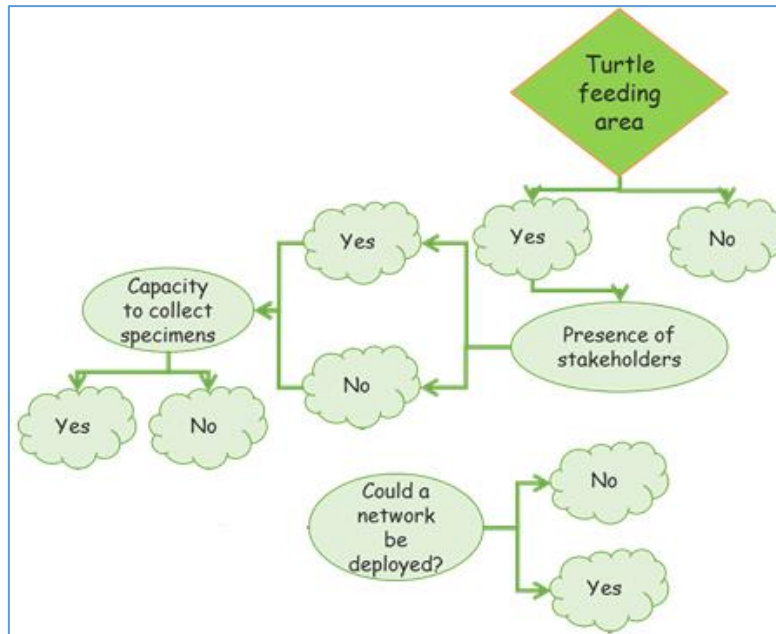


**Fig. III.1a.** Part of the decision tree with information on pollution pressure level in the selected area.

- *Entrance “Turtles”:* it concerns the sea turtles’ activity (e.g. feeding activity) and their population dynamics in the selected area and the capacity to collect information (e.g. ingestion/entanglement) in this area (e.g., presence of stakeholders and capacity to collect specimens (dead or live), with active stranding networks and rescue centres, capacity to perform laboratory analyses, human and



material means). Note that in case of absence of stakeholders or incapacity of collect turtle or to analyse ingested litter the possibility (e.g., network to be deployed, etc.) is provided on the right of the fig. III.1b. Indeed, the decision tree should help to reinforce the monitoring capacities by targeting the possible weaknesses locally.



**Fig. III.1b.** Part of the decision tree with information on sea turtles in the selected area.

- *Entrance “PoM”*: This third entrance should provide information on the implementation of PoMs that potentially affect the quantity/quality of litter that is ingested/causing entanglement (Fig. III.1c). The identification of PoM in each country has been provided in the D.2.7, and it has been updated for this final report and used for this part of the decision tree (Appendix 2).

The INDICIT protocol for collecting data on litter ingestion (more specific categories have been included for assessing impacts by entanglement, especially related to fishing and agriculture activities, See Activity 3) differentiate litter categories according to INDICIT protocol adapted from MSFD guidelines (Matiddi et al., 2019), as:

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

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

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

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

**USE POTH:** Any other plastic type of plastics, including elastics, dense rubber, balloon pieces, soft air gun bullets

**USE SHE:** The plastic bags are included in this category, which are regularly ingested by sea turtles. Soft packaging such as for candy or cake are observed ingested by sea turtles and are included in USE SHE. These specific items were not detailed in the MSFD nor INDICIT protocol. They may have been noted in column “Notes”

by the stakeholders in charge of data collection. The PoMs related to the “**Ban of plastic bags**” can be related to this category.

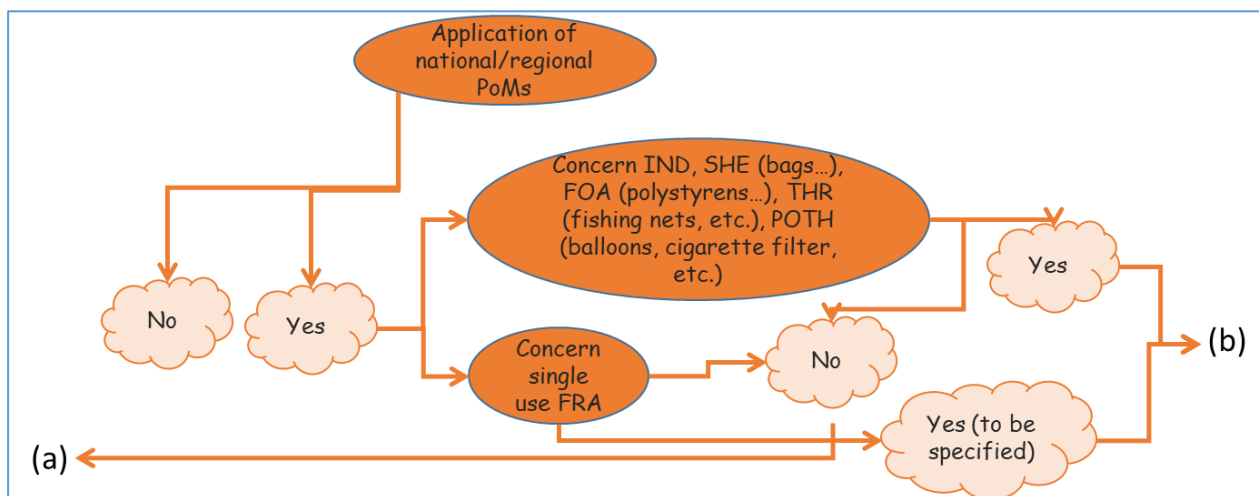
**USE FRA:** The PoM “**Ban of single use plastic**” is related to this category. Single use plastics have been included in this category, such as parts of cotton bud, plastic straws, fragments of plastic cups and corks, which are regularly ingested by sea turtles. These specific items were not detailed in the MSFD nor INDICIT protocol. They may have been noted in column “Notes” by the stakeholders in charge of data collection. Hard plastics (even not for a single use) are included in USE FRA. We noticed that ingested items are generally not specified by stakeholders (exempt sometime in the column “note”).

**USE FOA:** Items of this category are ingested by sea turtles (in low frequencies of occurrence). Polystyrenes are subject to specific measures. Spatial and temporal variations can be evaluated in order to assess risks related to polystyrene and future influence of PoMs targeting this category (e.g. synergy with CleanAtlantic which specifically focuses on this kind of pollution including cigarette butts).

**USE THR:** Items of this category are often fishing lines or mixed of fishing lines and nets (threadlike materials, e.g. pieces of nylon wire and net-fragments), and less frequently textile fibres. These items are ingested by sea turtles and involved in entanglement. The PoM allowing the reduction of this pollution (related to the **Ban of fishing gear deposition at sea**, facility to trash litter in harbour) is related to this category.

The efficiency of the PoMs will be evaluated by recording the relationships between litter ingestion and its availability in the environment according to the main categories (both in occurrence and quantity).

The “PoM” part of the decision tree is filled in (Fig. III.1c).



**Fig. III.1c.** Part of the decision tree with information on PoMs implemented in the selected area. (a) this arrow is connected with the “pollution level” part of the tree, (b) lead to the result of the decision tree (Pilot area with high or low pressure).

**Results:**

The decision tree methodology has been applied in France (Mediterranean facade and Corsica), Italy (Tyrrhenian Sea), Spain (Mediterranean facade and Balearic Islands), Spain (Canary Islands), Portugal (Azores), Tunisia (Gulf of Gabès), Greece (National Marine Park of Zakynthos) and Turkey (Dalyan area) (Appendix 3). An example is provided below (Fig. III.2) showing the selected area at Northern Corsica. In this area, the presence of a large coastal city (with high tourism activities) in this part of Corsica, the Levantine current, the proximity with Tuscany (also with high touristic frequentation) lead to a high pollution pressure as confirmed by empirical data (Darmon et al. 2017). The area is known as a feeding area for sea turtles (observations at sea from ferries and aerial

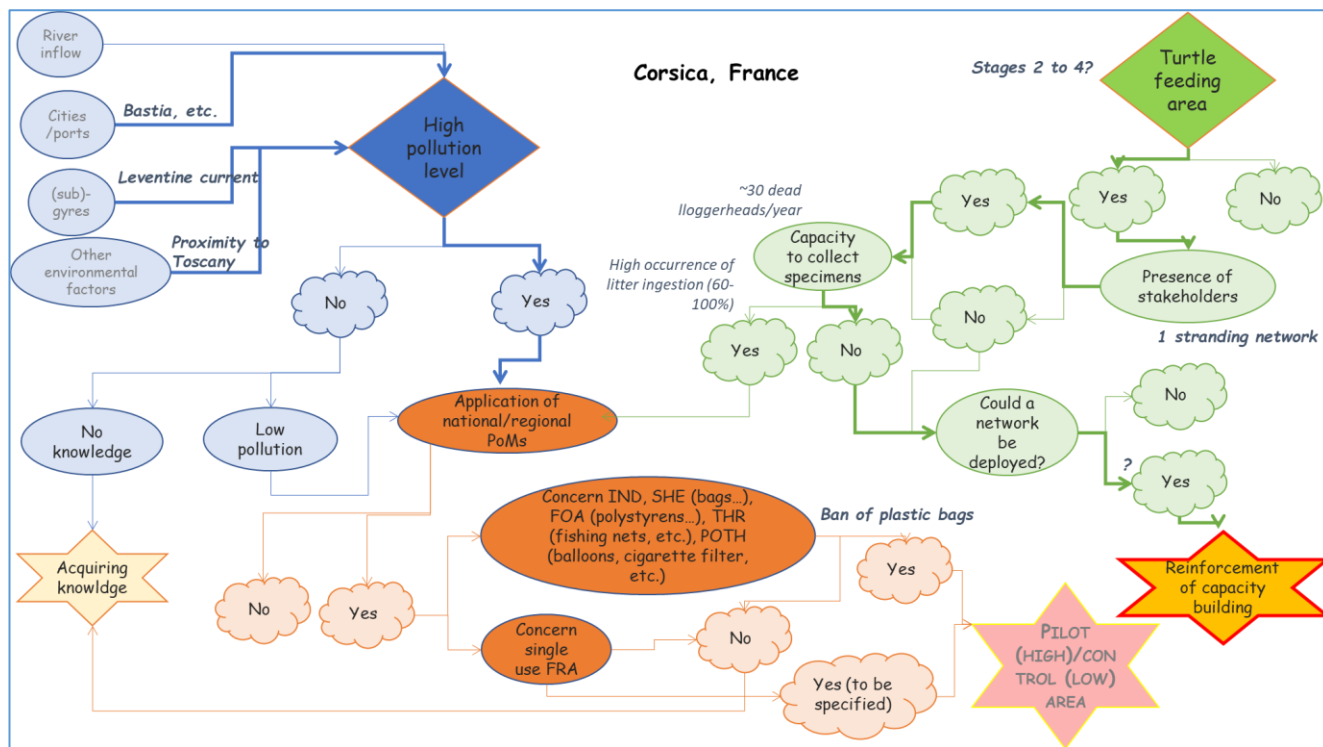
surveys (e.g. SAMM; ACCOBAMS). About 30 dead turtles can be analysed each year by the CARI stranded network NGO. A frequency of occurrence of litter ingestion reaches 100 % in juveniles and adults. While the network was mobilised for analysing digestive contents during the INDICIT I and II programs, the human and material means for collecting dead and live (rescue centre now in development) specimens in a sustained and sustainable manner need to be reinforced. The decision tree thus underlined the interest of the area as a pilot area but highlight the need of « Reinforcement of capacity building ».

The main outputs of this methodology applied in the 7 countries (Appendix 3) are provided in the table III.1.

(a)



(b)



**Fig. III.2.** An application of the decision tree to North-East Corsica. (a) the area (red area) concerns by this analysis. (b) the global analysis leading to the proposal “reinforcement of the capacity building”.

**Table III.1.** Selection of pilot area for the monitoring of the indicator “ingestion” and PoM.

area	Pollution level	turtle feeding area	presence of stakeholders	capacity to collect specimen	capacity to analyse samples	other	PoM	recommandation
Golf of Lion	high	yes	yes	yes			ban of plastic bag and single use plastic	Pilot area for high pollution
North Corsica	high	yes	yes	no		stranding network need development	ban of plastic bag and single use plastic	capacity building
Golf of Gabes	high	yes	yes	yes	yes		ban of plastic bag	Pilot area for high pollution
Azores	high	yes	yes	yes	yes	Large influence of oceanic currents i.e. litter coming form extra-european countries	ban of plastic bag and single use plastic	Pilot area for high pollution (see text)
MPA Zakynthos	high	yes	yes	no	yes	means for samples analysis present, but low capacity to collect specimens	ban of plastic bag	capacity building
Tyrrhenian sea	high	yes	yes	yes	yes		ban of plastic bag and single use plastic	Pilot area for high pollution
Continental facade	high	yes	yes	yes	yes		ban of plastic bag and single use plastic; navigation and fisheries litter detectionand removal; ghost nets identification and removal	Pilot area for high pollution
Balearic Islands	high	yes	yes	yes	yes	capacity of collecting and analysing turtles can be improved	ban of plastic bag and single use plastic; navigation and fisheries litter detectionand removal; ghost nets identification and removal	Pilot area for high pollution
Canary islands	high	yes	yes	yes	yes	Large influence of oceanic currents i.e. litter coming form extra-european countries	ban of plastic bag and single use plastic; navigation and fisheries litter detectionand removal; ghost nets identification and removal	Pilot area for high pollution (see text)
Dalyan area	low	yes	yes	yes	yes	capacity of collecting and analysing turtles can be improved	Plastic bag charged ain shops, ghost-net bins implemented at three ports	Pilot area for low pollution

The selection of the pilot area previously proposed (D2.7) led to the proposal of 3 areas in the Mediterranean Sea and 2 potential areas in the Atlantic (Fig III.3).

In addition, the results on litter ingestion by the loggerhead turtles collected during the INDICIT I and II projects, in relation with simulation models on litter monthly distribution (Mansui et al., 2020), showed that litter ingestion was minimum in the Eastern Mediterranean and maximum in the Western sub-basin, and intermediate in the central basin. In the Atlantic, although more data is needed for accurate analyses, the first results showed an increase of litter ingestion from North to South in the prospected areas (INDICIT ongoing publication).

Thanks to the newly performed analysis (Table III.1), we propose:

### **Pilot area for low-pressures**

- Turkish waters were proposed as pilot area as the frequency of occurrence of litter ingestion by sea turtles was the lowest observed in the studied areas of the Mediterranean Sea. This lower level is confirmed with the new results obtained during the INDICIT-II project (occurrence = 38%, table IV.2, p. 7), and simulation models. There is a clear variation of litter density between winter and summer season in Turkish waters, litter being concentrated by currents in the Western part of the Mediterranean in Summer. Adult sea turtles frequent these waters for the breeding season (presence of important nesting beaches in Turkey), when adults could reduce their feeding activity. These concomitant factors could explain the lowest percentage of litter ingestion observed in this area.

The decision tree (Appendix 3) confirms the presence of involved stakeholders with the capacity to collect specimens and analyse the data collected. This area was thus proposed as pilot areas with “low litter pressure”. The collection of further data in this area will enable to confirm the percentage and quantities of litter ingested by the sea turtles, which may vary locally according to spatial configuration and river inputs.

### **Pilot area for moderate and high -pressures:**

The Italian waters (frequency of occurrence of 62% in the Tyrrhenian, Ionian and Sardinian Seas), were considered as moderate pressure area along with the Tunisian waters (occurrence = 45% in the Gulf of Gabès, table IV.2, p. 7). As both capacities to collect specimen and analyse samples exist in these two countries, these two areas were proposed as pilot areas for “moderate-pressure”.

The French waters and Spanish waters presented the highest litter ingestion frequencies (respectively 78% in the Gulf of Lion and Corsica and 75% in the Spanish Mediterranean waters, including Balearic Islands). As both capacities to collect specimen and analyse samples exist (to be reinforced locally such as in certain parts of Corsica), these two areas were proposed as pilot areas for “high-pressure”.

### **Other cases:**

Greece waters (frequency of occurrence 64 % in INDICIT final report, now 66%, table IV.2, p. 7) was not previously proposed as pilot area (D2.7). Acquiring more data in this area will enable to confirm the area as low or moderate pressure and better understand litter impacts on sea turtles considering both litter and turtle distributions. The decision tree analysis highlighted the need of “Reinforcement of capacity building” in this area (Table I.4): a relevant number of stranded turtles is available each year for analysis, several stakeholders are present, but there is a need to reinforce and organise a stranding network to collect and store the specimens.

In the Canary Islands (Spain), oceanic juveniles are observed and the area is a feeding region. The frequency of occurrence is high (occurrence = 100%, table IV.2, p. 7), and the presence of NGOs (8 stranding networks - 1 per island-, 2 rescue centres and 1 research lab) allows data collection. However, the local density of litter is highly influenced by important oceanic currents. Litter can come from non-European countries (USA, Caribbean, Africa), transported by currents such as the Gulf stream and the Canary current). A large amount of data is

therefore required for an accurate assessment and interpretation allowing the correlation of the pressure observed (e.g., ingested litter or litter causing entanglement) with the European, national or regional PoMs.

- In Azores, the frequency of occurrence was high (occurrence = 82%, table IV.2, p. 7), but the number of available sea turtles for analysis is low each year. It is also difficult to connect these occurrences with the litter in the environment (and PoM) as this litter may come from both the western north Atlantic currents. The simulations performed in the framework of the CleanAtlantic project showed that a significant flux of litter is also coming from the South Western Europe. More accurate analyses will be further possible thanks to the current active engagement of stakeholders (stranding networks, rescue centre) in the Azores.

In conclusion, this study confirms the gradient of pressures (minimum in the Eastern Mediterranean, intermediate in the central basin and maximum in the Western sub-basin). This gradient from low to high pressure was considered for performing ecotoxicological analyses (i.e. choice of location for sampling) and evaluating the relationships between litter ingestion and toxins in the tissues (see I.V.7, p. 17).



**Fig. III.3.** Consolidation of pilot areas proposed at the end of the INDICIT-II project. (1) France (Gulf of Lion), (2) France (Corsica), (3) Tunisia (Gulf of Gabes), (4) Portugal (Azores), (5) Greece (MPA Zakynthos), (6) Italy (Tyrrhenian sea), (7) Spain (continental façade), (8) Spain (Balearic Islands) and (9) Spain (Canary Islands). Blue area = “high-moderate pressure” area, red area = “low-pressure” area; Green area: pressure related to litter of extra-European origin; yellow arrow: area where capacity building is needed (see table I.4).

## III.2. Validating the pilot areas for indicator “entanglement in sea turtles”

A similar methodology was applied for the indicator “Entanglement”, considering sea turtles as target taxa (because of the presence of existing networks). The proposed pilot areas are compatible for evaluating both pressures by ingestion and entanglement. However, the feedbacks from INDICIT II partners and the new data collected during INDICIT-II following the feasibility study performed during the INDICIT project, show that the quantity of data collected each year (see V, p. 64) is strongly lower than for the indicator “ingestion” (Table III.2).

**Table III.2.** Summary of the evaluation for proposing pilot areas for the indicator “entanglement” in sea turtles.

Country	Area	Pressure (floating litter from fishing/aquaculture activities)	Availability of data on entanglement	Recommendation
France	Gulf of Lion	low, to be evaluated	high number of turtle available	Continue the recording of data (including social media)
France	North Corsica	low, to be evaluated	high number of turtle available	Continue the recording of data (including social media)
Tunisia	Gulf of Gabès	high	high number of turtle available	Continue the recording of data (including social media)
Portugal	Azores (Sao Miguel)	low, to be evaluated	Low number of turtle available	Continue the recording of data (including social media)
Greece		to be evaluated	high number of turtle available	Continue the recording of data (including social media)
Cyprus		to be evaluated	high number of turtle available	Continue the recording of data (including social media)
Spain	8 Islands in Canarias Islands	high	high number of turtle available	Potential pilot area for this indicator

Globally, data collected from different regions are very diverse, with important differences in time periods included, where most accurate data were collected from 2017 to date.

Looking by regions / countries:

### France – Atlantic:

- Standard data on entanglement have not yet been collected.

### Portugal (Azores):

- Few records of loggerhead turtle per year, even if entanglement impact is high in this area.
- Area influenced by important currents (Gulf Stream, Labrador current), bringing litter from non-European countries (difficulties to quantify the effect of European POMs).

### Spain – Atlantic (Canary Islands):

- Very detailed analysis of entanglement of sea turtles has been conducted (historical and current data).
- High impact of entanglement in the area (entanglement hot spot).



- Area influenced by important currents (Canary current connected with Gulf Stream and Labrador current), bringing litter from non-European countries (difficulties to monitor European POMs).
- Important influence of the proximity of western Africa coast (Morocco) (difficulties to quantify the effect of European POMs).

**Spain - Mediterranean:**

- Detailed analysis of entanglement has been conducted (historical and current).
- Important impact of entanglement in the area.
- Influence of bycatch in the area, where many loggerhead turtles are collected by fishermen in their fishing gears.

**France – Mediterranean:**

- Few standard data on entanglement were collected.
- Great influence of bycatch in the area, where most part of the loggerhead turtles are collected by fishermen in their fishing gears.

**Italy:**

- Enough and accurate data to consider pilot areas in the region.

**Greece:**

- Standard data on entanglement have not yet been collected.
- Systematic data collection on entanglement by stakeholders is not yet performed.

**Turkey:**

- Standard data on entanglement have not yet been collected.

**Cyprus:**

- Few data on loggerhead turtle were recorded.
- Important impact of entanglement of the green turtle. This region could be proposed as pilot area to monitor litter on neritic ecosystems, through entanglement on green turtle.

**Tunisia:**

- Standard data have not yet been collected.

The selection of pilot areas makes it possible to better assess the relationship between litter ingestion and entanglement and litter abundance in the environment, in connection with the PoMs. It also enables to identify the areas where means and actions should be implemented/improved for the acquisition of data and more detailed assessments, as in mainland Atlantic Spain and Portugal, and in the South Mediterranean countries. , A more specific analysis would be needed in Turkey, Greece and Cyprus where the islands and spatial configuration can influence the local litter abundance and pressures. This does not preclude continuing analyses at all sites and collecting specimens in an opportunistic manner.

## IV. Indicator litter ingested by sea turtle

### IV.1. Strengthening the implementation of the indicator

#### IV.1.1. Supporting the network of stakeholders in charge of data collection

Following the high mobilisation of the stakeholders during the INDICIT project, the new INDICIT-II project has allowed to pursue the strengthening of the networks in charge of collecting specimens and data on litter ingestion. The global covid-19 pandemic has made difficult to travel and to meet stakeholders. No training session could be given except locally. Short training sessions could be given by email (dissemination of protocols) or online conferences, especially to provide answers to specific requests related to the conduct of dissection of dead animals or the identification of items ingested by turtles, for examples. The open-access peer-reviewed publication of the INDICIT protocol in the JOVE Journal (Matiddi et al., 2019), in which it can be automatically translated into 17 languages, has facilitated considerably the dissemination of the protocol. A video-tutorial is joined with the publication, providing a visual support for readers. Another video on the necropsy procedure, in French language, already available, was disseminated to stakeholders using French language.

The google map providing the 120 stakeholders' locations and contact details (some of them being also included in the monitoring of entanglement and micro-plastic ingestion indicators) has been further developed and could be completed in the future (Figure IV.1). This map aims easily targeting the contact institution for collecting live or dead turtle or for recording data, it also aims to identify areas where the monitoring should be improved (targeting, training, reinforcing the means of existing or new stakeholders). The list of stakeholders includes 28 stranding networks, 34 rescue centres, 11 national institutions, 14 veterinary clinics and laboratories, 26 research institutions and 7 "Observers" (e.g. NGOs in charge of other taxa than sea turtles). Table IV.1 details the implementation of these stakeholders in the respective countries.

**Table IV.1.** Numbers of stakeholders involved in the monitoring of the indicator "ingestion". Note that some stranding networks can include rescue centres.

	stranding network	Rescue centre	Veterinary clinic	Research institution	National institution
Portugal (Azores)	1		1	1	1
Portugal (mainland)	1	1			1
Spain (Canaria Islands)	5				1
Spain (Atlantic)	2				
Spain (Balaric islands)	2				
Spain (Med)	4	5	1	2	1
France (Atlantic)	4	1		1	2
France (Med)	1	2	1	2	1
France (Corsica)	1			1	
Italy	1	1	3	5	1
Italy (Sardinia)	1	7	2	2	1
Greece	4	1		3	
Turkey	4	2	4	4	1
Cyprus	1			1	
Tunisia	3	3	1	1	



**Figure IV.1:** Google map with restricted access providing the stakeholders' locations and contact details, with part of the legends. This map aims to be completed by stakeholders in charge of specimen or data collection, or by representatives, in the future (<https://www.google.com/maps/d/viewer?mid=1uGPARrPl6wEsqmfx8nSXclevck&ll=40.33355333017604%2C3.7828229999999863&z=4>).

#### IV.1.2. Protocols and data recording

The standard protocol developed during the INDICIT project has been widely distributed and used, and the collaborations have been pursued for collecting and recording data (see section III.3).

In partnership with the INDICIT II consortium and stakeholders in charge of data collection, EPHE cleaned and homogenised the necropsy data in order to compile them in the INDICIT's Excel template. The dataset from necropsies has been harmonised for considering some possible discrepancies among stakeholders in charge of data collection. The total masses and abundances have been validated after careful verification of the values recorded per litter/food type and digestive sections. The units (mass, volume and abundances of ingested litter, par category of items, size and colour, carapace lengths (cm) and body weights (g)) have been harmonized. The individual growth stages have been evaluated using the standard curved carapace length. The decomposition status, circumstances of discovery, health proxy parameters (fat reserves, plastron shapes, injuries) have also been standardized. The INDICIT II consortium recommends to train dedicated people for data cleaning and recording in the sharing platform.

A collaboration has been made with IFREMER (CleanAtlantic project, see Activity 5) for pursuing the development of the DALI platform, which will now be used by stakeholders of the OSPAR RSC area.

Considering stakeholders' feedback, results from data cleaning and analyses (see section III.4), the INDICIT II consortium recommends to provide some slight modifications to the protocol. As proposed during INDICIT

project, it is suggested that dedicated teams, professionalised for data collection, could intervene in support to local stakeholders.

The mass and volume of the content of the separate digestive sections, which aims to evaluate the individuals' digestive capacity, were recorded separately. Several other parameters could be collected in the framework of research studies. However, recording these parameters appears to be highly time-consuming for a systematic monitoring of litter ingestion and impacts on sea turtles especially for stakeholders not dedicated to monitor litter ingestion by sea turtles. The interest for the systematic collection of these data is questioned:

- As it is now recognised that most litter is found in the intestines (particularly in *Caretta caretta*) and for simplifying the dissection procedures, we propose to suppress the recording of data by digestive sections. Data "ingested litter" can be collected for the entire digestive tract.
- The volume of ingested plastics seems to be difficult to be evaluated because e.g. light plastics, which composed most of the ingested litter in loggerhead turtles, are floating materials. This parameter can be collected by researchers and optionally by stakeholders for evaluating the ratio of volume of ingested litter on volume of digestive tract.
- Some parameters should be harmonized, with encouragement to collect similar measures, such as the standard carapace length, in priority to the minimum and maximum lengths (even if the standard length can be evaluated *posteriori* with the minimum or maximum lengths).
- Stakeholders should be encouraged to harmonised the way "Health" parameters are collected (injuries, fat reserves, plastron shape), and other parameters which could be collected after discussing with biologist in the field, veterinarians, researchers and managers. Specific workshops should be organised on this important topic.
- We encourage stakeholders to note the type of plastic items they find, in particular for the hard fragments (USE FRAG category), in order to better make the relationships with PoMs related to the ban of single-use plastics. The category FRAG could be more detailed in the future, trying to refer to items to the new MSFD joint list.

The stranding networks, rescue centres, veterinary clinics and research laboratory were mobilised to collect different tissue samples (dead turtles) and faeces (live turtles) for the ecotoxicological analysis. A specific protocol has been built in collaboration with Plastic Busters MPA in the framework of Activity 5. The objective of this protocol is to make the link between litter ingestion and toxins in the tissues of living and dead turtles (See section IV.7).

## IV.2. Quantification and characterization of the ingested litter

Necropsies and litter extraction from the 3 separated digestive sections (oesophagus, stomach, intestines) were performed following Matiddi et al. (2019). The presence, the dry mass and the abundance of ingested litter and of plastics specifically were the elementary parameters to note, and other parameters, especially related to body condition were proposed as optional. All litter fragments were then grouped into categories simplified from MSFD guidance (Galgani et al., 2013), differentiating plastics from industry (thereafter noted "IND PLA") or from users' origin as follow: sheet like (USE SHE), hard fragments (USE FRAG), threadlike (USE THR), foams (USE FOA) and other (USE POTH). Litter other than plastics (OTHER), natural food (FOO) and natural no food items (NFO) were also noted. They were dry weighted (precision 0.01 g) and numbered per category (except FOO and NFO which could not be numbered), then differentiated into three colour classes (dark, light, white/transparent) and optionally three size classes (micro-litter from 1 to 5 mm, meso (5 mm to 25 mm) and macro-litter (from 25 mm)). Contains which would have a weigh below the minimum precision would be attributed to 0.001 g.

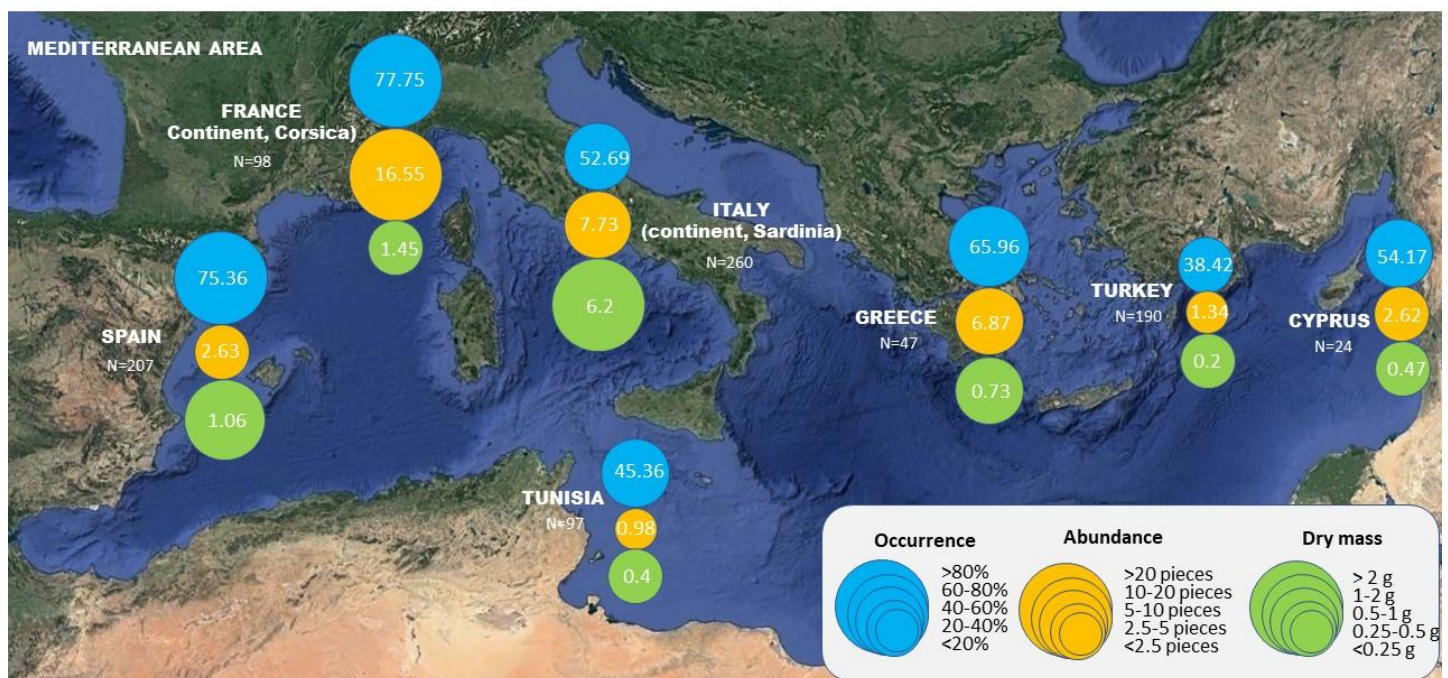
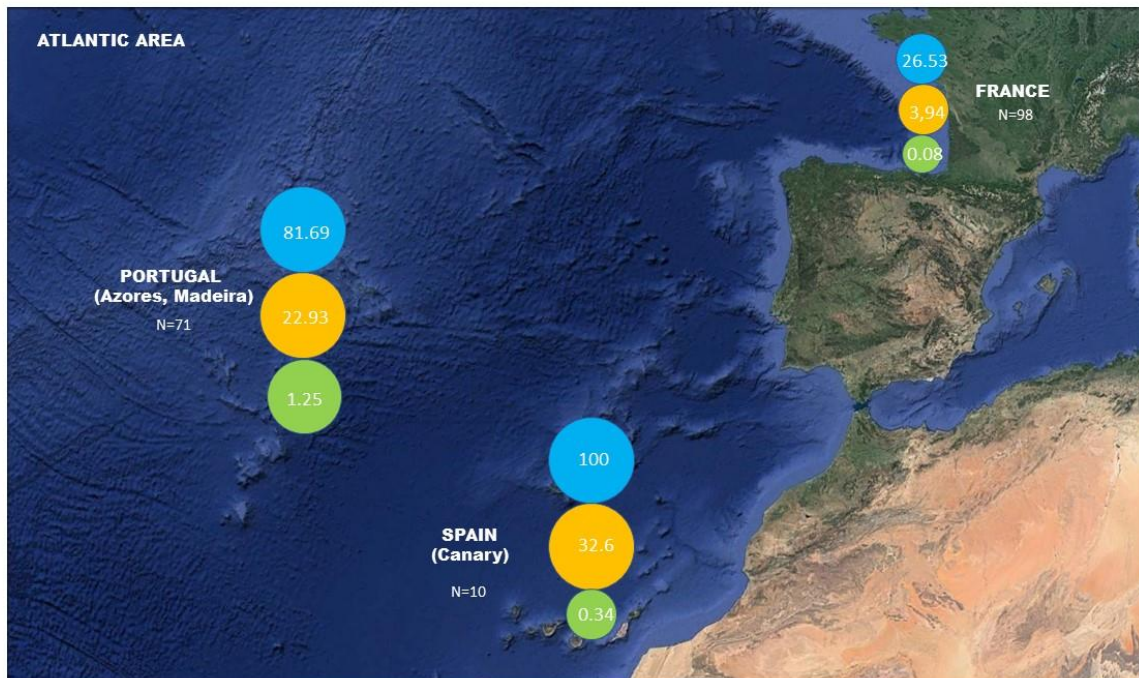
The mummified individuals (conservation status 5) were removed because of the too severe autolysis of the digestive tract that prevented an accurate evaluation of the ingested material and of the body condition. We calculated the occurrence of litter and specifically plastics ingestion as the frequency of litter (all categories except FOO and NFO) found in the digestive tract among all collected individuals. The population mean for

abundance and dry mass of ingested litter, with standard errors, considers all individuals including those with an empty digestive tract or without ingested litter. We also described the ingested material by differentiating the mean mass and the mean abundance per litter category. As proposed during INDICIT project, for better discussing the possible impacts of litter ingestion on individual's capacity to feed, we also calculated the ratio of the dry mass of natural food (category FOO) on the dry mass of litter.

**Among the 1103 individuals collected since 1988 with the 110 collaborator institutions, 69.24% were found to ingest litter, and 56.62 % when considering plastics only. Among the 802 individuals collected since 2013 (date of the publication of MSFD guidance with procedures to extract litter in turtles' digestive tracts), these percentages increased respectively to 73.46% and 58.99% (fig. IV.2).**

The individuals ingested an average of  $31.56 \pm 4.61$  g of both synthetic and natural materials. The natural food (FOO) constituted  $42.38 \pm 1.37$  % ( $22.64 \pm 4$  g on average among individuals) of the total mass of ingested material ( $59.66 \pm 1.32$ % ( $1.75 \pm 0.41$  g) along with the natural no food items (NFO). Plastics ( $1.94 \pm 1.26$  g on average; max=1400.25 g) accounted for  $38.77 \pm 1.3$  % of the mass of all ingested material and  $93.89 \pm 0.64$ % of the ingested litter. In terms of abundance, the litter represented  $6.67 \pm 0.55$  pieces on average, with plastics corresponding to  $95.52 \pm 0.51$  % of them ( $6.27 \pm 0.53$  pieces; max=200, fig. IV.2). Adjusted to the individuals' mass and size (standardized standard carapace length), plastics represented  $0.1 \pm 0.033$  g/kg turtle (N=454) and  $1.6 \pm 0.38$  pieces/kg of turtle (N=473) or  $0.16 \pm 0.013$  pieces/cm (N=1003).

USE SHE, USE FRAG and USE THR were the categories the most regularly found ingested in terms of both dry mass and abundance (Table IV.2). The ingested debris items were generally soft and hard plastics, such as fragments of food packaging, bags, cups, caps, cotton buds, lollipop sticks, balloons, finger rinse wipes, sanitary napkins or filters from waste treatment plants for example. The threadlike litter were generally items from fishing activities, especially fragments of lines and nets. Micro-plastics (1-5 mm) counted for an average of  $0.77 \pm 0.12$  pieces (N=682; 26% of the total number of ingested plastics). The majority of plastic pieces was white or transparent ( $3.42 \pm 0.34$  pieces), dark ( $1.09 \pm 0.12$  pieces) and more rarely light coloured ( $1.01 \pm 0.11$  pieces, Fig. IV.3).



**Figure IV.2.** Occurrence (number of turtles with ingested litter/total turtles studied), abundance of ingested litter and mass (dry mass) of ingested litter in the Atlantic area (above) and Mediterranean Sea (below). N = 802 individuals collected since 2013 (from Darmon et al. submitted).



**Figure IV.3:** Examples of litter items found ingested by the loggerhead turtles. Example in Corsica, France, from excretion by live turtles in Accupulata centre, A) Balloon with wire excreted, B) soft and hard fragments, including media filters from waste water treatment, C) water bottle packaging, E) diverse packaging and soft items, and D) examples being extracted from digestive tract.

Plastic ingestion did not vary significantly over years since 1988 when considering both mass ( $R^2=10^{-4}$ ;  $p=0.71$ ) and abundance ( $R^2=3 \times 10^{-5}$ ;  $p=0.86$ ), and the relationship although significant, was almost null with occurrence ( $R^2=0.007$ ;  $p=0.004$ ). The occurrence of plastic ingestion did not differ between the Atlantic and the Mediterranean (68.11% and 58.99% after 2013;  $t=1.53$ ;  $p=0.14$ , Figure III.6, Tables III.5 and III.6) as well as the dry mass ( $0.25 \pm 0.09$  g and  $3.15 \pm 1.83$  after 2013;  $t=-1.31$ ;  $p=0.19$ ;  $0.25 \pm 0.09$  g and  $3.15 \pm 1.83$  after 2013;  $t=-1.31$ ;  $p=0.19$ ), but the abundance of ingested litter appeared significantly higher in the Atlantic compared to the Mediterranean area ( $t = 2.45$ ;  $p = 0.01$ ;  $2.62 \pm 0.52$  pieces and  $8.06 \pm 0.61$  pieces after 2013;  $t = -5.56$ ;  $p < 0.01$ ).

**Table IV.2.** Sample and litter ingestion in the loggerheads necropsied between 1988 and 2019, per country and area. N: Sample size (number of individuals; starting date in brackets); mean standard curve carapace length; occurrence of plastic ingestion (percentage of turtles found with ingested litter) and population means of the dry mass of ingested plastics, the abundance, the mass per cm of standard curved carapace length (StCCL), the abundance per cm of standard curved carapace length ( $\pm$  standard error) (*adapted from Darmon et al, publication in progress*).

	Atlantic			Mediterranean						
	France	Portugal (Azores, Madeira)	Spain (Canaries)	Cyprus	France	Greece	Italy	Spain	Tunisia	Turkey
<b>N (date)</b>	98 (1988)	71 (1996)	10 (2016)	24 (2019)	98 (2007)	47 (2017)	260 (2008)	207 (1995)	97 (2004)	190 (2016)
<b>Standardized StCCL (cm)</b>	37.94 $\pm$ 1.34			56.85 $\pm$ 0.47						
	31.31 $\pm$ 1.49	41.32 $\pm$ 2.2	51.79 $\pm$ 5.22	62.53 $\pm$ 3.28	49.82 $\pm$ 1.45	58.28 $\pm$ 2.48	56.4 $\pm$ 0.83	49.8 $\pm$ 0.96	59.2 $\pm$ 1.1	66.76 $\pm$ 0.72
<b>Occurrence (%)</b>	52.51%			57.42%						
	26.53	81.69	100	54.17	77.75	65.96	52.6 9	75.3 6	45.36	38.42
<b>Dry mass (g)</b>	0.57 $\pm$ 0.1 g			2.44 $\pm$ 1.56 g						
	0.08 $\pm$ 0.04	1.25 $\pm$ 0.23	0.34 $\pm$ 0.13	0.47 $\pm$ 0.26	1.45 $\pm$ 0.25	0.73 $\pm$ 0.42	6.2 $\pm$ 5.38	1.06 $\pm$ 0.19	0.4 $\pm$ 0.24	0.2 $\pm$ 0.14
<b>Abundance (pieces)</b>	11.72 $\pm$ 1.89 pieces			6.76 $\pm$ 0.54 pieces						
	3.94 $\pm$ 1.75	22.93 $\pm$ 4	32.6 $\pm$ 6.11	2.62 $\pm$ 0.87	16.55 $\pm$ 2.74	6.87 $\pm$ 3.01	7.73 $\pm$ 1.27	2.63 $\pm$ 0.45	0.98 $\pm$ 0.4	1.34 $\pm$ 0.2
<b>Dry mass/StCCL (g/cm)</b>										
	0.003 $\pm$ 0.001	0.03 $\pm$ 0.004	0.008 $\pm$ 0.003	0.007 $\pm$ 0.003	0.02 $\pm$ 0.004	0.02 $\pm$ 0.01	0.09 $\pm$ 0.08	0.02 $\pm$ 0.003	0.007 $\pm$ 0.004	0.003 $\pm$ 0.002
<b>Abundance/StCCL (pieces/cm)</b>										
	0.2 $\pm$ 0.07	0.56 $\pm$ 0.11	0.15 $\pm$ 0.74	0.07 $\pm$ 0.04	0.31 $\pm$ 0.05	0.21 $\pm$ 0.11	0.13 $\pm$ 0.02	0.05 $\pm$ 0.01	0.02 $\pm$ 0.01	0.03 $\pm$ 0.007



**Table IV.3.** Dry mass (grams) and abundance (number of pieces) of ingested material (litter and natural food and no food items), litter, plastics only and according to litter categories (population means  $\pm$  standard errors). The percentages are calculated according to the total ingested litter. Total abundance for all materials is not calculated since it could not be evaluated for food (FOO) and natural no food items (NFO) (*adapted from Darmon et al, publication in progress*).

Category	Ingested material	Ingested litter	Ingested plastics	IND	USE SHE	USE THR	USE FOA	USE FRAG	USE POTH	No plastics
Dry mass	26.52 $\pm$ 4.23 g	2.02 $\pm$ 41.27 g	1.95 $\pm$ 1.27 g	3.51 $\pm$ 0.53% (0.03 $\pm$ 0.015 g)	38.63 $\pm$ 1.17 % (0.21 $\pm$ 0.03 g)	17.75 $\pm$ 1% (0.12 $\pm$ 0.03 g)	4.97 $\pm$ 0.53 % (0.04 $\pm$ 0.009 g)	24.06 $\pm$ 1.03% (1.5 $\pm$ 1.26 g)	4.97 $\pm$ 0.53% (0.03 $\pm$ 0.007 g)	6.1 $\pm$ 0.64% (0.07 $\pm$ 0.02 g)
Abundance	-	6.7 $\pm$ 0.56	6.3 $\pm$ 0.53	3.51 $\pm$ 0.53%; 0.13 $\pm$ 0.03 pieces	45.75 $\pm$ 1.15%; 3.34 $\pm$ 0.32 pieces	22.64 $\pm$ 1.05 %; 1.19 $\pm$ 0.19 pieces	3.71 $\pm$ 0.4%; 0.18 $\pm$ 0.03	16.86 $\pm$ 0.83%; 1.27 $\pm$ 0.17 pieces	3.43 $\pm$ 0.41 %; 0.16 $\pm$ 0.03 pieces	4.48 $\pm$ 0.51%; 0.4 $\pm$ 0.11 pieces

### IV.3 Ingested litter and individuals' health

One of the major challenges in the development of the indicator is to establish the link between litter ingestion (occurrence and quantities) and health in order to meet the definition of GES for Criteria D10C3 which targets "no impact caused by litter on the health of the species concerned" (Commission Decision 2017/848/EC). How health could be defined depends on the objective, and the parameters to measure may differ at the individual or population level or that of the "species" as stated in the official Criteria definition. The link between individual biology and population dynamics, and by extension, the species viability, depends on parameters related to survival and reproduction.

The indicator mainly depends on data collected on dead individuals, on which we have prioritised our analyses although we have also collected data on living individuals, which are currently being analysed. We collected very few living individuals who subsequently died, on which we could compare body condition analyses, including behaviour and blood chemistry which are no longer possible on dead individuals. We therefore collected as much information as possible at the individual level, with biometric measurements, visual evaluations of injuries that could have prevented normal eating behaviour (subjective injury severity: from severely (amputation, fracture, sectioning of carapace), moderately (carapace cut) and not or slightly injured (abrasion of the carapace, delamination of part of the scales, scar)), or of fat reserves and plastron shape (Thomson et al., 2009) that could have cause-and-consequence relationship on litter ingestion. The causes of morbidity and mortality were evaluated, with a complete description of the external and internal injuries and lesions. In partnership with the School of Agrifood and Forestry Science and Engineering, University of Lleida (Spain), EPHE carried out a literature review (from biology, ecology, veterinary disciplines) on the parameters used to assess living and dead individual's health and body condition in different animal taxa (mammals, birds, reptiles) in order to possibly be inspired. To do this, we tested different body condition indices (Peig and Green, 2010) and tested those more commonly used in the literature on sea turtles, more regularly the green species (Fulton's K index ( $\text{kg}\cdot\text{cm}^{-3}$ ), ratio of body mass (g) on cubic standardised curved carapace length ( $\text{cm}^3$ ), expected to be lower when the reserve accumulation decreases (Bjorndal et al., 2000; Nash et al., 2006). All these parameters were tested in relationship with litter ingestion and we considered the possible confounding factors that may influence the probability of litter ingestion, either related to litter availability in the environment and sea turtles' distribution and energetic requirement: spatial variations, seasons, growth stages, observation pressure, circumstances of

discovery. For doing such analyses, we first harmonised the data among stakeholders and performed linear modelling in order to standardize the carapace lengths into standard curved carapace length. Finally, we also tested the ratio of litter and food remains, with the hypothesis that individuals should ingest more natural food than litter if 1) they are in good health and 2) food is more available than litter and individuals are able to select for food.

#### IV.4 Factors acting on litter ingestion

For evaluating the factors which may contribute to litter ingestion, we used permutational linear models, robust against non-normal distributions of residuals and outlier values. All the analyses were performed in collaboration with Marcus Schulz (AquaEcology, Germany). We built different types of models, with the occurrence of litter ingestion as response variable (noted as presence (1) /absence (0) for an individual) considering a Binomial error distribution. Other response variables were also modelled using a Normal distribution error: the dry mass of plastics, the abundance of plastics items and the ratio of plastic mass on StCCL ( $\text{g.cm}^{-1}$ ) as well as on body mass ( $\text{g.kg}^{-1}$ ). Rather than a complete model which could be less statistically powerful and difficult to interpret because of all possible interactions or addition of factors, we considered simpler models for exploring three scenarios:

1) Influence of the spatial and temporal variability of resource (country/area (Cyprus, France Atlantic, France Mediterranean, Greece, Italy, Portugal, Spain Atlantic, Spain Mediterranean, Tunisia, Turkey) in interaction with season (winter, from January to March; spring, from April to June; summer, from July to September; autumn, from October to December));

2) energetic requirements which vary among sexes, growth stages (body weight or standardised curved carapace length) and seasons;

3) Individuals' body condition evaluated either by fat reserves, plastron shape, biometric measures (standardized curved carapace length or body weight), Fulton's index or severity of lesions. We also considered the ratio FOO/plastics as response variable, by removing the turtles with an empty digestive tract (with neither litter nor food) because of a possible confusion with individuals who could be chronically ill (Casale et al., 2016).

We tested all the models with the entire dataset (from 1988) and with data collected after 2013, presuming that litter ingestion was not systematically evaluated before the publication of MSFD guidance (Galgani et al., 2013). The best model was selected using the lowest Akaike Information Criterion (AIC), two models with a difference of AIC less than 4 being considered as equivalent, the more parsimonious being selected. Analyses were performed with the R software version 4.0.1 (R Core Team, 2020), with the library "lmPerm" (Wheeler and Torchiano, 2016).

#### IV.5 Analysis and results

All models, whatever the data period, appeared either insignificant or showed significant relationships but with determination coefficients ( $R^2$ ) almost null regardless of the response variables (Table IV.4). Some factors explained a slight part of the observed variability, but depending on the response variable: Spatial (country / area) and seasonal variables on occurrence and abundance, energetic requirements, taken into consideration with sex, growth stage, body weight or StCCL on occurrence, mass and abundance of ingested plastics, and for body condition, only the Fulton index contributed to explain a very slight part of the abundance of plastics. Similar results were obtained with data from 2013. Only the spatial factor (Country/Area) appeared significant when considering only the individuals collected during summer (N=522).

**Table IV.4.** Results of the selected models built from our hypotheses on the factors which may influence litter ingestion among loggerhead turtles in the study area. For each hypothesis, all tested models came from a full (complete) model, then selected based on AIC criterion and the parsimony principle. Only the selected model is presented, along with its correlation coefficient ( $R^2$ ) and p-value. Significant  $R^2$  (p-value < 0.05) appear in bold and response variable with an asterisk (From Darmon et al., *in progress*).

Hypothesis	Full model	Response variable	Selected model	Adjusted $R^2$	p-value
1. Plastic ingestion is influenced by spatial and temporal variations in resources	<i>Country/area x Season</i>	Occurrence*	Country/Area + Season	<b>0.15</b>	<<0.001
		Mass	Season	0.005	0.06
		Abundance*	Country/Area + Season	<b>0.16</b>	<<0.001
		Plastic mass/StCCL	Season	0.002	0.22
		Plastic Nb/StCCL*	Country/Area + Season	0.12	< 2.2e-16
		Plastic Nb/Body mass*	Country/Area	<b>0.03</b>	0.001
		Plastic mass/FOO mass	Season	-0.008	0.6
2. Plastic ingestion is influenced by variations in energetic needs among individuals	<i>Sex x Growth stage x Season</i>	Occurrence*	Sex + Stage x Season	<b>0.04</b>	0.002
		Mass*	Sex x Stage + Season	<b>0.03</b>	0.002
		Abundance*	Sex x Stage + Season	<b>0.05</b>	<<0.001
		Plastic mass/Body mass	Sex	0.001	0.29
		Plastic Nb/ Body mass	1	-	-
		Plastic mass/FOO mass	Sex + Season	-0.03	0.73
	<i>Sex x Body weight x season</i>	Occurrence*	Sex x Weight+ Season	<b>0.03</b>	0.02
		Mass*	Sex x Weight+ Season	<b>0.06</b>	<<0.001
		Abundance*	Sex x Weight+ Season	<b>0.04</b>	0.001
		Plastic mass/StCCL	Season	0.002	0.22
		Plastic Nb/StCCL	Sex + Weight + Season	0.02	0.07
		Plastic mass/FOO mass	Sex + Weight	-0.06	0.91
	<i>Sex x StCCL x season</i>	Occurrence*	Sex + StCCL x Season	<b>0.04</b>	0.001
		Mass	Sex + StCCL + Season	$-6.92 \times 10^{-4}$	0.46
		Abundance*	Sex + StCCL * Season	<b>0.08</b>	<<0.001
		Plastic mass/Weight	Sex	0.001	0.29

		Plastic Nb/Weight	1	-	-
		Plastic mass/FOO mass	Sex + StCCL	-0.01	0.57
3. Plastic ingestion is influenced by individuals' body condition	<i>Fat reserves</i>	Occurrence	Fat reserves	-0.009	0.8
		Mass	Fat reserves	0.009	0.16
		Abundance	Fat reserves	-0.009	0.83
		Plastic mass/StCCL	Fat reserves	0.001	0.34
		Plastic mass/Weight	1	-	-
		Plastic nb/StCCL	Fat reserves	-0.008	0.74
		Plastic nb/Weight	1	-	-
		Plastic mass/FOO mass	Fat reserves	0.65	-0.04
	<i>Plastron shape</i>	Occurrence	Health status	-0.003	0.61
		Mass	Health status	-0.002	0.49
		Abundance	Health status	-0.009	0.83
	<i>StCCL</i>	Occurrence	StCCL	0.002	0.06
		Mass	StCCL	$1.27 \times 10^{-4}$	0.29
		Abundance	StCCL	$4.94 \times 10^{-4}$	0.22
	<i>Body weight</i>	Occurrence	Body mass	$3.18 \times 10^{-4}$	0.36
		Mass	Body mass	-0.001	0.52
		Abundance	Body mass	0.001	0.19
	<i>Fulton's index</i>	Occurrence	Fulton's index	$8.91 \times 10^{-4}$	0.43
		Mass	Fulton's index	-0.002	0.79
		Abundance*	Fulton's index	<b>0.02</b>	0.002

The cause of death appears generally difficult to determine. Most of deaths were probably caused by an interaction with human activities (e.g. bycatch, 27.94%, N=273) and anthropogenic trauma such as propellers (5.42%, N=53), and entanglement in litter (2.25%, N=22), but death was attributed to litter ingestion due to digestive occlusion or perforation, for 14 out of 977 recorded cases (1.43%). Natural causes and trauma (e.g. shark attack) counted for (2.45 %, N=24).

It has been suggested that stranded individuals would be removed from the data used for an indicator of ingestion (Casale et al. 2016) because ingestion could be one cause of death and potentially biases the sample used to calculate the indicator. Our results showed that stranded and bycaught turtles have overall a similar body condition (from Fulton index, fat reserves and plastron shape) and bycaught turtles ingested more plastics than stranded turtles (69.48% and 47.6% respectively;  $p < 0.001$ ) with a greater abundance of items (12.95 and 5.81 pieces;  $p < 0.01$ ) while the difference in dry mass was not significant (0.9 and 3.29 g;  $p = 0.36$ ). We thus considered that using stranded individuals do not bias the evaluation of the indicator.

While we were not able to prove direct relationship with body condition, our results regarding the ratio food/litter appear highly alarming according to models published in the scientific literature. Among individuals for which the presence of food in the digestive content was recorded, 235 turtles had food remains in their digestive tract. Almost a third of the individuals (30.5%) were found with more litter ingested than natural food (in terms of mass). Literature and personal experience show that the dilution of ingested nutrients with other

litter (plastics) could influence individuals' movements and feeding behaviour, and can induce a sensation of satiety (Machovsky-Capuska et al., 2019). Such individuals could reduce the acquisition of nutrient needed for body maintenance, growth and reproduction. The observed rate (30.5%) is much higher than evaluated in other studies or species (1.2 to 3.4%, Clukey et al. 2017, Frick et al. (2009)), although it has rarely been evaluated in the literature and the methodology to assess it is likely to differ among authors.

Several models show that the absence of detection at the short term can however have repercussions at the longer term. Marn et al. (2017, 2020)'s mechanistic models show that even if individuals have the capacity to grow up and become as large as individuals not exposed to plastics, long term or occasional starvation would decrease reproduction output. The models predict that a plastic quantity from 3 to 25% of the digestive content could cause up to 20% reduction of energy available for body maintenance, resulting in 10-15% lower condition index and almost 11% lower egg production compared to unaffected turtles. Beyond 25%, the authors expect that energy left for individuals to ensure their energy expenditure related to growth, reproduction and reserve accumulation becomes very weak and the seasonal reproduction output would be impacted to 88%. Beyond 30% of plastics in the digestive content, turtles may no longer be able to mature and reproduce. We found **38.77 ± 1.3 %** of plastics among all ingested material on average. According to mechanistic models, **this high ratio could impact population dynamics.**

#### IV.6. Conclusion and recommendations

- The **absence of detection of health impact does not mean that there is no impact** of litter on turtle health.
- First, the parameters that we considered are possibly not adapted to detect a cause-and-effect relationship with litter ingestion at the short term, especially because the loggerhead turtle is a robust species. Other species, the green or the leatherback, could potentially be more highly impacted. It is also possible that other parameters should be considered for better highlighting the individual factors influencing litter ingestion and the consequences on health. To this purpose, we conducted a complementary ecotoxicological analyses in order to evaluate the link between the quantity of ingested litter and the toxins in the tissues (see IV.7).
- Secondly, it is possible that due to the high abundance and extended distribution of plastics in the environment (e.g., Mansui et al., 2020), all individuals are potentially impacted by the ingested litter, which prevented from showing a link with their biological characteristics.- For pursuing such evaluations, **the training of as many stakeholders as possible** for the collection of systematic and standard data, using these same methodologies, should be encouraged, especially in missing areas, and ideally on a larger spatial scale.
- **We recommend to collect standard measurements, such as the dry mass and the number of pieces of litter.**
- For acquiring more knowledge on litter impacts on health, collecting data on fat, plastron shape, body mass, lengths (especially Standard curved carapace length, injuries) should ideally be systematic and not optional. This would allow more robust analyses in the future.
- A collaboration with veterinarian is also encouraged to better identify the parameters to collect for evaluating body condition and health before death, with a comparison with living individuals, and between individuals in bad condition and supposed to be healthy, a comparison with individuals in clean area (control area) being unfortunately impossible.
- **Dry mass**, instead of number of items, should be considered as main data to be collected, as this parameter is able to show local differences in marine litter ingestion and is less susceptible of operator's bias (items counting).
- Regarding our results, we recommend **to consider all individuals** for evaluating the indicator, whatever their growth stage/size/mass, sex, origin, circumstances of discovery, thus with no data stratification.

## IV.7 Evaluating the relation between litter ingestion, bio-indicators and toxins in the tissues

The ecotoxicological analysis carried out in this project aimed to understand the possible ecotoxicological effects of litter ingestion on the loggerhead sea turtles *C. caretta*. Since marine litter, and in particular, plastic litter can exert physical and chemical impacts a multiple approach should be applied to understand the possible effects on sea turtle health. On this regard, the three-fold approach developed in the Interreg-Med project “Plastic Busters MPAs” has been applied and improved.

This approach combines an accurate measure of marine litter and microplastic loads in organisms, the evaluation of plastic additives levels in tissues and the related toxicological effects.

The monitoring approach should rely on the following three types of data:

i) analysis of digestive content to evaluate the marine litter ingested by the organisms. The results of this analysis must focus on assessing the occurrence (%), abundance (n), weight (g), colour, polymer type of the marine litter ingested by the different species;

ii) quantitative and qualitative analysis of plastic additives (e.g. phthalates and Polybrominated diphenyl ethers - PBDEs) and Persistent, bio-accumulative and toxic (PBT) compounds used as plastic tracers in the tissues of bioindicators;

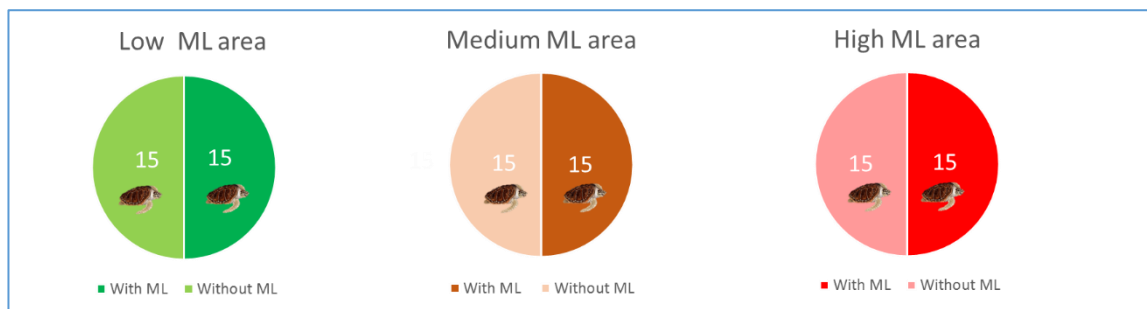
iii) analysis of the effects of litter ingestion by biomarker responses at different levels of biological organisation.

Based on the sea turtle samples available from the INDICIT II database, the approach has been applied when possible (all the partners were not able to performed or manage the sampling of all the tissues required).

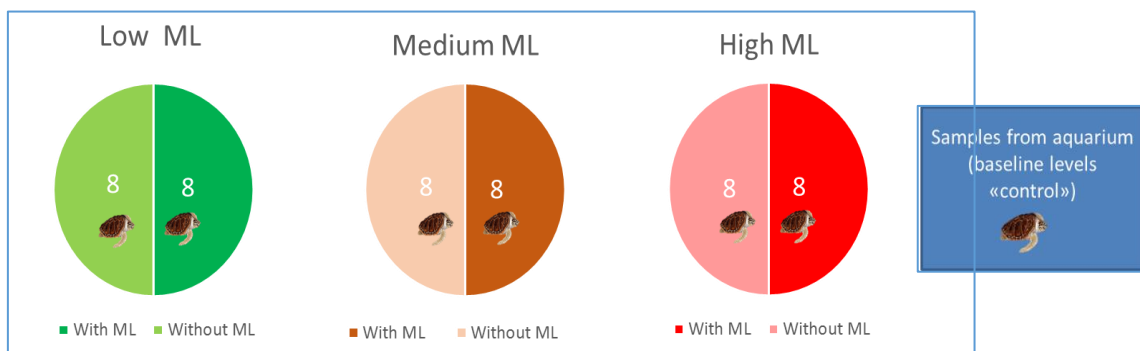
Since a gradient of litter ingestion have been identified from West to East (from most polluted to less polluted organisms, in terms of both occurrence (%) and quantities (dry mass and abundance)), the Turkish area has been classified as “Low impact area”, Sardinia as “Medium impact area” and France and Spain as “High impact area (Table IV.2. p 7).

According to this classification, each INDICIT partner has been asked (Fig. IV.4) to provide samples from stranded and hospitalised sea turtles. In addition, also captive sea turtles living in aquarium have been proposed to be used as hypothetical baseline controls for biological responses and contaminants.

## Stranded sea turtles



## Hospitalized/Captive organisms



**Figure IV.4.** Experimental design agreed with INDICIT II partners with number of expected samples per area and per turtles with and without litter found ingested

The methods (e.g. sampling material and tool kits sent to each partner) are described in details in the deliverable D2.11. The samples available by the end of July 2021, used for statistical analyses, are provided in Table IV.5.

**Table IV.5.** Summary of the samples arrived and available by the end of July 2021. In red the number of samples obtained, less than expected, in green the samples obtained in the expected number.

	PAU-DEKAMER			ISPRA-CNR IAS			EPHE/Marineland			UVEG			ULPGC		INSTM		
	Expected	Obtained		Expected	Obtained		Expected	Obtained		Expected	Obtained		Expected	Obtained	Expected	Obtained	
<b>Live Turtles</b>	16	11	<b>11 Excreta (multiple sampling)</b>	16	11	<b>11 Excreta</b>	8	15	0 Excreta (30 arriving)	8	12	5 Excreta	0	0	6 Excreta	0	0 (cites issues)
0 Blood			0 Blood			<b>15 Blood *</b>			<b>8 Blood **</b>			0 Blood					
0 Skin biopsy			0 Skin biopsy			<b>14 Skin biopsy *</b>			<b>11 Skin biopsy**</b>			0 Skin biopsy					
11 Blood smear			0 Blood smear			<b>13 Blood smear</b>			<b>11 Blood smear</b>			0 Blood smea					
<b>Dead Turtles</b>	30	0	0 Liver	30	10	<b>8 Liver</b>	16	14	<b>14 Liver</b>	14	8	<b>8 Liver</b>	0	6	<b>6 Liver</b>	0	0 (cites issues)
0 Fatty tissue			<b>6 Fatty tissue</b>			0 Fatty tissue			<b>8 Fat tissue</b>			<b>5 Fat tissue</b>					
<b>Captive</b>	0	0	0 Skin biopsy	3	0	0 Skin biopsy	8	9	<b>9 Skin biopsy</b>	0	0	0 Skin biopsy	4	8	<b>8 Skin biopsy</b>	0	0 Skin biopsy
0 Blood			0 Blood			<b>9 Blood</b>			0 Blood			<b>8 Blood</b>			0 Blood		
0 Blood smear			0 Blood smear			<b>9 Blood smear</b>			0 Blood smear			<b>0 Blood smea</b>			0 Blood smear		
<b>Total (104)</b>	46	<b>11</b>		49	<b>21</b>		32	<b>38</b>		22	<b>20</b>		4	<b>14</b>		0	



## IV.7.1 Summary of the methodology (detailed presentation provided in D2.11)

### a) Phthalate ester analysis (PAEs)

The analysis has been performed in different tissues in stranded and hospitalised/captive turtles. Regarding dead turtles, the analysis has been carried out mainly from liver and, when available in sufficient amount and quality, also in fat tissue. Regarding the hospitalised/captive sea turtles, the analysis has been carried out on blood and, for few samples, in excreta. This low number comes from the poor conservation status and/or the use of plastic tubes to preserve them. Eleven Phthalate ester congeners have been analysed (Table IV.6):

**Table IV.6.** Eleven phthalates esters and relative CAs number. In green the compounds listed as reprotoxic category 1B substances under EU Regulation (EC) 1272/2008.

11 phthalates	CAS#
Dimethyl phthalate	131-11-3
Diethyl phthalate	84-66-2
Diallyl phthalate	131-17-9
Dipropyl phthalate	131-16-8
Diisobutyl phthalate	84-69-5
Dibutyl phthalate	84-74-2
Benzyl butyl phthalate	85-68-7
Dicyclohexyl phthalate	84-61-7
Bis(2-ethylhexyl) phthalate	117-81-7
Diisononyl phthalate	28553-12-0
Di-n-octyl phthalate	117-84-0

### b) Porphyrins analysis

Porphyrin accumulation was quantified in the liver and in the faeces (excreta).

### c) Gene expression analysis

The evaluation of the expression levels of a set of genes selected on the basis of their biological functions, was conducted through the use of droplet digital PCR, allowing the quantification of the target molecules, specifically the expression of 6 genes of interest in the skin biopsy samples. As with any gene expression experiment, total RNA was extracted from the tissue sample (skin biopsies), retrotranscribed into cDNA (single-stranded DNA), which is then used in the quantification of the expression levels of each individual gene by amplification using specific primers.

### d) Selection of target genes

The analysis of data in the literature has shown that marine litter and microplastics can generate an inflammatory state in target tissues, causing oxidative stress potentially correlated with the set of physiological responses of inflammation. If the cellular damage resulting from inflammatory processes is excessive, mechanisms of cell death and programmed cell death (apoptosis) may also be activated. Some studies report metabolic alterations at biochemical and transcriptional level, in particular of lipid metabolism and energy metabolism. Finally, the possible transport and release of contaminants associated with ingested microplastics

should not be overlooked, whether these are environmental contaminants adsorbed to the particles or plasticizers and other additives added to the polymer during production. Therefore, eleven genes of interest were identified and primers accurately designed and their expression level in the skin biopsy of *C. caretta* specimens was investigated. Three house-keeping (HK) genes, beta actin (actb), glyceraldehyde triphosphate dehydrogenase (gapdh), ribosomal protein L4 (rpl4), which being constitutive genes, present a stable expression level even under stress conditions, were selected for normalization of the data.

## IV.7.2. Results

The analysis has been carried out, whenever possible, to obtain more data and endpoints in the same individuals for which several tissues were provided in order to have enough data to accurately define the health status of each individual and the population to which it belongs to, then to obtain and propose possible indices to be applied to define the effects of marine litter ingestion on sea turtle.

However, the complete assessment has not been possible on the whole dataset due to the scattered distribution of the tissues and samples available, some missing information on the health condition of the organisms, and the presence or absence of data on marine litter ingestion for alive organisms. This data is being completed.

### IV.7.2.1. Dead Sea turtles

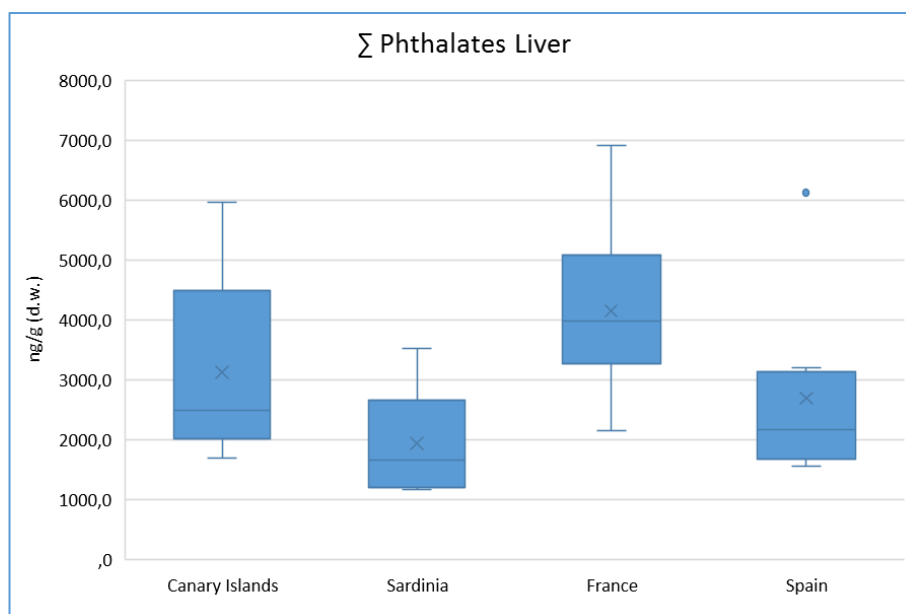
A total of 38 organisms have been analysed. The 38 liver samples have been used both for the analysis of the phthalate levels and the quantification of porphyrins. Five fatty tissue samples from the Canary Islands (from 5 of the 38 organisms) were also analysed to compare the possible different distribution and accumulation of these compounds in liver and fat tissue (Table IV.7).

**Table IV.7.** Summary of the samples available per area and per analysis.

Area	Phthalates Liver	Phthalates Fat	Porphyrins
Canary Islands	6	5	6
France	16		16
Sardinia	8		8
Spain Med	8		8
<b>Total number</b>	<b>38</b>	<b>5</b>	<b>38</b>

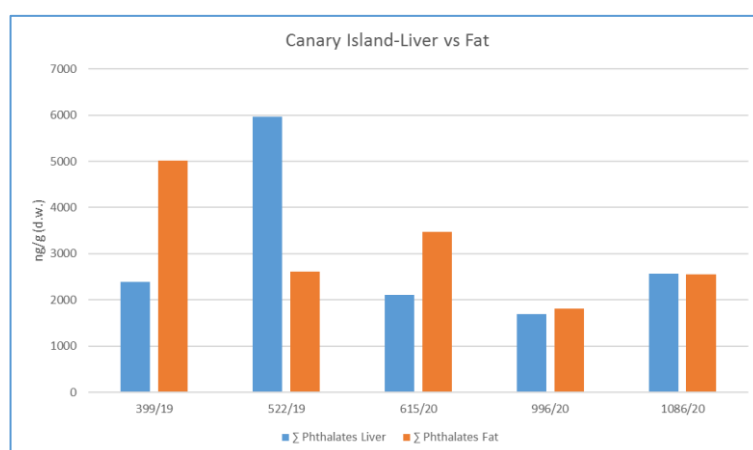
#### a) Phthalates esters concentrations in liver

The levels of eleven PAEs were quantified in the liver of stranded sea turtles. The comparison among the samples were firstly performed among countries, which significantly differ (Kruskal-Wallis chi-squared test = 14.437, df = 3, p-value = 0.002). Specifically, the levels of PAEs are higher for the organisms stranded along the France coast, while Sardinian sea turtles have the lowest concentration (Pairwise comparisons using Wilcoxon rank sum exact test and Benjamini and Hochberg correction, p-value=0.002). Sea turtles from France (Mediterranean) also differ from the Spanish (Mediterranean) ones (p=0,039). The levels for the sea turtles found dead in the Canary Islands are very variable, and do not differ from the other groups (Fig. IV.5).



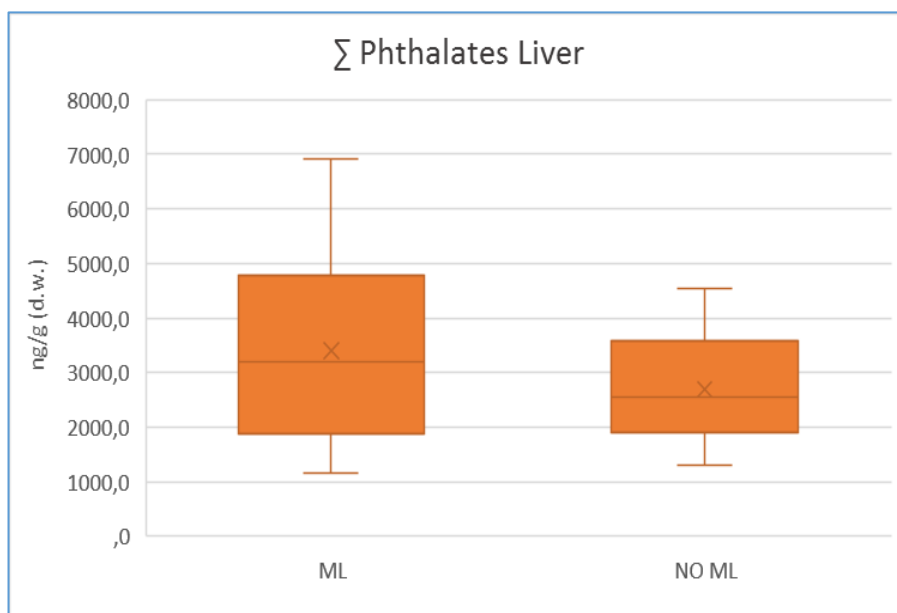
**Figure IV.5.** Phthalates esters concentration in the liver of stranded sea turtles. The data are expressed in ng/g dry weight. Canary Islands (n=6); Sardinia (n=8), France (n=16), Spain (n=8).

An additional comparison has been carried out to evaluate the different levels of PAEs in fat tissue and liver of the same individuals (Fig. IV.6). The results show variability and further investigation are needed, also considering the status of the organisms, level of conservation, the cause of death and the age status which are currently not available for the samples analysed.



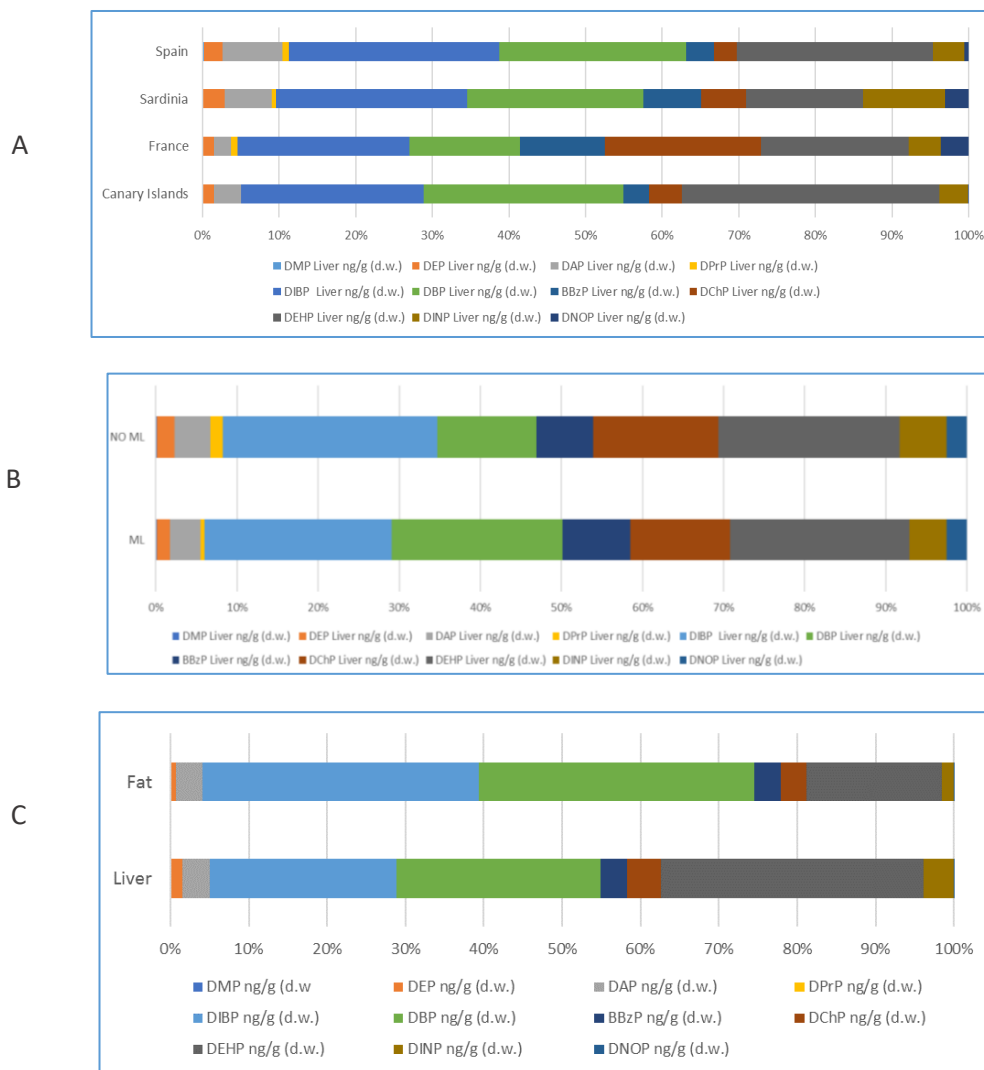
**Figure IV.6.** Comparison between the phthalate's esters concentration in the liver and in the fat of the same individuals of stranded sea turtles sampled in the Canary Islands. The data are expressed in ng/g dry weight.

Although the concentrations are slightly higher in organisms with marine litter in their digestive tract, any statistical difference exists (Fig. IV.7). According to the data provided by the partners, 28 out of 38 sea turtles had marine litter in their digestive tract. A further analysis on the organisms with and without marine litter in the different countries has been performed but the levels, also within the different areas (Atlantic and Med), are similar and any statistically significant difference has been found (details in D2.11).



**Figure IV.7.** Comparison between the phthalate esters concentration in the liver of the individual with (n=28) and without (n=10) marine litter (ML) analysed in the digestive tract of dead sea turtles sampled in the four study countries. The data are expressed in ng/g dry weight of tissue.

Analysing the fingerprints of the eleven PAEs, some variations among countries and among individuals with or without ingested marine litter occurred. In the samples from the Canary Islands, which presumably belong to the Atlantic population, DPrP and DNOP were not detected whereas they were detected in the other areas. In addition, the concentrations of both DEHP and DPB vary among countries (Fig. IV.8A). Slight differences in the fingerprints among individuals with and without marine litter are shown, mainly related to the percentage of DIBP and DBP (Fig. IV.8B). The profiles of DEHP and DPB concentrations were also compared between the tissue (Fat and liver). More data are needed to make some recommendations on the tissue to target for these analyses in sea turtles (Fig. IV.8C).



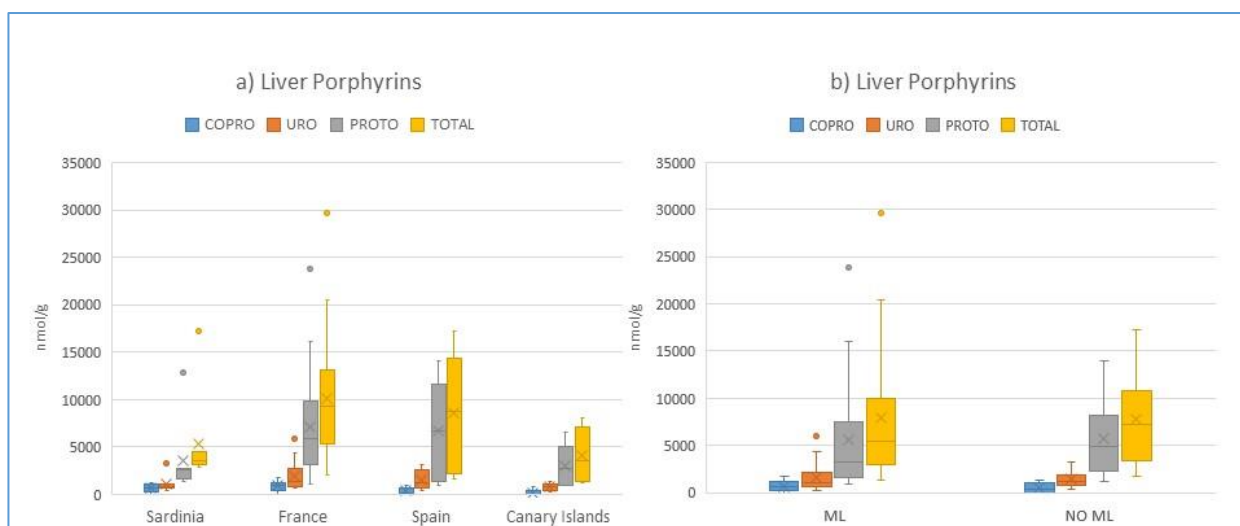
**Figure IV.8.** Phthalate esters fingerprint in the four different countries A) and in the individuals with and without ingested marine litter B) and in different tissues C).

## b) Porphyrins in liver

The levels of hepatic porphyrins have been measured in dead sea turtles since they represent biomarkers of exposure to various classes of contaminants (e.g. PAHs, PCB, heavy metals, etc.). The alteration of porphyrins is related to the activation of the metabolism of these compounds. On this regard, these biomarkers have been selected as possible signals of exposure to the ingestion of marine litter and related contaminants.

The analysis has been carried out in the liver of the same individuals whose PAEs concentrations were measured. The results have been analysed per country and according to the presence of marine litter in the gastro-intestinal tract (Fig. IV.9). Despite lower levels of Total Porphyrins in the organisms sampled in Sardinia and in the Canary Islands, the difference among region was not significant (Kruskal-Wallis test). However, the trend observed (less concentration of Total Porphyrins in the Sardinian samples) are in line with the gradient observed with the Phthalates esters concentrations (PAEs) results.

Analysing the data disregarding the country but grouping the results for the presence or absence of marine litter in the gastro-intestinal tract, the levels are very similar, indicating that the ingestion of marine litter seems to not affect the levels of porphyrins in liver.



**Figure IV.9.** Porphyrin concentrations in liver from the stranded sea turtles in four study areas (a) and on the basis of presence (ML) and absence (NO ML) of marine litter (b) of the dead sea turtles.

#### IV.7.2.2. Hospitalized and Captive sea turtles

A total of 120 samples from 4 areas (France, Sardinia, Spain, Turkey) and 2 aquariums (Controls; Canary Islands and France) have been analysed. Due to the scattered distribution of the samples, all analyses of the foreseen endpoints have been carried out only in the samples from Spain Med (UVEG). For the other countries, the analyses have been carried out according to the tissue available (Table IV.8).

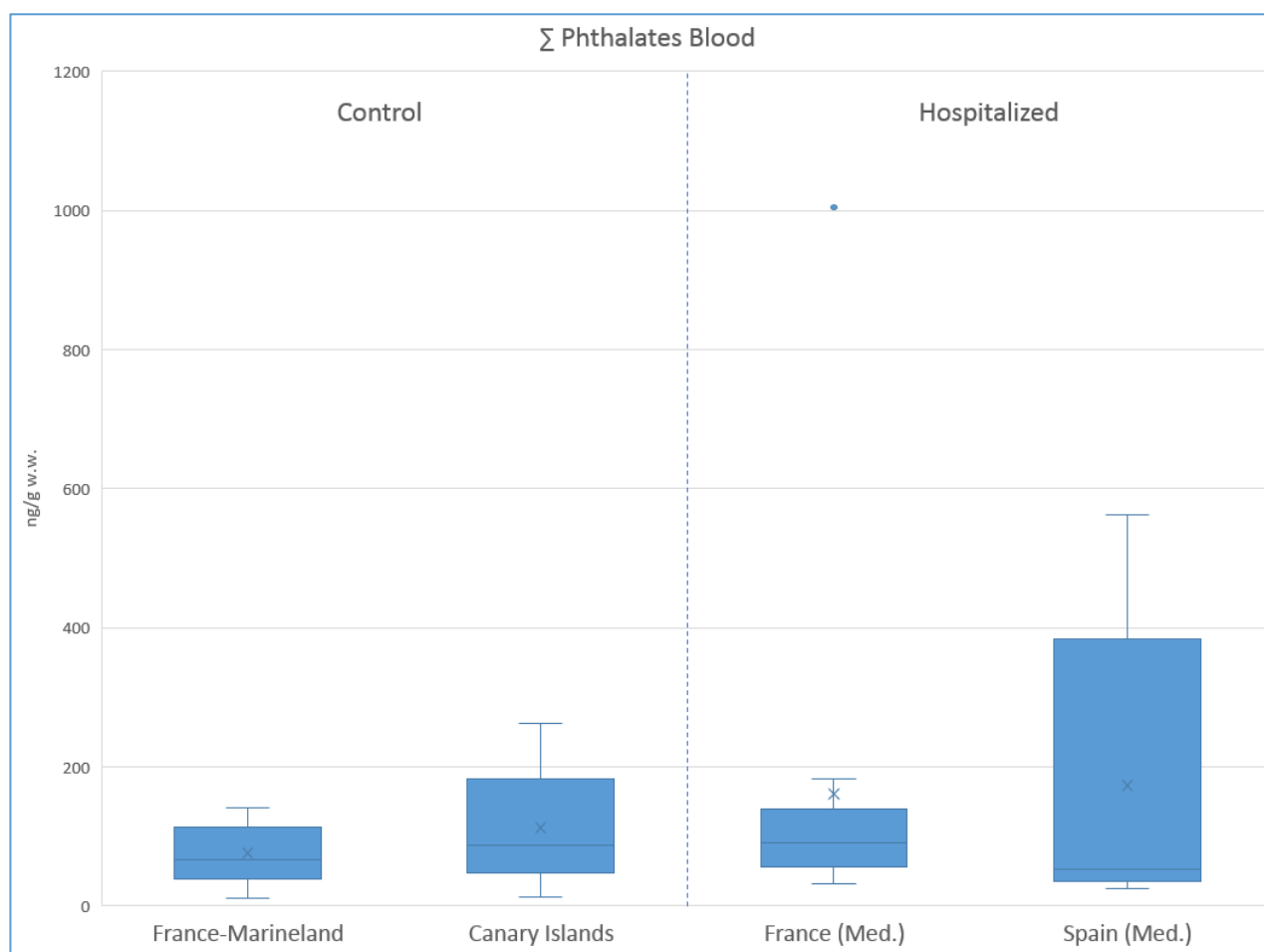
Despite the request to provide data for the presence of Marine Litter in excreta, often the data were not provided so, in some cases, this issue seriously affected the analysis of the data oriented by the ingestion of marine litter as per the experimental design.

**Table IV.8.** Summary of the samples available for area and for the type of analysis.

Area/Condition	Number of Samples	$\Sigma$ Phthalates Blood	$\Sigma$ Phthalates Excreta	Porphyrins	ENA Assay	WBC	Gene Expression
<b>Canary Islands</b>	<b>8</b>	<b>8</b>					<b>8</b>
Captive	8	8					8
<b>France</b>	<b>23</b>	<b>22</b>			<b>22</b>	<b>22</b>	<b>14</b>
Captive	9	9			9	9	9
Hospitalised	14	13			13	13	5
<b>Sardinia</b>	<b>11</b>			<b>11</b>			
Hospitalised	11			11			
<b>Spain</b>	<b>15</b>	<b>8</b>	<b>6</b>	<b>6</b>	<b>11</b>	<b>11</b>	<b>10</b>
Hospitalised	15	8	6	6	11	11	10
<b>Turkey</b>	<b>63</b>			<b>54</b>	<b>13</b>	<b>13</b>	
Hospitalised	59			52	12	12	
Hospitalised/dead	4			2	1	1	
<b>Total</b>	<b>120</b>	<b>38</b>	<b>6</b>	<b>71</b>	<b>46</b>	<b>46</b>	<b>32</b>

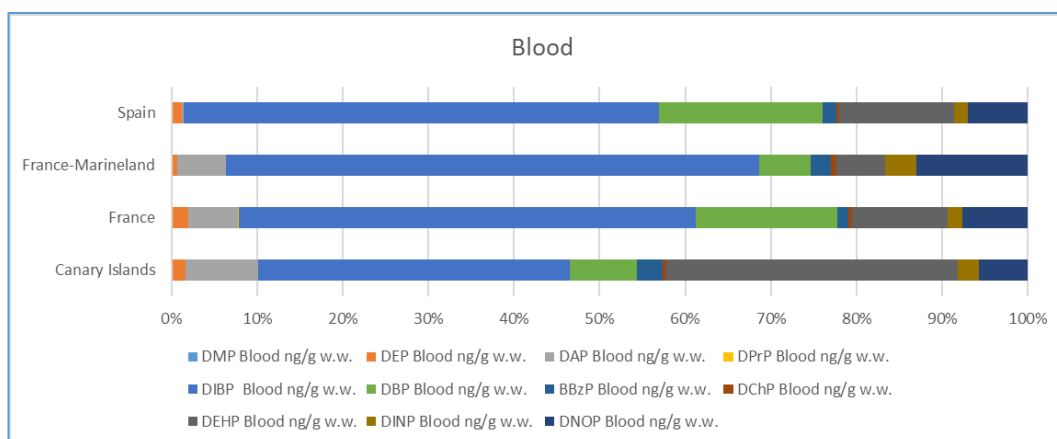
### a) Phthalates esters concentrations

PAEs have been analysed in whole blood samples obtained from hospitalised and captive sea turtles. The levels of PAEs in the blood were lower than in the liver of dead organisms. No statistical difference was observed between the hospitalized sea turtles from the Mediterranean France and Spain (Fig. IV.9). No statistical difference was observed between the hospitalised and captive organisms (Fig. IV.10). The absence of differences among captive and wild animals did not allowed to establish the baseline levels in terms of contaminant concentration as hypothesised in the experimental design. In addition, the lack of information on the ingestion of marine litter by the organisms analysed did not allowed to perform a comparison of accumulation of plastic additives between the organisms that may have ingested marine litter before the hospitalisation.



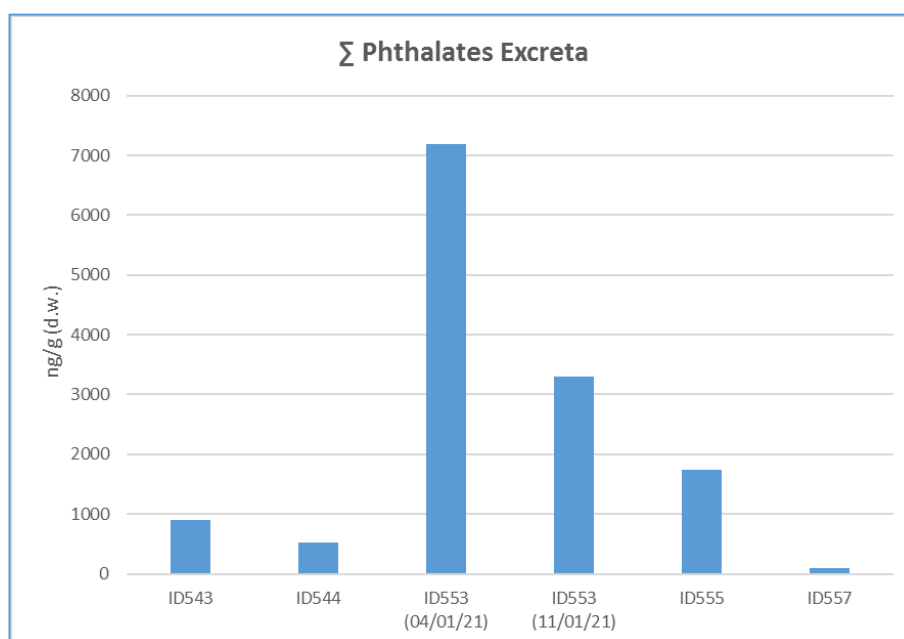
**Figure IV.10.** Phthalates esters concentration in the blood sampled from hospitalised and captive sea turtles (control). The data are expressed in ng/g dry weight. France-Marineland (n=9), Canary Islands (n=8), France (n=5), Spain (n=9). Note that the contamination can come either from the food (plastic ingested) and water (environmental pollution).

The analysis of the fingerprint of the 11 PAEs in the sea turtles shows that the DIBP compound is the most abundant in the hospitalised and captive organisms. The relative abundances of the other PAEs are similar among the different areas, apart from the captive organisms from Canary Islands where DEHP percentage is higher than in the other samples (Fig. IV.11).



**Figure IV.11.** Phthalates esters fingerprint in the blood sampled from hospitalised and captive sea turtles. The data are expressed in ng/g dry weight. France-Marineland (n=9), Canary Islands (n=8), France (n=5), Spain (n=9).

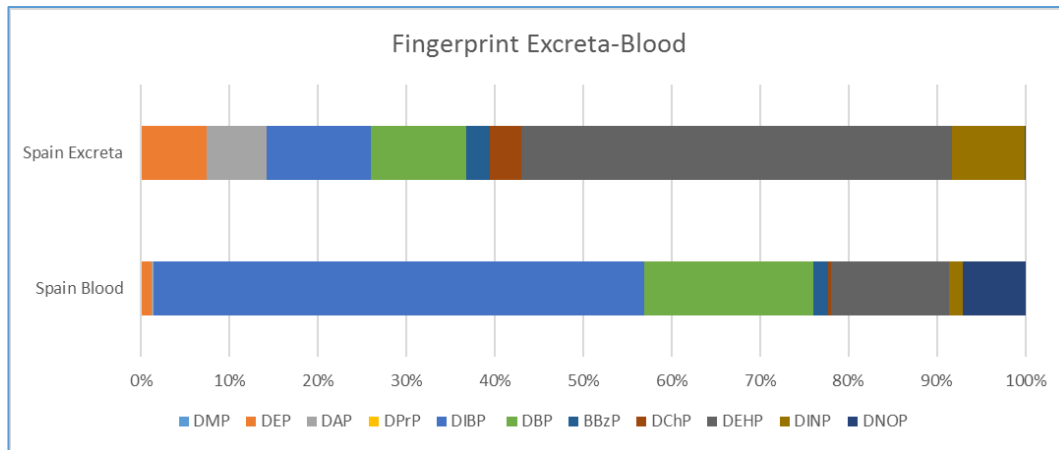
A preliminary analysis was carried out on 6 excreta sampled from hospitalised turtles in Spain (Fig. IV.12). The levels of total phthalates in these samples are highly variable, with specimen ID557 having the lowest levels and ID553 having the highest. Analysing specimen ID55,3 it was also possible to observe a decrease in the levels of phthalates from the date of the first sampling (04/01/2021) to the second sampling (11/01/2021), suggesting an excretion of these compounds through the excreta.



**Figure IV.12.** Phthalates esters concentration in the excreta sampled by hospitalized and captive sea turtles. Data are expressed in ng/g dry weight.

Due to the low sample number it was not possible to verify if there was a correlation between PAEs levels in excreta and blood (only 3 specimens available). A comparison of fingerprints was however performed (Fig. IV.13), showing high differences between the two profiles and in particular a prevalence of DIBP in excreta and DEHP in blood.





**Figure IV.13.** Phthalates esters fingerprint in the excreta and blood in the same organisms from hospitalized sea turtles.

### b) Gene expression analysis

The alteration of mRNA levels in skin biopsy collected from hospitalised and captive organisms has been quantified by ddPCR. Fourteen genes for *C. caretta* and 3 reference genes (GAPDH, RPL4, ACTB) have been selected. Two out of fourteen genes did not work properly (CYP1B and IL1B) to obtain reliable results so they were excluded from the analysis.

As the Turkish samples (skin biopsy and blood) have been carried out with another method (qRT-PCR), the results are presented separately see deliverable D.2.11). The data are shown grouped for the main biological function of the endpoints measured (Table IV.9) which have been selected according to limited literature studied on sea turtles and on the main possible effects of marine litter, both physical and chemical.

**Table IV.9.** Selected gene of interests and their main biological function.

Genes of interest (GOI)	Gene Symbol	Main biological function
<b>Cluster of Differentiation 83</b>	CD83	Inflammation/immune responses
<b>CC-chemokine receptor 7</b>	CCR7	Inflammation/immune responses
<b>Lysozyme</b>	LYZ	Inflammation/immune responses
Interleukin 1 beta	IL1B	Inflammation/immune responses
<b>Thyroid hormone receptor alpha</b>	THRa	Lipid/Energy metabolism
<b>Retinoid X Receptor Alpha</b>	RXRa	Lipid/Energy metabolism
<b>Proxisome proliferator receptor alpha</b>	PPARa	Lipid/Energy metabolism/xenobiotic
<b>Acyl-coa dehydrogenase</b>	ACADL	Lipid/Energy metabolism
<b>Cytochrome P450 1A</b>	CYP1A	Response to xenobiotics
Cytochrome P450 1B	CYP1B	Response to xenobiotics
<b>Glutathione S-transferase</b>	GST	Response to xenobiotics
<b>Heat shock protein 60</b>	HSP60	Response to cellular stress
<b>Progesteron receptor</b>	PR	Endocrine signaling/xenobiotic
<b>Estrogen receptor alpha</b>	ERa	Endocrine signaling/xenobiotic

### c) Inflammation and Immune Responses biomarkers

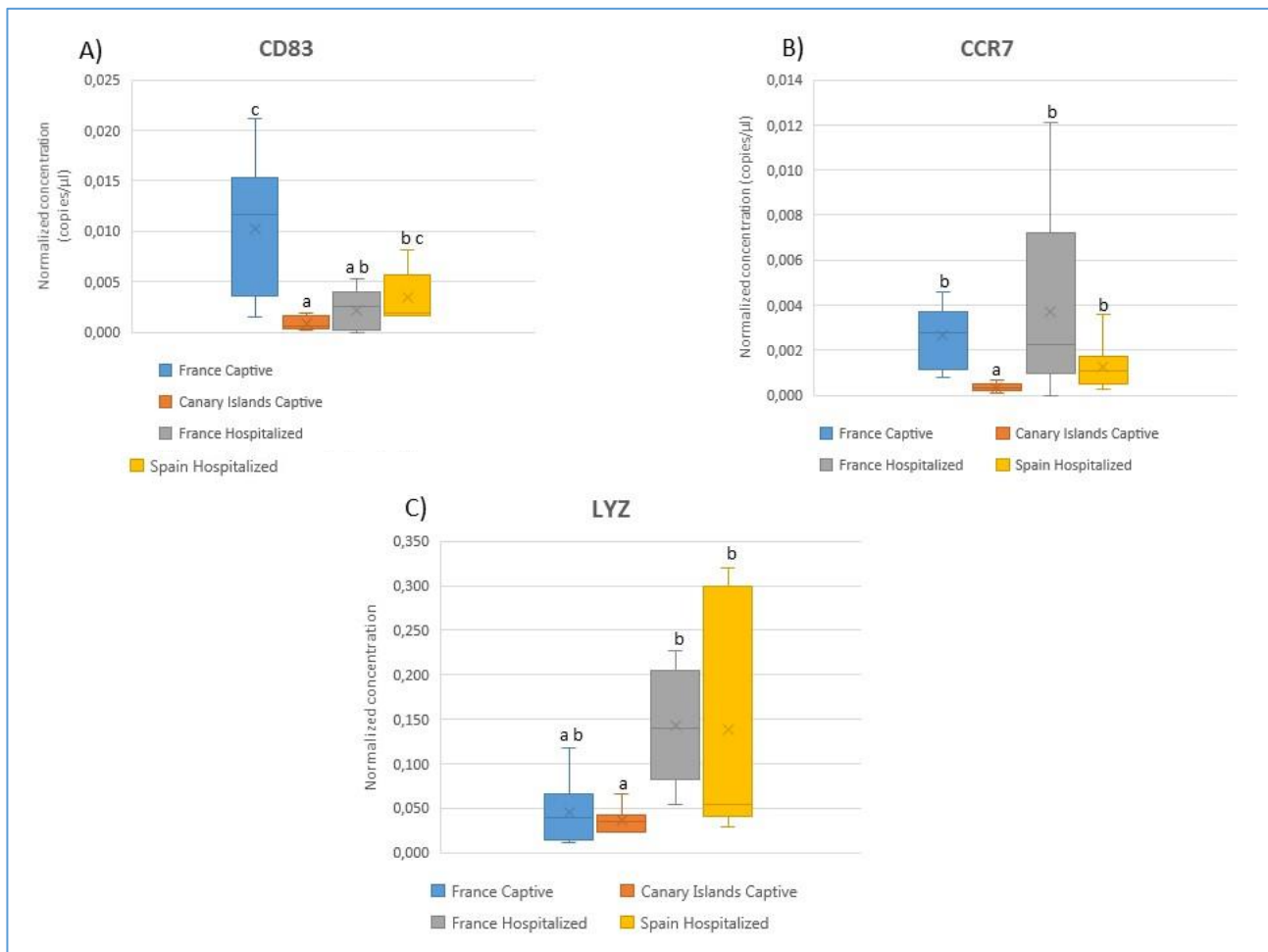
The variation of mRNA levels of three biomarkers genes has been evaluated in order to evaluate the possible inflammatory effects and activation of immune responses.

The Cluster of Differentiation 83 (CD83) gene is a marker of immune system activation in several immune cells. The upregulation of this gene is a signal of the activation of immune response by stressors. The French captive organisms present the highest up-regulation of this gene while the captive sea turtle from Canary Island shows baseline levels, which presents statistically significant differences with the Med Spanish hospitalised sample ( $p=0.017$ ) (Fig. IV.12A).

Regarding the CC-chemokine receptor 7 (CCR7), which is expressed in several immune cells (e.g. lymphocytes B and T) and regulates immune responses, Canary Island captive sea turtles show the lowest mRNA concentration, statistically different from French captive organisms ( $p=0.0005$ ) and the other hospitalised organisms ( $p=0.0237$ ) (Fig. IV.12B).

The lysozyme coding gene, related to the innate immune response, is a marker of exposure to pathogens. The expression of this gene is higher in the hospitalized organisms, both from Spain and France, and differs statistically from the expression levels measured in the captive organisms ( $p=0.046$ ,  $p=0.019$ ) (Fig. IV.12C).

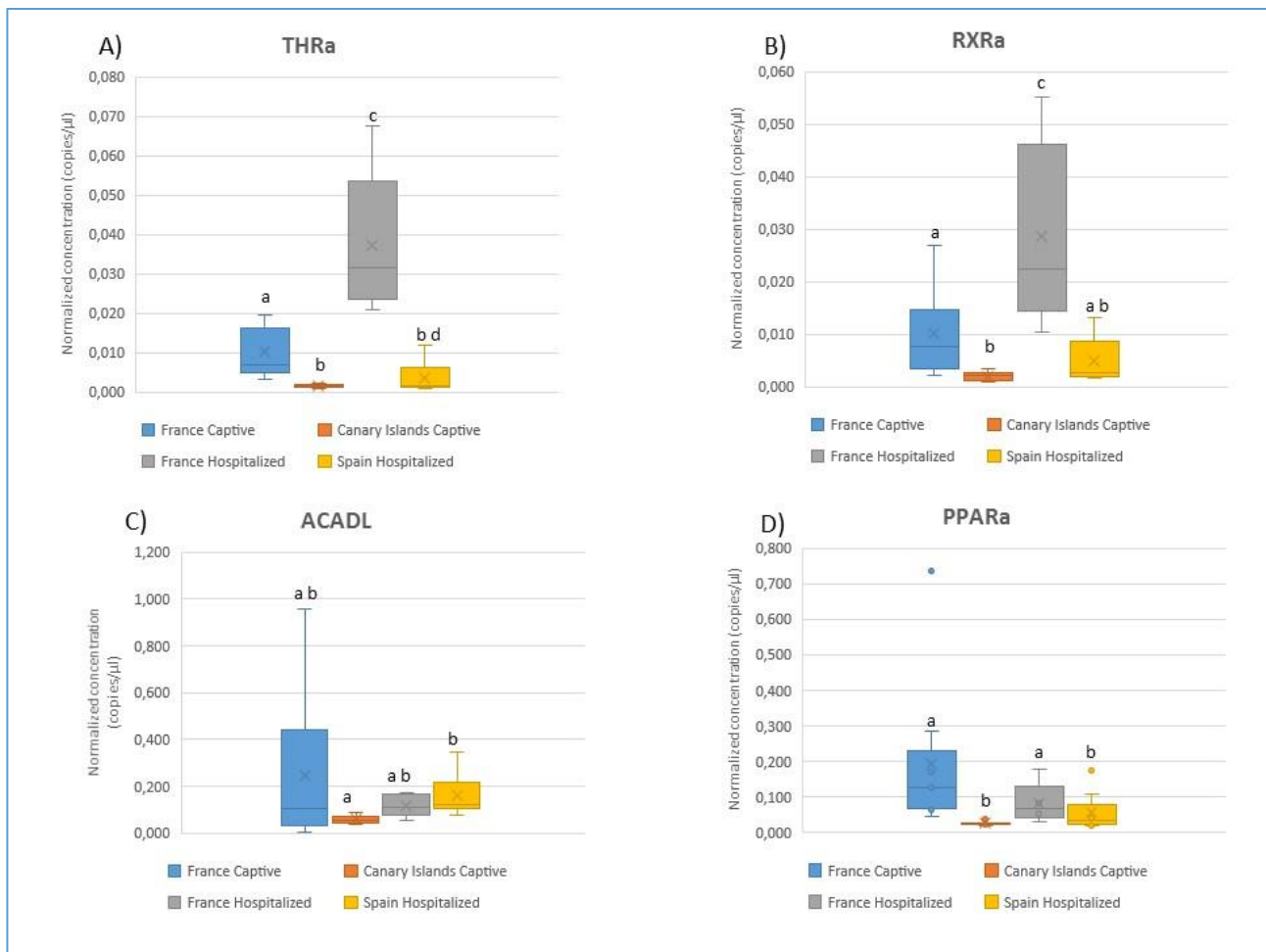
Inflammation and Immune Responses biomarkers can be related to individual stress. The precise conditions have to be described to explain e.g. the differences observed between the captive turtles in France and Canary Islands.



**Figure IV.14.** Normalized concentrations of the genes CD83 (A), CCR7 (B) and LYZ (C) expressed as copies/ $\mu$ l measured in sea turtles skin biopsy. The data are normalized to the HKG GAPDH. Statistical differences tested with the Kruskal-Wallis test followed by Wilcoxon-Mann Whitney test for pairwise comparison. Different letters indicate statistical significance ( $p < 0.05$ ).

#### d) Lipid and Energy Metabolism biomarkers

The genes encoding for the thyroid hormone receptor alpha (THRa) and Retinoid X Receptor Alpha (RXRa) follow the same trend. The captive organisms present lowest mRNA levels while the French hospitalised organisms show the highest concentration of both genes ( $p = 0.002$ ). The upregulation of these genes can be related to the activation of lipid and energy metabolism after the exposure to endocrine disrupting chemicals (Fig. IV.13A-B).



**Figure IV.15.** Normalized concentrations of the genes THRa (A), RXRa (B), ACADL (C) and PPARa (C) expressed as copies/ $\mu$ l measured in sea turtles skin biopsy. The data are normalized to the HKG GAPDH. Statistical differences tested with the Kruskal-Wallis test followed by Wilcoxon-Mann Whitney test for pairwise comparison. Different letters indicate statistical significance ( $p < 0.05$ ).

The PPARa gene, instead, which is linked to the lipid metabolism shows the highest mRNA levels in the captive French organisms which are statistically different if compared to the Canary Island captive sea turtles ( $p = 0.0005$ ) and Spanish hospitalised organisms ( $p = 0.015$ ) (Fig. IV.13C).

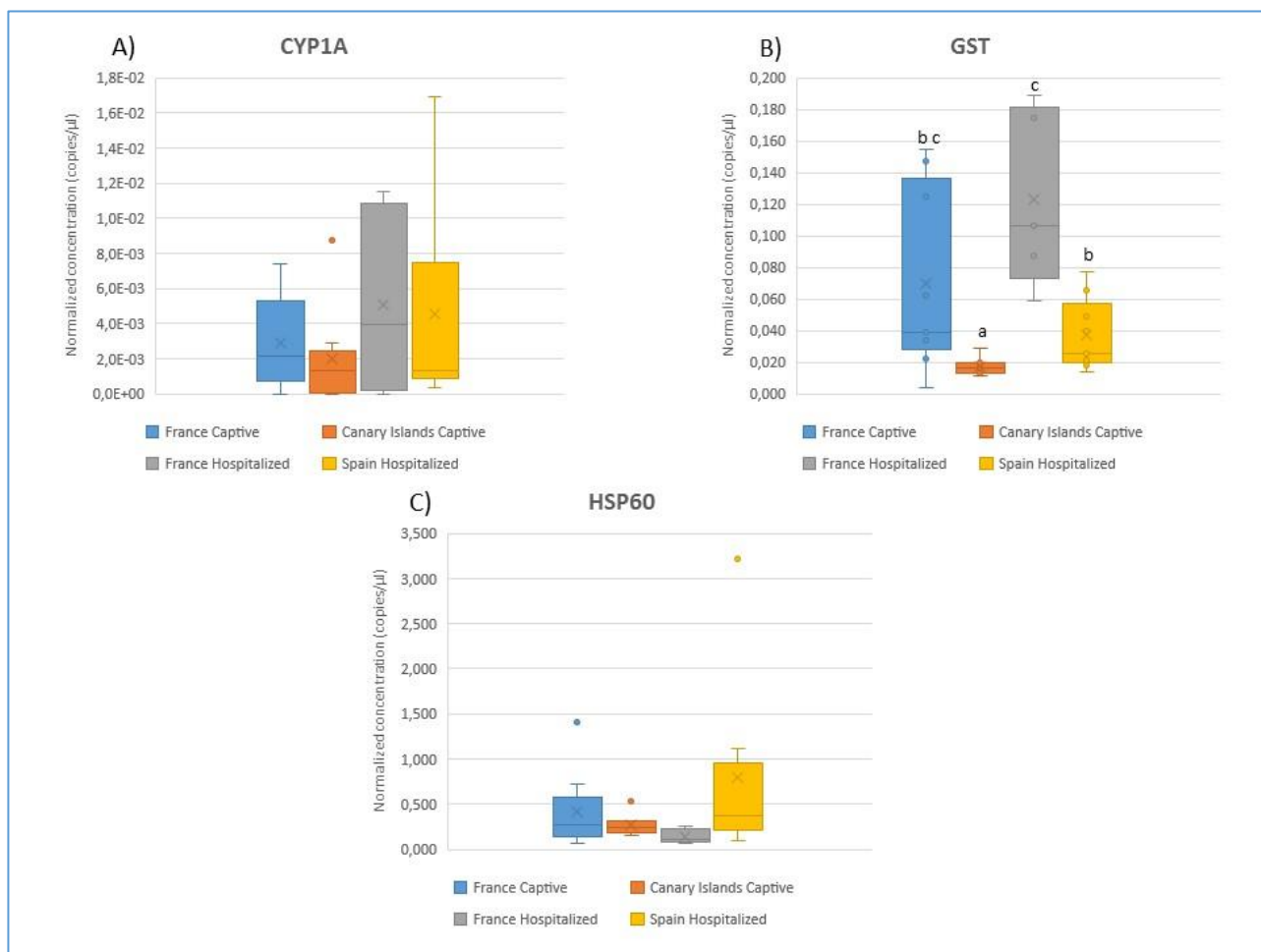
The Acyl-coA-dehydrogenase (ACADL) is the main enzyme involved in the beta-oxidation of the fatty acid, thus is responsible for energy production. The Spanish hospitalised organisms show the highest mRNA concentrations, which are statistically higher than the Canary Island captive sea turtles (Fig. IV.13D).

#### e) Xenobiotic metabolism and cellular stress biomarkers

The Cytochrome P450 1A has been considered as biomarkers of exposure to xenobiotic compounds. The four groups of sea turtles have similar levels of CYP1A mRNA. No statistical difference has been found among the groups (Fig. IV.14A).

The Glutathione S-transferase (GST) is a gene encoding for the GST enzyme, involved in the catalysis of xenobiotic substrates. The highest mRNA levels have been found in the French Hospitalised organisms (Fig. IV.14B) suggesting an exposure to higher concentration of xenobiotics. These levels are significantly different from captive ( $p = 0.015$ ) and Spanish hospitalised sea turtles ( $p = 0.012$ ).

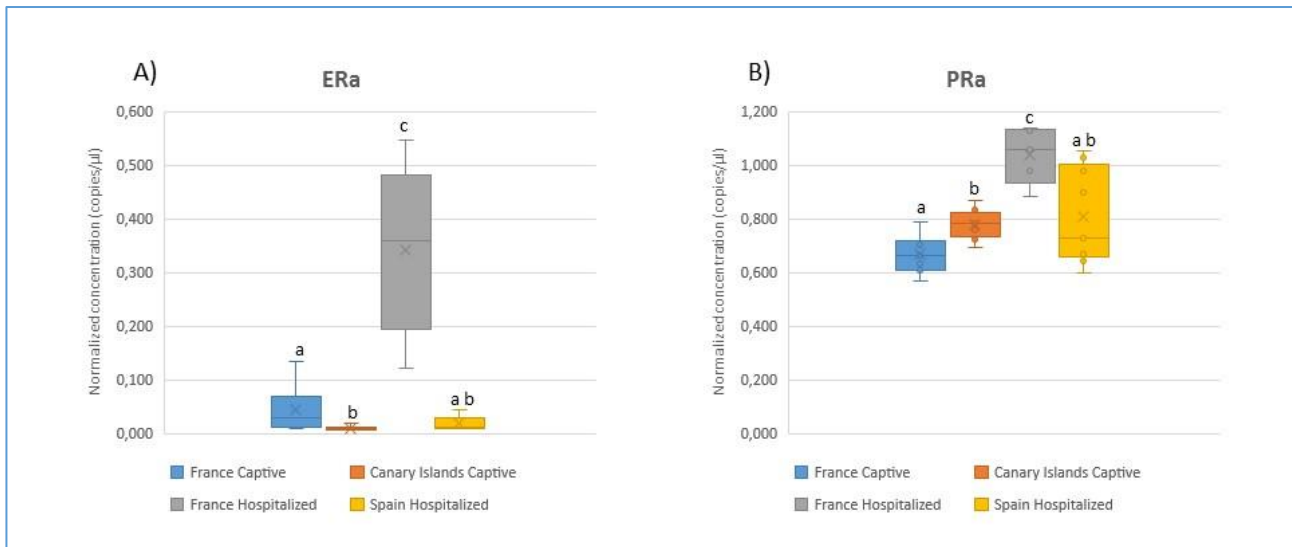
A general stress marker as the heat shock protein 60 gene did not show significant alterations in the levels of expression in the four groups of organisms analysed (Fig. IV.14C).



**Figure IV.16.** Normalized concentrations of the genes CYP1a (A), GST (B) and HSP60 (C) expressed as copies/ $\mu$ l measured in sea turtles skin biopsy. The data are normalized to the HKG GAPDH. Statistical differences tested with the Kruskal-Wallis test followed by Wilcoxon-Mann Whitney test for pairwise comparison. Different letters indicate statistical significance  $p < 0.05$ .

#### f) Endocrine signalling and xenobiotic biomarkers

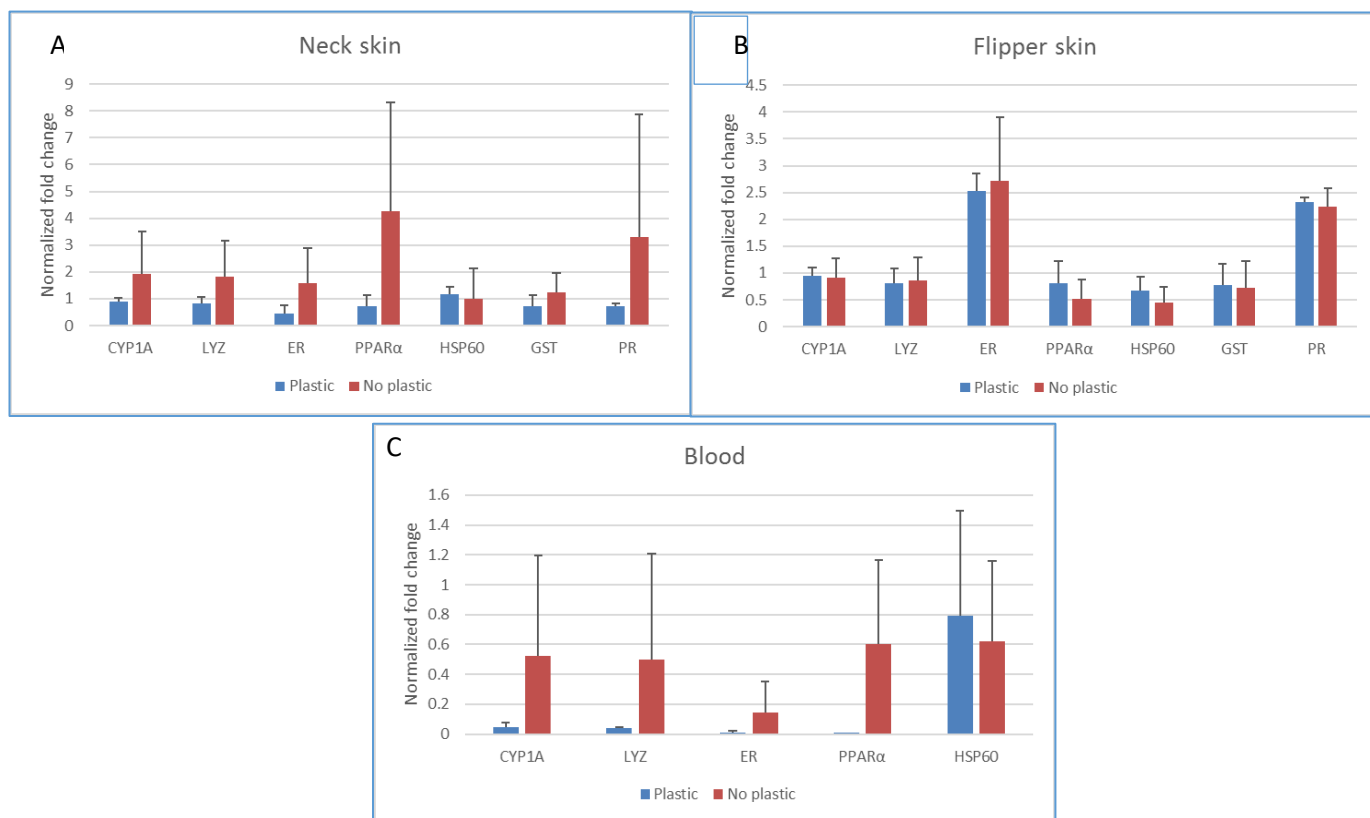
The oestrogen receptor alpha (Era) and the progesterone coding genes are marker of exposure to endocrine disrupting chemicals with estrogenic effects. The expression of these two genes follows a similar trend among the four groups of organisms with the wild (hospitalized) French sea turtles showing the highest mRNA concentrations for both genes (Fig. IV.15).



**Figure IV.17.** Normalized concentrations of the genes ERa (A) and PRa (B) expressed as copies/ $\mu$ l measured in sea turtles skin biopsy. The data are normalized to the HKG GAPDH. Statistical differences tested with the Kruskal-Wallis test followed by Wilcoxon-Mann Whitney test for pairwise comparison. Different letters indicate statistical significance  $p < 0.05$ .

#### g) Gene expression analysis by qRT-PCR in the Turkish samples

The analysis of the variation of the expression of seven genes was evaluated in the samples collected from hospitalized turtles in the Turkish rescue centre. The analysis has been carried out in blood and skin sampled from the same individuals. In addition, skin samples were collected in two different body parts (neck and flipper). The presence of plastic in the excreta was also recorded. The mRNA levels vary according to the tissues for all of the genes analysed. Generally, the expression of the seven target genes is higher in the organisms, which did not excreted plastics during the hospitalization period apart for the flipper skin biopsy where the expression is similar among the individuals with and without plastics. Due to the low number of samples analysed, a statistical analysis cannot be performed, but the presence of plastics seems not affecting the expression of the endpoints analysed (Fig. IV.16).



**Figure IV.18.** Normalized gene expression measured by qRT-PCR in sea turtles. A) Neck skin biopsies (plastic n=2, no plastic n=6); B) Flipper skin biopsies (plastic n=2, no plastic n=3) and C) blood (plastic n=2, no plastic n=2). The data are normalized to the HKG GAPDH, ACTB and RPL4. The values are expressed as mean  $\pm$  SD.

#### h) White blood cell count

The different categories of white cells were counted in the blood of sea turtles collected in different rescue centres and in the aquarium (France). Eosinophils, lymphocytes and basophils show highest values in sea turtles in captivity (without statistically differences), indicating a potential stress condition and involvement in parasitic infections and other types of antigenic stimulation. Heterophils and thrombocytes are highest in French turtles rescued, in agreement with ratio H:L values. The highest level of monocytes observed in the Turkish animals indicate the presence of an inflammation in the animals as reported by Flower et al. (2015). Highest values of lymphocytes in Spain turtles rescued were observed (figures are provided in D2.11, p. 27).

#### i) Erythrocytes Nuclear Abnormalities Assay

Other abnormalities were counted in the blood of sea turtles collected in three rescue centres (France, Spain, Turkey) and in an aquarium. The lobed shape is the most frequent in all the samples. The frequency of micronuclei and the total of nuclear abnormalities are reported in table IV.10. Regarding micronuclei, French sea turtle from aquarium shows highest values and animals rescued have values near to zero. No statistical differences were found among the different countries of sampling for the frequencies of total abnormalities, although in the samples from France (both hospitalised and in captivity) are observed higher values compared to those of all the other areas. The micronucleus test is widely used in ecotoxicology to evaluate the genotoxicity damage in erythrocytes of different species of fish, amphibian but only some papers are available for this aim on reptiles such as sea turtles. Furthermore, other erythrocyte nuclear abnormalities, such as lobed nuclei, kidneyshaped nuclei and segmented nuclei (Attademo et al., 2011; Ghaffar et al., 2015; Lajmanovich et al., 2013) have been observed in erythrocytes of fish, amphibian and reptiles (Schaumburg et al., 2012), included sea

turtles (Caliani et al., 2014; Casini et al., 2018). These abnormalities are considered to be indicators of genotoxic damage, although the mechanism responsible for its formation is unclear (Ghaffar et al., 2015). In this study, micronuclei and total abnormalities values are lower than to the scientific literature (MN= range from 5 to 10 ‰) (ENAtot= range from 150 to 200 ‰).

**Table IV.10.** Erythrocytes Nuclear Abnormalities frequency.

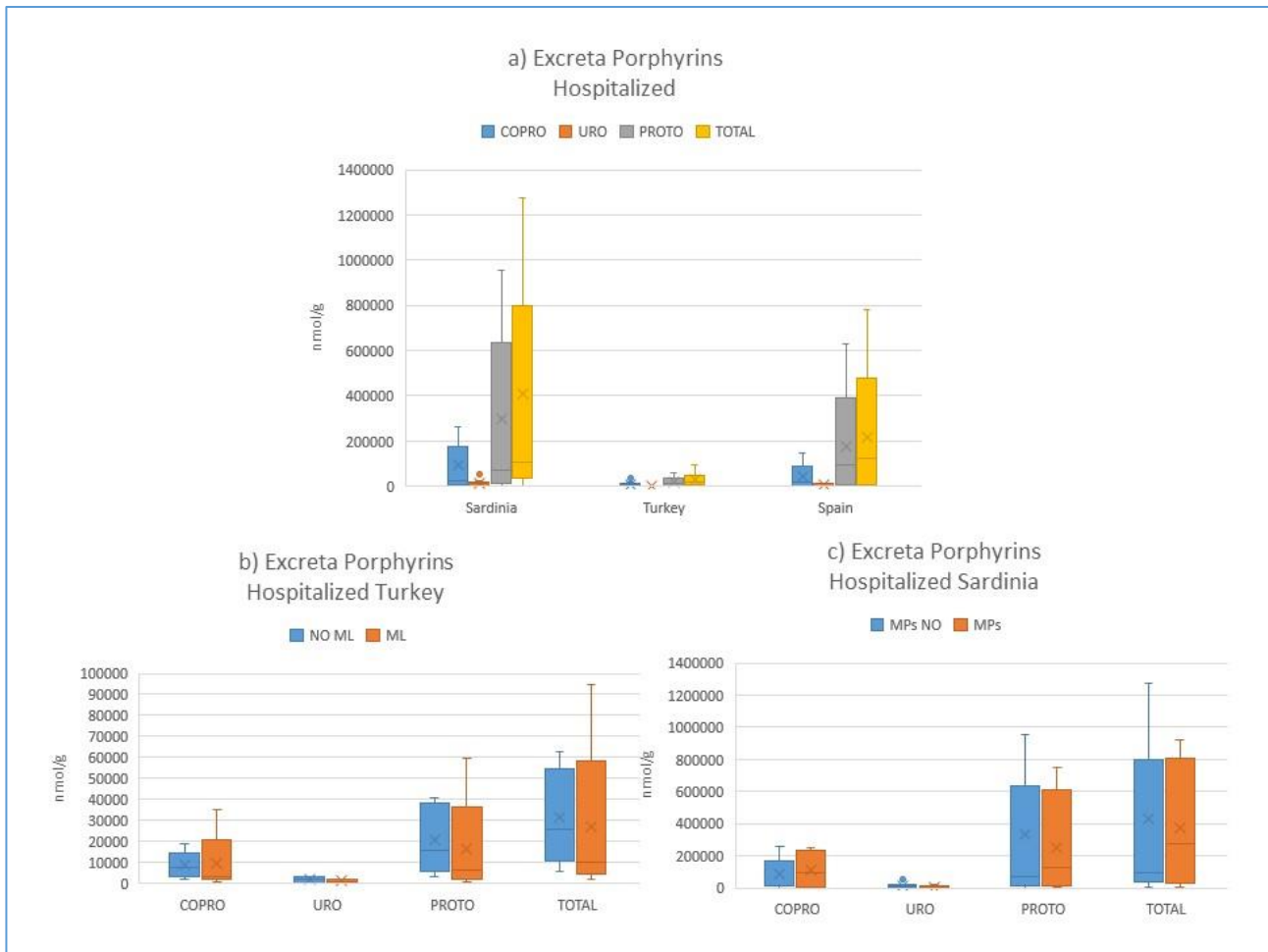
	<b>Kidney</b>	<b>Lobed</b>	<b>Segmented</b>	<b>Micronucleus</b>	<b>Total</b>
<b>France</b> (captive)	0,111±0,333	53,667±13,12	0,000	1,000±1,732	54,333±12,884
<b>France</b> (wild)	0,000	51,308±13,36	0,000	0,154±0,666	51,462±13,833
<b>Spain</b> (wild)	0,091±	23,909±8,845	0,091±	0,182±0,145	24,273±8,845
<b>Turkey</b> (wild)	0,167±0,390	38,167±27,42	0,000	0,000	38,333±27,294

#### j) Porphyrins levels in excreta

The levels of porphyrins in the excreta of the sea turtles collected in three rescue centres and in an aquarium are reported in Figure IV.17. Protoporphyrins are the most abundant type among all the categories, without statistically differences among the countries. No differences between excreta with and without plastics were observed in the samples from Turkey and Sardinia. These data are higher than those found by Guerranti et al. (2014). The increase in protoporphyrins could be due to the presence of contaminants, such heavy metals or organochlorines.

The data on marine litter presence in excreta were available only for some samples from hospitalized sea turtles in Sardinia and Turkey (more data are available in other countries for future analysis). A slight difference in coproporphyrins between organisms with and without marine litter was detected, despite any statistical difference was found between the areas of origin of the turtles (Figure IV.17).





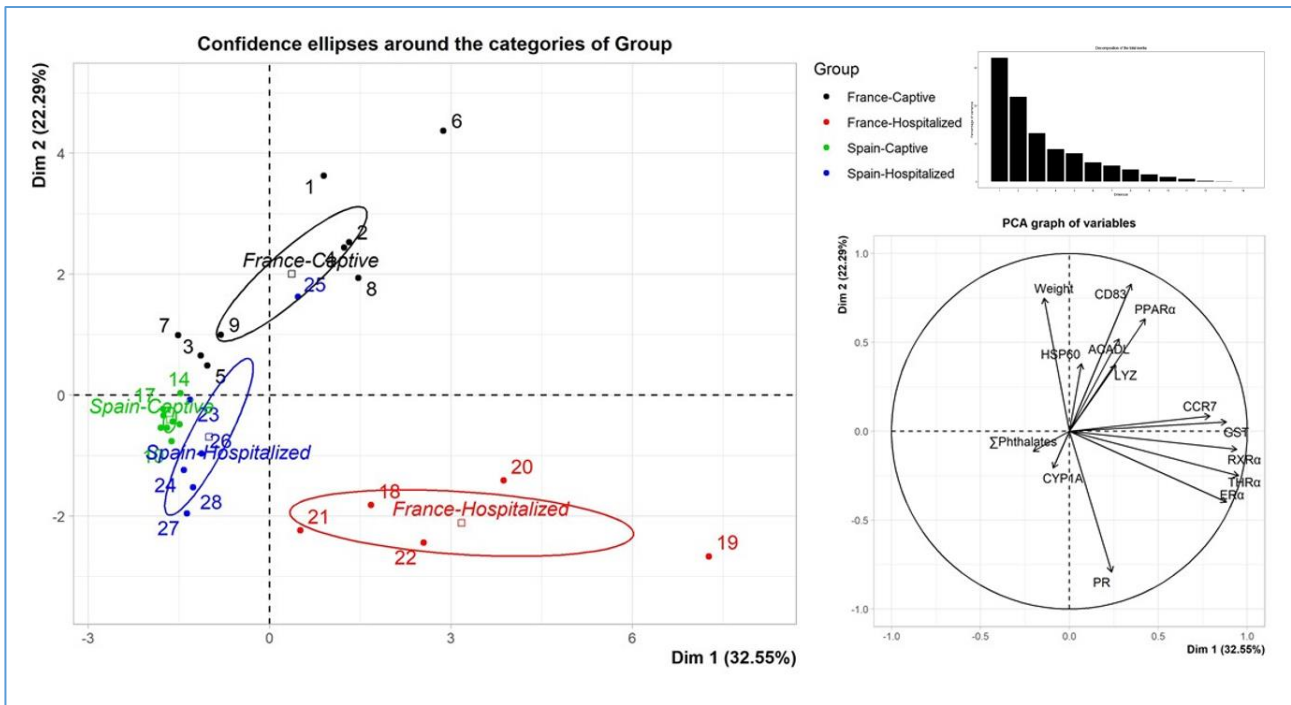
**Figure IV.19.** Levels of porphyrins in excreta of sea turtles collected in three different rescue centres (Spain, Italy, Turkey) and in an aquarium (France). a) copro-, uro-, proto-porphyrins and total porphyrins in sea turtles; b-c) porphyrins from hospitalized sea turtles from Turkey and Sardinia divided in individual with and without marine litter.

The multiple samplings of excreta over time after the hospitalisation of the sea turtles in the Turkish rescue centres reveals a high level of porphyrins in the first days of hospitalisation for several individuals (INDTR577, 579, 580, 581) probably related to the excretion of pollutants accumulated during the life in the wild (figure available in D2.11, p. 31).

### IV.7.3. Principal component analysis of the results on alive organisms: perspectives for the definition of ecotoxicological status

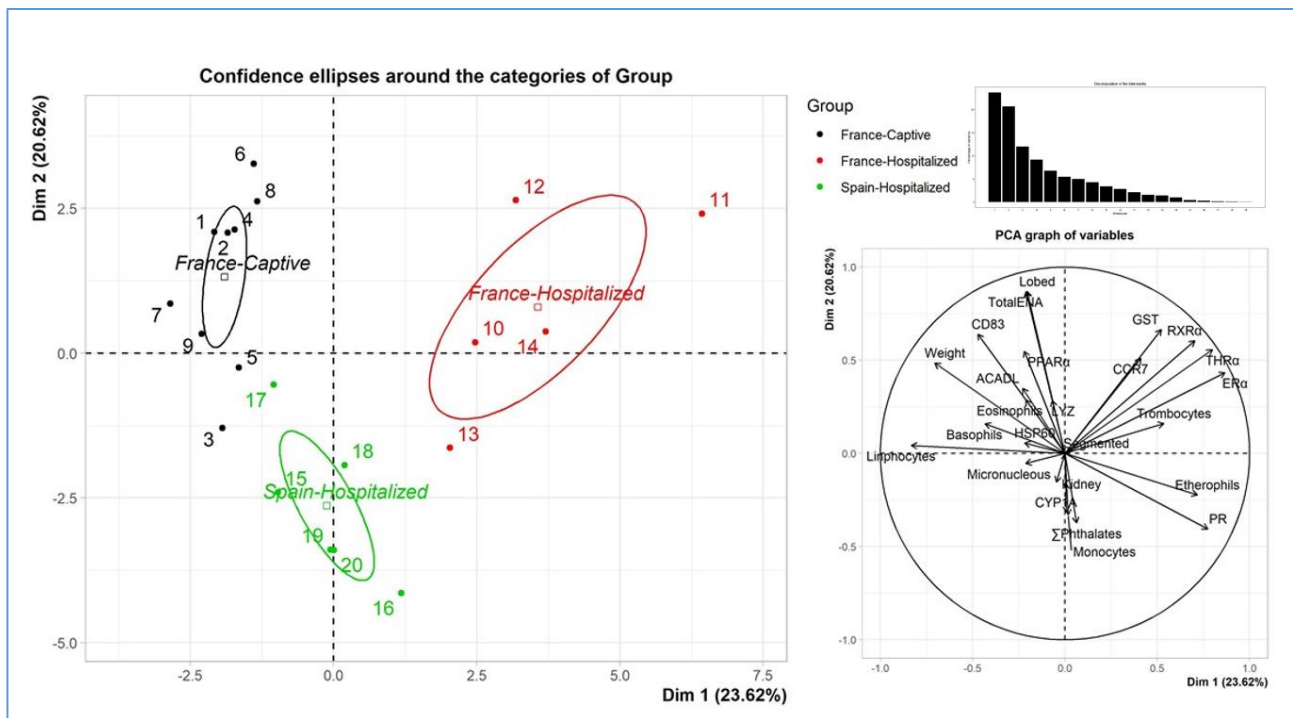
A principal component analysis has been performed to comprehensively analyse the data obtained from the alive sea turtles for which multiple data were present for the same specimen.

A first analysis was performed using the dataset of the twelve biomarker genes and the concentration of total PAEs in the blood of the same individuals. The samples available allowed to compare hospitalised (France and Spain) and captive organisms (Canary Island and French) (Fig. IV.18). Secondly, increasing the endpoints analysed (but decreasing the number of organisms due to the lack of appropriate tissues), a further analysis has been performed including also WBC count, ENA assay and biometric data. In this latter case, the Canary Island has been excluded from the analysis due to the lack of blood smear and excreta samples (Fig. IV.19).



**Figure IV.20.** Principal component analysis on the data from gene expression and PAEs.

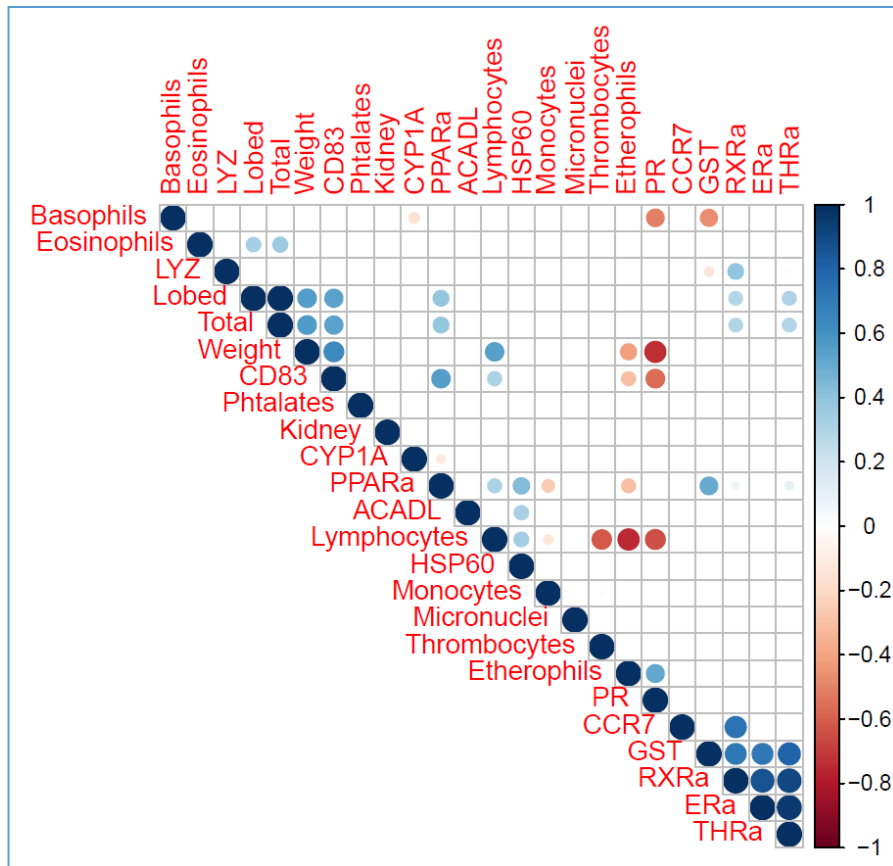
Both PCA analyses reveal clear clusters in the whole dataset according to the area (French/Spain) and condition (hospitalized/captive) and highlight which parameters mainly influence the segregation of the groups. The captive specimens show a narrow distribution of the data in comparison to the hospitalized specimen. Moreover, the French hospitalised specimens are statistically different for most of the endpoint investigated compared to the Spanish ones, suggesting a higher exposure to environmental stressors than the other Mediterranean specimens.



**Figure IV.21.** Principal component analysis on the data from gene expression, PAEs, WBC, ENA.

The correlogram built on the whole dataset from the analysis of living organisms in figure IV.20 shows the correlation among several endpoints. All the signals related to endocrine system (e.g. RXR $\alpha$ , THRa, Era) are correlated. The number of monocytes and the CCR7 genes are strictly correlated to the activation of the immune responses. Most of the alteration in the organisms analyzed are thus related to the immune system and the alteration of the endocrine-related genes being correlated, it is highly probable that effects are acting at different levels of the endocrine system.

The multiple-tier approach applied in such extensive way on this species appear to be successful in the definition of the ecotoxicological status of this species.



**Figure IV.22.** Correlation among all the endpoints analysed merging together all the data from alive samples. Blank square means the lack of statistically significant correlation. The circle means statistical correlation higher on the basis of dimension and intensity of colour. Blue: positive correlation, Red: negative correlation.

#### IV.7.4. Recommendations on ecotoxicological investigations

The main outcome of this research can allow drawing recommendation for the future investigation on this bioindicator species:

- The **multi-tier approach** (detection of marine litter ingestion, accumulation of PAEs, biological endpoints) allows **separating different populations** living in area characterised by different marine litter impact;
- The evaluation of the marine litter ingestion should **be complemented by the analysis of biological endpoints and health parameters** combined with plastic additive concentrations in order to define the “health status” of this species;
- The investigation on **both dead organisms and hospitalised organisms** can allow the evaluation at different level of the threat to a specific sea turtle population;
- **Cumulative stress** (including marine litter, climate change, exposure to legacy and emerging contaminants, etc.) to the sea turtles should be considered **for a correct definition of the health status** of this species.

## IV.8. Evaluating the relationships with the Programs of Measures for restoring the Good Environmental Status

Plastics constituted the most part of the ingested litter. USE SHE, USE FRAG and USE THR were the categories the most regularly found ingested in terms of both dry mass and abundance (Table IV.11). The ingested debris were soft and hard plastics, highly diverse (see fig. IV.2, p. 9, e.g., packaging, bags, cups, caps, cotton buds, lollipop sticks, balloons, finger rinse wipes, sanitary napkins, filters from waste treatment plants). The threadlike litter were generally items from fishing activities, especially fragments of lines and nets.

**Table IV.11.** Dry mass (grams) and abundance (number of pieces) of ingested material (litter and natural food and no food items), litter, plastics only and according to litter categories (population means  $\pm$  standard errors). The percentages are calculated according to the total ingested litter. Total abundance for all materials is not calculated since it could not be evaluated for food (FOO) and natural no food items (NFO). From Darmon et al., *publication in progress*).

Category	Ingested material	Ingested litter	Ingested plastics	IND	USE SHE	USE THR	USE FOA	USE FRAG	USE POTH	No plastics
Dry mass	26.52 $\pm$ 4.23 g	2.02 $\pm$ 41.27 g	1.95 $\pm$ 1.27 g	3.51 $\pm$ 0.53% (0.03 $\pm$ 0.015 g)	38.63 $\pm$ 1.17 % (0.21 $\pm$ 0.03 g)	17.75 $\pm$ 1% (0.12 $\pm$ 0.03 g)	4.97 $\pm$ 0.53 % (0.04 $\pm$ 0.009 g)	24.06 $\pm$ 1.03% (1.5 $\pm$ 1.26 g)	4.97 $\pm$ 0.53% (0.03 $\pm$ 0.007 g)	6.1 $\pm$ 0.64% (0.07 $\pm$ 0.02 g)
Abundance	-	6.7 $\pm$ 0.56	6.3 $\pm$ 0.53	3.51 $\pm$ 0.53%; 0.13 $\pm$ 0.03 pieces	45.75 $\pm$ 1.15%; 3.34 $\pm$ 0.32 pieces	22.64 $\pm$ 1.05 %; 1.19 $\pm$ 0.19 pieces	3.71 $\pm$ 0.4%; 0.18 $\pm$ 0.03 pieces	16.86 $\pm$ 0.83%; 1.27 $\pm$ 0.17 pieces	3.43 $\pm$ 0.41 %; 0.16 $\pm$ 0.03 pieces	4.48 $\pm$ 0.51%; 0.4 $\pm$ 0.11 pieces

The characteristics of the ingested litter can be used to assess the effectiveness of the associated Programs of Measures. For example, the frequency of occurrence and the quantity of USE SHE and USE FRA found in the turtles' digestive tract can be related to measures relative to the ban on single-use plastics (EU Directive 2019/904) (see table III.1 with the pilot areas). Differentiating the types of ingested litter will enable to assess the efficiency of several PoM with more accuracy. For this, we recommend to encourage stakeholder to note both the dry mass and the abundance (number of pieces) for each category and comment in the column "Notes" the type of item when it can be defined, especially for the category USE FRAG which is too simplified at this stage (e.g., fragment of plastic cork...). This category could be sub-divided in the near future in relation to the results obtained from turtles' digestive tracts and from objectives of PoMs. The abundance (number of pieces) can also be recorded, but with the highlighted caution regarding the counting of pieces (p. 61).

### IV.8.1 Updating GES scenarios

We tested various Good Environmental Status scenarios:

- i) the "Fulmar approach" (OSPAR Ecological Quality Objective scenario - OSPAR EcoQO) in the format "There should be less than X% of turtles with more than Y g (or pieces) of ingested plastics",
- ii) the "INDICIT approach" ([https://indicit-europa.eu/cms/wp-content/uploads/2019/09/INDICIT-Final-report\\_Final.pdf](https://indicit-europa.eu/cms/wp-content/uploads/2019/09/INDICIT-Final-report_Final.pdf)), which aims to consider the food remains in food remains as a proxy of health, which

can be written as “There should be not more than a ratio of x of ingested plastic litter / food remains in the digestive tract”. Other scenarios, derived from this one, were also tested (e.g., relative to the total ingested materials). Only the dry mass was considered since the natural food could not be numbered.

- iii) The “Descriptor 8 Contaminants” approaches which aims to make the link between health and litter ingestion (dose-response relationship). They can be written as “There should be less than X g (or pieces) of ingested plastics”, X being the threshold at which an impact on health is detected, according to GES definition.

In order to test these scenarios, we considered all data without stratification because our previous analyses showed that no biological factors had a significant influence on the probability to ingest litter (Table IV.4, p. 13-14). We considered only data after 2013 because 1) we aimed to limit the possible biases before this period, since the procedures for data collection were possibly not known (publication of MSFD guidance in 2013, Galgani et al., 2013) and necropsies for evaluating litter ingestion were probably not systematic, and 2) we based on MSFD 6-year cycle. We thus used the data from 2013 to 2018 for evaluating the thresholds (values X and Y of GES scenarios). The data collected during a new period (2019-2024) will allow evaluating the distance needed to reach the GES. We performed power analyses for evaluating the feasibility of the proposed scenarios.

All analyses were performed in collaboration with Marcus Schulz (AquaEcology, Germany). These results are being written for international peer-reviewed publication.

#### IV.8.1.1 Evaluation of the scenarios

According to previous workshops with international experts on the methods to assess the GES thresholds (Berlin, 2019), we used four approaches for setting threshold values, i.e. 10 percentiles, lower quartile, median, and arithmetic mean with 95% confidence interval.

For **setting thresholds as OSPAR EcoQO**, we considered previous data on 1) litter ingestion in sea turtles (Table IV.2, p. 10) showing that the lowest occurrence of litter ingestion and quantities of ingested litter were found in Eastern Mediterranean sea (Turkey), 2) litter distribution, with simulation models (Mansui et al., 2020) showing that the litter would be excluded from the Eastern Mediterranean sub-basin in Summer. This selected area thus served as pristine area (i.e. reference) and we used only data from Greece and Turkey from May to October. Both raw data and standardised data by dividing the mass and abundance of ingested plastics by Standard curved carapace length, were used.

**For the INDICIT scenarios**, the data from the entire Mediterranean were employed. The Atlantic data was not considered for this test in order to avoid possible differences in environmental mechanisms of litter distribution and impacts (even if they are not evidenced with the current dataset) and because of a lack of data to evaluate it separately. Standardisations by individuals’ size or body mass were obsolete, because by calculating the ratios of ingested plastics and food remains, standardisations were inherently considered.

**For the dose-response scenario**, we used permutational linear models with the Fulton’s K index as dependent (response) variable and plastic abundance and plastic dry mass as independent (explanatory) variables, which were the most significant in our previous results (Table IV.4, p. 13-14). Two logistic regression models with fat reserves as dependent categorical variable and plastic abundance and plastic mass as independent variables, respectively, were still tested. We also tested with data only from sea turtles of growth stages 2 and 3, in order to limit the differences among countries, regions and seasons. Regions and seasons, coded as integers, were used as additional explaining factors in all models.

Results for the OSPAR EcoQO approach are given in Table IV.12. For both, mass and abundance of ingested litter, the 10 percentiles, lower quartiles, and median values correspond to a zero value. Therefore, arithmetic means were selected for defining standardised and non-standardised threshold values. The scenario can be written as follows:

- There should be not more than 26% of individuals with > 2 pieces of ingested plastic litter
- There should be not more than 26% of individuals with > 0.32 g of ingested plastic litter (dry mass)

Considering the size of the individuals (unit of ingested litter in pieces or grams per size of the individual (in standard curved carapace length in cm)

- There should be not more than 25% of individuals with > 0.035 pieces of ingested plastic litter (abundance) / 10 cm (size of the individual)
- There should be not more than 25% of individuals with > 0.091g of ingested plastic litter (dry mass) / 10 cm (size of the individual)

**Table IV.12:** Descriptive statistics of the OSPAR Ecological Quality Objective approach (OSPAR EcoQO). CI is the confidence interval, N is the number of input data (From Darmon et al., *in progress*).

Parameter of ingested plastic litter	Period	10 percentiles	Lower quartile	Median	Arithmetic mean	95% CI of mean lower boundary	95% CI of mean upper boundary	N [-]
Abundance [counts]	2013-2018	0.0	0.0	0.0	1.69	0.85	2.52	105
	2019	0.0	0.0	1.0	3.76	-	-	127
Mass [g]	2013-2018	0.0	0.0	0.0	0.32	-0.016	0.65	105
	2019	0.0	0.0	0.002	0.9	-	-	127
Occurrence [%]	2013-2018	-	-	-	25.7	-	-	105
	2019	-	-	-	49.6	-	-	127
Standardized abundance [counts 10 cm <sup>-1</sup> ]	2013-2018	0.0	0.0	0.0	0.35	0.085	0.61	105
	2019	0.0	0.0	0.3	1.0	-	-	127
Standardized mass [g 10 cm <sup>-1</sup> ]	2013-2018	0.0	0.0	0.0	0.091	-0.027	0.21	105
	2019	0.0	0.0	0.00026	0.18	-	-	127

Deviations of arithmetic means in the year 2019 from the baseline period (2013-2018) are high: from 62.1% for occurrence to 185.7% for standardized abundance of ingested plastic litter (Table IV.13). There are high differences among countries. For most countries, such threshold values are far below the present levels of plastic ingestion found in turtles collected in 2019. Greece and Tunisia would have reached the threshold values given for mass and abundance of plastic in 2019. However, the number of replicates per country was very low and therefore, results are very uncertain. While a scenario considering the ratio of ingested plastic mass / food remains in the digestive tracks could provide a good proxy for evaluating the impacts of litter on individuals' health, the number of data on "food remains" was low (rarely collected and weighted/counted by stakeholders). Further data collection is needed to validate this approach. The model selection analysis shows that the scenarios based on OSPAR EcoQO approach provides the most powerful results (according to the quantity of

available data at the time of this selection). Using the abundance (number of pieces of litter) as predictive variable instead of the mass (dry mass) would also provide most accurate results. However, there was a possible misunderstanding in the methodology of enumeration that was underlined by INDICIT partners. For evaluating abundance (number of items) some stakeholders counted the number of all litter fragments found in the digestive tracks. Whereas other stakeholders counted these fragments but grouped them after having gathered the pieces which could maybe originate and have fragmented from a same item. INDICIT II consortium thus proposes to use the OSPAR scenario with the mass (because it was well standardised) as monitoring parameter. We are encouraging stakeholders in charge of data collection to also collect further data on both abundance (all fragments without reconstruction) and dry mass of food remain for future analysis.

**Table IV.13.** Distances to the OSPAR Ecological Quality Objective (EcoQO) given in Table IV.12 for each country region in the year 2019 (From Darmon et al., *in progress*).

Country region	Arithmetic mean of occurrence of plastic [%]	Arithmetic mean of mass of plastic [g]	Arithmetic mean of abundance of plastic [-]	Number of replicates [-]
Cyprus	26.30	-0.27	-1.69	25
France	60.01	5.83	18.81	7
Greece	7.63	-0.32	-1.52	6
Italy	74.30	0.37	6.31	1
Spain	74.30	2.61	5.25	17
Tunisia	24.30	-0.32	-1.69	22
Turkey	25.24	0.28	3.20	27

For the INDICIT scenarios (Table IV.14), 10 percentiles, lower quartiles and median values of both mass and abundance of ingested plastics correspond to the zero. Arithmetic means and median values were thus used to define the following three scenarios:

- According to mean values, there should be not more than a ratio of 0.0023 of mass of ingested plastic litter / total ingested materials,
- According to mean values, there should be not more than a ratio of 0.0215 of mass of ingested plastic litter / empty digestive tract,
- According to median values, there should be not more than a ratio of 0.028 of mass of ingested plastic litter / food remains.



**Table IV.14.** Descriptive statistics of the three INDICIT Scenario. N is the number of input data.

Ratio	Period	10 percentiles	Lower quartile	Median	Arithmetic mean	95% CI of mean lower boundary	95% CI of mean upper boundary	N [-]
Plastic litter / total ingested material [-]	2013-2018	0.0	0.0	0.0	0.0023	0.000092	0.0036	141
	2019	0.0	0.0	0.0	0.00082	-	-	72
Plastic litter / empty digestive tract [-]	2013-2018	0.0	0.0	0.0	0.021	-0.004	0.034	143
	2019	0.0	0.0	0.0	0.0025	-	-	72
Plastic litter / food remains [-]	2013-2018	0.0	0.0	0.028	181.3	38.3	324.3	260
	2019	0.0	0.0	0.02	1127	-	-	40

Deviations of ratios in the year 2019 from the baseline period are negative and range from -28.6% to -65.1%, indicating an amelioration of the situation in the recent past. However, the number of data collected in 2019 was very low (Covid-19 pandemic) and prevent an accurate assessment and interpretation. The arithmetic means appears to be a poor approach for setting the threshold values, because they would have already been achieved in 2019. The lower quartiles and median values are also not relevant, because they correspond to zero, which is not realistic within the next decades. Therefore, the INDICIT scenarios appear not relevant for setting threshold values at this stage because of the lack of data.

The Dose-response relationships scenarios could not be derived because of the weak performance of the models (Table IV.4, p. 49). All  $R^2$  were not significant or the relationships appear extremely weak. Only the permutational linear model with abundance of plastics as explaining variable revealed a good model fit ( $r^2 = 0.96$ ,  $p < 0.001$ ) but in fact plastic abundance had no significant influence on the Fulton's K index ( $p = 0.74$ ). Models could lack of robustness because more data is needed for highlighting the relationships and considering interactions among environmental and biological variables (Table IV.4, p. 49).

#### IV.8.1.2 Power analyses

Given the previous results on the GES scenarios, power analyses were calculated for the OSPAR EcoQO approach and the three INDICIT scenarios. All power analyses were carried out with the statistical software GPower 3.1 © University of Kiel, Germany).

The number of samples required to detect significant tendencies was obtained from power analyses, for assessing either significant reductions of the frequencies of litter ingestion and quantities of ingested (e.g., by a certain percentage for a given statistical power), or significant slopes for a given statistical power (10 %, 20 %, 30 %, 40 %, and 50 % decrease within six years). A randomised approach was used to calculate the minimum number of replicates necessary for the derivation of baseline values of abundance, mass and occurrence of ingested plastics. Data on abundance, mass and occurrence of ingested plastics were randomly selected from 2 to total number of data in the dataset, with 500 iterations for each variable in order to calculate means. The coefficients of variation (CV [%]) were calculated while increasing numbers of input data. The threshold of an acceptable CV was set to a value as low as 10% (Schulz et al., 2019) for ensuring a sufficient stability of the

baselines. Plotting CV against the numbers of input data resulted in minimum numbers of replicates necessary to obtain a maximum CV of 10%.

Given a threshold of a maximum CV of 10%, the numbers of minimum replicates (i.e. samples), necessary to obtain stable baselines, were calculated to 330, 740 and 55 for abundance, mass and occurrence of ingested plastics respectively. The monitoring efforts (number of samples to collect) to define baselines thus appears as mediocre for abundance, high for mass and low for occurrence of ingested plastics. For each of the three variables, the required monitoring effort is in a feasible range although abundance and occurrence appear as better indicators of plastic ingestion than dry mass, as agreed with the results of power analyses (see below).

A Bayesian approach (i.e. Markov Chains Monte Carlo (MCMC)) was performed to determine such baseline values including ranges of credibility. Three linear models with abundance, mass, and occurrence of ingested plastics as dependent variables, respectively were considered with region (Atlantic/Mediterranean) as regressor, and the intercept of each model was set to zero. MCMC were performed assuming Laplace distributions as priors, taking the data on ingestion as likelihood information, and calculating 20,000 randomised simulations for each model. Thus, estimates of mean and median values, as well as ranges of credibility of mean values were obtained.

The MCMC approach resulted in converged solutions of the simulations with Monte Carlo standard errors of 0.0 for each linear model. For the Mediterranean, mean baseline values of abundance, mass and occurrence of ingested plastics were calculated to 6.7 items, 2.9 g and 55.4%. For the Atlantic, the same values were 14.4 items, 0.6 g and 67.1%. Ranges of credibility, as given by the 10 and 90 percentiles of simulation results, were mediocre for abundance, wide for mass and narrow for occurrence of ingested plastics. Consequently, baseline values of occurrence and abundances are less uncertain than those of the dry mass of ingested plastics. In turn, from the viewpoint of reliability, occurrence and abundance are better indicators than mass of ingested plastics. However, ranges of credibility could even have been narrower with informative priors, such as normal distributions.

## IV.8.2 Recommendations

- The “OSPAR” scenario appears the most relevant at this stage (Table IV.12). Although the abundance of ingested plastics provides better powerful tests compared to the dry mass of ingested plastics, the INDICIT II consortium **recommends to conserve the dry mass for setting the threshold values** at this stage because this parameter is the most commonly collected by stakeholders with less confusion biases. We also encourage collecting data on abundance (number of pieces) with a careful training. The power analyses can be updated after collecting more data on all parameters.

- **GES update: There should be not more than 26% of individuals with > 0.32 g of ingested plastic litter (dry mass).**

- The “dose-response” scenarios do not yet provide accurate results, either because we are not able to properly assess health at this stage or because all individuals are affected and it is difficult to make the cause and effect relationship. To better assess the dose-response (or dose-effect) relationship, more data is needed and the definitions and modalities of health assessment should be discussed among experts and decision-makers. An assessment of the relation between litter ingestion, bio-indicators (e.g. fat, plastron shape, Fulton’s K index, etc.) and toxins in the tissues of Sea turtles is provided at IV.7. Even if some promising bio-indicators are proposed (e.g. porphyrin levels, immune system activity, p. 82), the selection and ability to record health parameters for a monitoring program remains to be tested.

- The INDICIT scenario appears relevant although there is a lack of data at this stage to provide powerful results. Our study on health, in connection with the models published in the literature (e.g., Marn et al., 2020), shows that the percentage of individuals with food remains in the digestive tract and the ratio of ingested litter relative to natural food could be relevant for evaluating long-term impacts on individuals in relation with population dynamics, and by extension species viability. We highly recommend to train stakeholders and systematically collect data on natural food for providing more accurate results in the near future.

- The assessments we have provided are based on the country level. The country does not necessarily result as a relevant unit with regard to the distribution of litter or that of the turtles. We had evaluated the seasonal variations in litter distribution from simulations at the entire Mediterranean scale (synergy with the MedSeaLitter project; Mansui et al., 2020), and highlighted sub-regions, according to different spatial scales (see INDICIT 1 final report: [https://indicit-europa.eu/cms/wp-content/uploads/2019/09/INDICIT-Final-report\\_Final.pdf](https://indicit-europa.eu/cms/wp-content/uploads/2019/09/INDICIT-Final-report_Final.pdf)). For example, **differentiating the Mediterranean into three (western, central and eastern basins) would possibly be more relevant.** Although we have established a collaboration with ACCOBAMS to consider data from aerial and boat observations during summer 2018 in almost the entire Mediterranean, we were not able to test this approach with the time allotted in the project. We were also unable to test this approach for the Atlantic region. A more powerful study will be possible when more data will be available in these separate areas.

## IV.9. Micro litter ingested by sea turtles

Although the ingestion of microplastics in marine turtles was included in Activity 4 in the proposal of the INDICIT II project, it was decided after the kick-off meeting to separate this analysis from the microplastics in fish due to large differences in methodologies and because not every partner was able to carry out this task: the first steps of the process need adequate facilities to avoid environmental contamination of the samples and these facilities were not available or operational in each country.

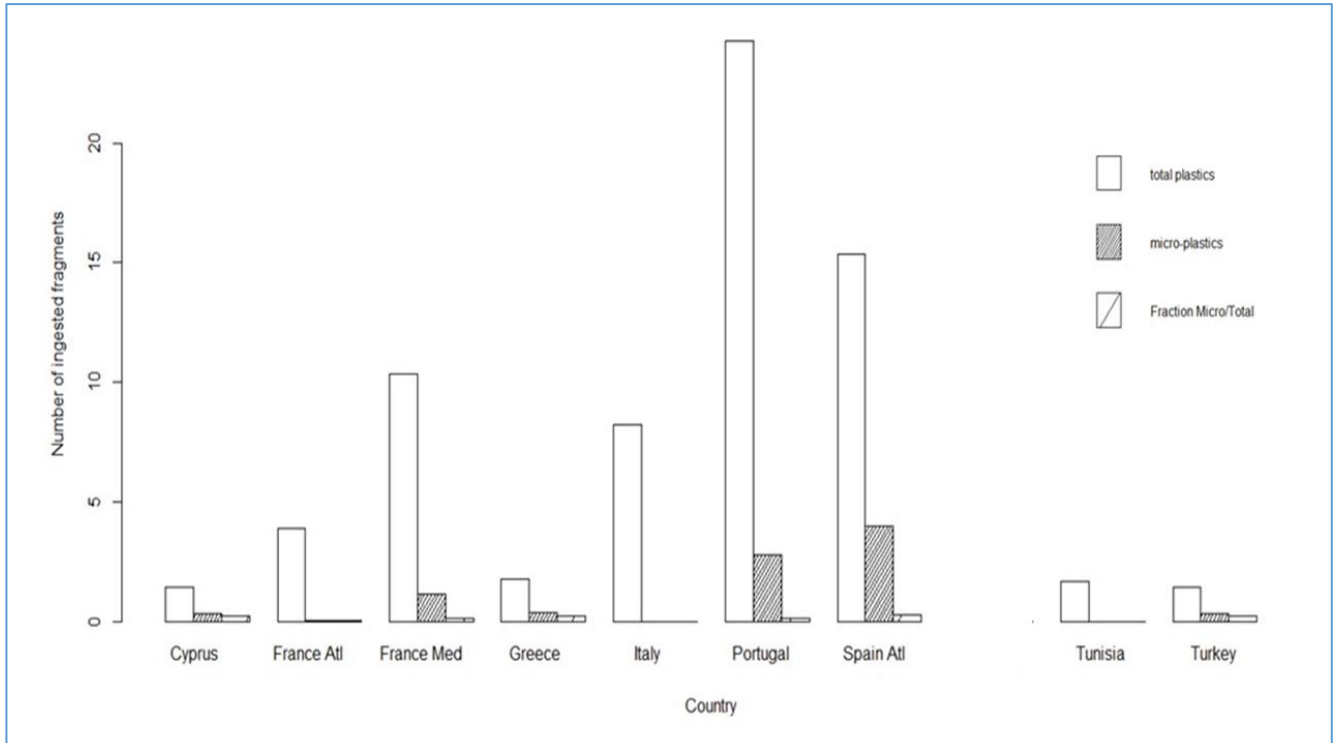
For these reasons, the study about microplastics in marine turtles was moved to Activity 2.

The ingestion of microplastics by sea turtles has been evaluated through two ways:

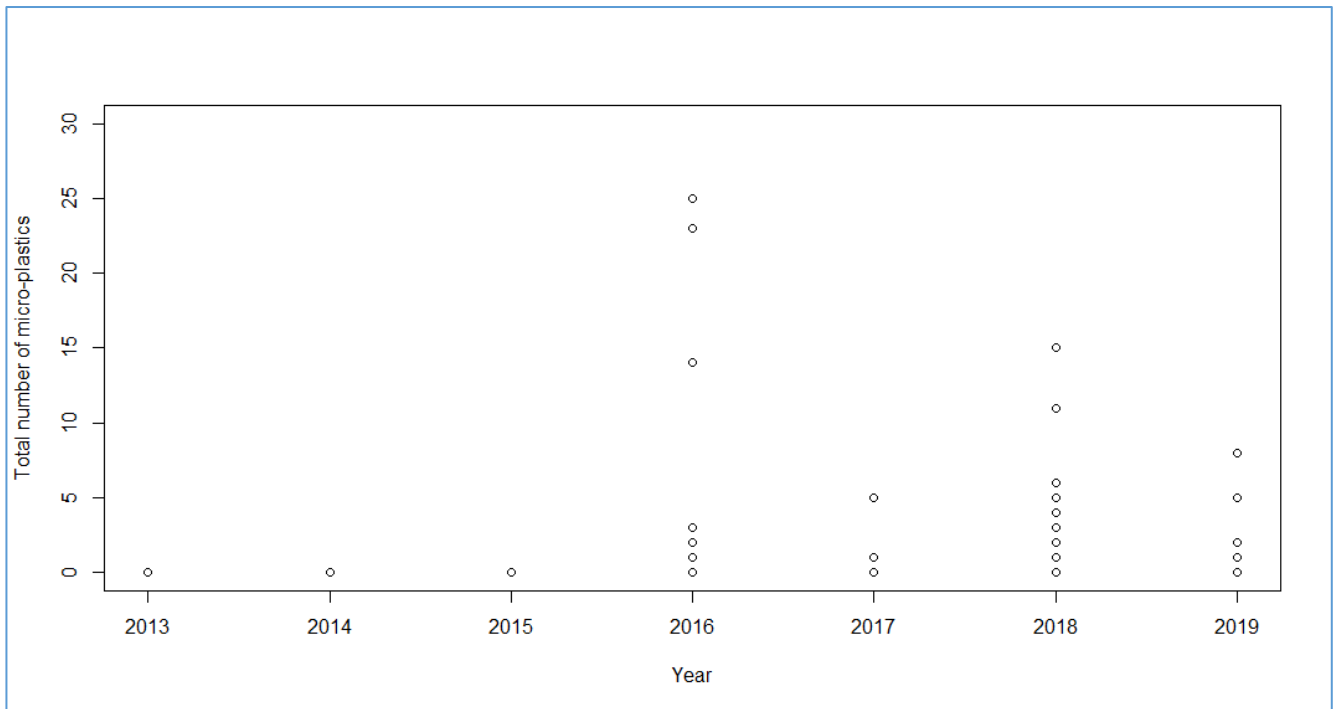
- 1) Collecting specifically the fraction 1-5 mm when evaluating the ingested plastics from the faeces and the digestive tract by affixing a second filter when rinsing the samples. This measure was proposed as optional for stakeholders in charge of data collection.
- 2) Performing dedicated laboratory analyses, with measures for avoiding environmental contamination of the samples. Not every partner was able to carry out this task because the material was not available or operational in each country.

### IV.9.1. Micro-litter ingested (fraction 1-5 mm)

The necropsied loggerhead turtles ingested an average of  $0.77 \pm 0.12$  pieces of microplastic (data from 1988; N=682), which represents 17.69% of the total abundance of ingested plastics. During the most recent period 2013-2019 (data continuing to be gathered after), they correspond to an average abundance of  $0.62 \pm 0.12$  pieces (15.75% of the total abundance of ingested plastics).



**Figure IV.23.** Total number of plastics (micro, meso, macro), total number of micro-plastics and fraction of micro-plastics on total plastics, per country. Averages from necropsied loggerheads (status 5, too putrefied was removed) collected during the period 2013-2019.



**Figure IV.24.** Total number of micro-plastics (minimum number of pieces) found in necropsied loggerhead turtles' digestive tract. Data have been collected only from 2016 encouraged with INDICIT project. The variability could be related to differences in methodologies for collecting data among stakeholders.

The fraction of microplastics among the total abundance of plastics ingested by sea turtles was not systematically collected. To differentiate the plastics from 1 to 5 mm, it is necessary to affix another filter and this adds a considerable time to data collection (and lab cleaning) while the whole procedure is already time-consuming. The number of microplastics is certainly a minimum number that is underestimated. Spatial (Fig. IV.28) or temporal (Fig. IV.29) variations are probably due to differences in methodologies between the stakeholders in charge of data collection, which is not systematic for all those involved.

#### IV.9.2. Micro-litter collection

Microplastics can appear in the digestive system either by direct ingestion or through the diet e.g. the ingestion of prey which have themselves ingested microplastics or through secondary contamination. The dissected digestive track has to come from recently died specimen.

Sieves of 5 mm, 1 mm and 200 µm mesh size were used during the washing of the content of the digestive track. The contents above the 5 mm were saved to study macro- and dietary debris. The contents below 5 mm were used to study microplastics.

The following standardised protocol was used:

- 1) Open the digestive tract and wash it on nested sieves. Basic sieves used were, 200 µm, 1 mm and 5 mm. The first one to retain parasites and microplastics, the second one for microplastics that measured more than 1mm and the last one in order to separate the macro-size fraction. When contents were very complex, an additional sieve (400 µm) was added.
- 2) Store contents in glass bottles with KOH 10% (potassium hydroxide). Amount of KOH solution added: three times the volume of the contents, approximately.
- 3) Filter under vacuum using a Büchner filter and Whatman GC/F microfiber filters in a type I laminar flow cabinet, ideally.

4) Identify and classify microplastics through observation with a stereomicroscope. It is especially important to discriminate between fibres, fragments (secondary microplastics) and primary microplastics.

5) Keep microplastics for further analyses (i. e. FT-IR spectroscopy) in small petri dishes or other suitable glass containers. If using petri dishes, close them with parafilm so air-borne contamination can be completely avoided.

6) Contamination and quality control: carry out procedural blanks. That is, some blank filters (one to three) should be treated as normal samples in order to assess contamination in the working space during the process.

Tap water is not free from microplastics. Filtering this water to wash the digestive tracts is not always possible, so samples of this water should be taken, filtered and observed to assess this contamination source. If microplastics are found, they should be subtracted from the samples accordingly.

If microplastics need to be cleaned with distilled/Milli-Q water or ethanol, they should be filtered previously.

Use natural fibre sponges to clean spaces and material to avoid contamination with plastic fibres.

Put a hood over the stereomicroscope when observing the filters to avoid air-borne contamination.

Avoid plastic material, use glass or stainless steel.

Very small microplastics and microfibers are difficult to manipulate. In this case, it is useful to stick a double-sided tape on the petri dish so you can immobilize them.

7) Micro plastic identification: Ideally, use a  $\mu$ -FT-IR or a FPA-FT-IR (Focal Plane Array – FT-IT). Otherwise, only the biggest microplastics could be analysed, as it was our case.

RAMAN spectrometers can also be used. However, environmental samples give a lot of fluorescence and it can interfere with the identification of microplastics. In addition, the lasers used can sometimes destroy the sample. By rinsing the microplastics with ethanol or distilled/milli-Q water, it is possible to reduce the interferences in the spectrometers.

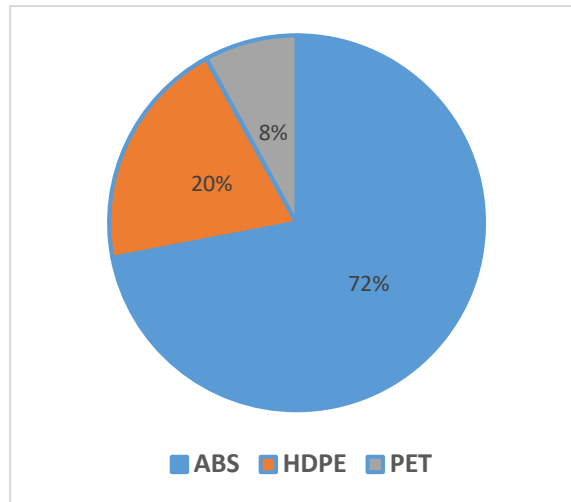
### IV.9.3. Identification of ingested polymers (Mediterranean Sea, Spain)

Once identified and classified, those items that were sufficiently large were analysed by Fourier transform infrared spectrometry (FT-IR), a spectrometer that allows the identification of the polymers of which the materials found are made. A library of reference spectra, developed in-house, was used for the identification. Only matches between the reference spectrum and the sample above 60% for turtles (70% for dolphins, Novillo et al., 2021) were accepted. Due to this criterion, two turtle samples that did not reach this percentage, and therefore had very low reliability, were excluded from the analysis.

Throughout the procedure, measures were taken to reduce possible external contamination of the samples by microplastics that could be found in the workspace.

The identification of microplastics was performed by FT-IR with 34 digestive track contents. Among them, 7 did not contain microplastics at all. The one with most microplastics had 21 items. The mean number of microplastics was  $4.88 \pm 6.86$  items/turtle.

Regarding the polymers analysed (Fig. IV.30), 72% were ABS (acrylonitrile butadiene styrene), 20% were HDPE (high density polyethylene) and 8% were PET (polyethylene terephthalate). These polymers are present in many plastic materials used by the population on a daily basis. This work is still in progress and new, more detailed results will be produced soon, including the increase of sample size and more detailed classification and quantification of the different types of items found.



**Figure IV.25.** Microplastic (< 5 mm) ingested by sea turtles (N=27) stranded and bycaught in waters of Valencia region (East Spain), in proportion of identified polymers (spectrometric analysis). ABS=acrylonitrile butadiene styrene; HDPE=high density polyethylene; PET=polyethylene terephthalate).

#### IV.9.4. Recommendations

- The INDICIT-II protocol used **recommend to select the range 1-5 mm to analyse the micro-litter**, as it was impossible to manage cross contamination for litter size < 1 mm.
- We recommend that **the indicator "Litter ingestion by sea turtles" includes all litter debris from 1 mm**, like the Fulmar indicator, in order to be able to compare the results of these two indicators in their common area (OSPAR). This litter size is also considering stakeholders' data collection habits.
- Applying the protocol well needs a specific facility which is not available (or operational) in each country. Evaluating the presence of micro litter in sea turtle is a complex task, and identification of micro plastic also need complex analysis (spectrometry).
- At this time, we consider that **this indicator cannot be implemented as a routine protocol** on most of the country considered.

## V. Implementation of the indicator “Entanglement in floating debris by sea turtles, birds and cetaceans” at the OSPAR and Barcelona RSCs and MSFD areas

### V.1. Resume of the pilot study

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”.

INDICIT project (2017-2019) conducted a feasibility study through the review of available grey, published literature and the responses of a questionnaire disseminated to experts in sea turtles, marine mammals, seabirds, fishes, or marine litter. The objectives were: 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 focused on identifying the networks and skills necessary to implement the monitoring of entanglement. All taxa were assessed 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. The main results obtained on the feasibility study are:

#### *a) Data on entanglement:*

Very few data on entanglement were reported in literature. The literature review showed that marine mammals, birds, fishes, and reptiles (sea turtles) have been recorded to suffer 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, life stage 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 for statistical analyses. We did not find any standard protocol and no standardised typology of the types and sizes of marine litter that cause entanglement.

#### *b) Types of litter:*

The litter causing entanglement came from various sources, but the fragments of abandoned fishing gear and human product packaging are the most frequent 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 gear 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.

#### *c) 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 seabed, 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.

#### *d) 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 and the existence of a network for the observation and the rescue of these species.
- In sea turtles: The characteristics described for seals were also true for sea turtles. Moreover, the presence of large network trained to collect data on litter ingested by the loggerhead turtle and the standard protocol



established and disseminated for the indicator “Litter ingested by sea turtles” (Indicator 1, INDICIT Project) should confer a direct benefit for the development of this new indicator on entanglement.

- In seabirds: 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.

*e) Networks:*

Entanglement was observed in a wide range of depths. The structures producing data in the European waters 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 needed 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.

*f) General resume of the feasibility study:*

Before INDICIT II project, the criteria D10C4 indicator « Entanglement » was not implemented in the MSFD area, as no standard protocol existed, nor specialised stakeholders were identified. The feasibility study of this Criteria D10C4 indicator was started during INDICIT program based on the review of available grey and published literature and the dissemination of a questionnaire between experts on sea turtles, marine mammals, marine birds, fish, and marine litter to identify potential networks and the state of the art about the indicator. At the beginning of INDICIT II, the assessment of entanglement was not accurate as partial information was based only on opportunistic data. There were constraints related to the methodology for the collection of specimens and intrinsic factors such as behaviour and life stages. There was also discrepancy on the classification of marine litter causing entanglement and particularly, differentiation between passive entanglement caused by litter and active entanglement due to bycatch on active fishing gear was unclear.

## V.2. Networking

### V.2.1. Situation at the beginning of the INDICIT II project

At the end of INDICIT project (February 2019), 106 stakeholders were contacted and involved on litter impact monitoring on sea turtles (indicator “litter ingestion”): 68 in the Mediterranean, 43 in the Atlantic and 6 in both basins. Several institutions which first role was the collection of specimens, were already involved in monitoring litter ingestion in sea turtles and sharing data. For those who provided information, they were:

- 36 stranding networks: in charge of the observation and recovery of dead or live turtles, sometimes managed by a rescue centre. Some institutions are 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, can report stranding events, such some NGOs (e.g. in Turkey), National parks in Greece or research and rescue centres in Italy.
- 28 rescue centres: in charge of medical care of live specimens, some also supervising stranding networks and performing themselves the necropsies of dead individuals. Several do a considerable work with fishermen to collect bycaught individuals.
- 10 transit centres: recovering specimens temporarily before rescue centres take charge of the individuals.
- 2 veterinarian centres: 1 veterinary school and 1 veterinarian laboratory, in charge of the necropsies of dead specimens and the evaluation of health status of the animals. Some research centres are also in charge of veterinarian analyses centres, such as the Istituto Zooprofilattico of Sicilia and Abruzzo/Molise, in Italy.
- 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 have also their rescue centre, such as IAMC-CNR in Sardinia Island in Italy, PAU-DEKAMER in Turkey. INSTM also managed a stranding network and a rescue centre in Tunisia.
- 2 institutions in charge of networking experts and of sharing information.

NOTE: some stakeholders are referenced 2 times due to double activities developed or activities conducted in different areas.

### V.2.2. Training sessions for collecting data on litter impacts on sea turtles

There were large difficulties in conducting training sessions with stakeholders during INDICIT II project due to COVID-19 pandemic situation. To solve this issue INDICIT II Partners disseminated the Standard protocol between stakeholders sharing the protocol by email and conducting several regional 4 online meetings (along 2021) and 1 face-to-face meeting (at the end of the project).

Italy-Sardinia (June 2021) - online meeting: Dissemination meeting focused on both indicators (ingestion and entanglement) for the Sardinian network, including all stakeholders and Vigilance Bodies. Coordinated by the INDICIT II Italian partner IAS-CNR.

Spain (14 June 2021) - online meeting: Dissemination meeting focused on both indicators (ingestion and entanglement), with national stranding networks, rescue centres and regional authorities. Coordinated by Spanish Minister (Marta Martínez-Gil, Marine litter Focal Point), in collaboration with INDICIT II Spanish partners (Ana Liria Loza, ULPGC and Jesús Tomás, UVEG).

Spain (13-14 October 2021) – face-to-face meeting (Valsaín, Segovia): Dissemination meeting focused on both indicators (ingestion and entanglement), with national stranding networks, rescue centres and regional authorities. Coordinated by Spanish Minister (Marta Martínez-Gil, Marine litter Focal Point), in collaboration with INDICIT II Spanish partners (Ana Liria Loza, ULPGC and Jesús Tomás, UVEG).

France (3 July 2021) - online meeting + live demo in a rescue centre (CESTMED): Dissemination meeting focused on entanglement indicator (Standard protocol dissemination) through national stranding networks. Coordinated

by the French stranding network and the Grau du Roi rescue centre in collaboration with INDICIT II French partners (Gaelle Darmon and Claude Miaud, EPHE).

Cyprus (June 2021) - online meeting: Dissemination meeting focused on both indicators (ingestion and entanglement), focus on improve data collection coverage. Coordinated by the Cyprus Wildlife Society (Simon Demetropoulos) and Meritta Rescue Centre.

### V.2.3. List of stakeholders involved on monitoring entanglement and conditions

INDICIT II partners contacted stakeholders involved in INDICIT project and with new stakeholders working with sea turtles, marine mammals and seabirds, throughout the project period, to try to involve them on data collection about entanglement.

This contact was conducted individually (email/phone, etc.) or during specific events such as conferences where INDICIT II was represented (e.g., ISTS in February 2019 in South Carolina, USA), or thanks to the facilitation by EAB and national representatives. INDICIT II consortium participated in several national and international workshops on the topic of Marine Litter, and co-organised some of them, e.g. ULPGC in MICRO 2020 international conference in November 2020 (online meeting). These events provided the opportunity to not only share INDICIT II outputs and NDICIT II protocol, but also enlarge networking.

Activity leaders supported by partners, listed all already or possibly involved stakeholders for monitoring and reporting data on “indicator Entanglement by sea turtles and other taxa” (Table 4, and table “Collaborators” on INDICIT website”). Moreover, activity leaders updated the Google map referencing all the stakeholders involved in monitoring entanglement on sea turtles and other taxa. This may show the spatial distribution of the networks and should facilitate contacts. This list and this map contain personal coordinates and thus cannot be shared in this report, but they are transmitted to DG Env. and national representatives.

**At the end of the project, 113 stakeholders have been contacted (69 in the Mediterranean, 41 in the Atlantic and 3 in both basins).** Several institutions which first role was the collection of specimens, were involved or interested to be involved in monitoring litter impact in sea turtles and sharing data. For those who provided information, they were:

- **38 stranding networks:** in charge of location and recovery of dead or live turtles, sometimes managed by rescue centres. Some institutions are structured in networks with voluntaries trained to carry out the specimens and conduct the first cares after the alert (e.g., RTMFF and RTMAE in France), or not directly structured in a network, but reporting stranding events, such some NGOs (e.g. in Turkey), National parks in Greece or research and rescue centres in Italy.
- **31 rescue centres:** in charge of medical care of live specimens. Some also supervising stranding networks and perform themselves the necropsies of dead individuals. Several do a considerable work with fishermen to collect bycaught individuals.
- **10 transit centres:** recovering specimens temporarily before rescue centres take charge of the individuals.
- **5 veterinarian institutions:** in charge of necropsies of dead specimens and the evaluation of health status. Some of them are also in charge of veterinarian analyses, such as Istituto Zooprofilattico Sicilia and Istituto Zooprofilattico Abruzzo/Molise, in Italy, or the University of Las Palmas (ULPGC) and University of Valencia (UVEG) in Spain.
- **34 research institutions,** in charge of data collection and analysis of samples focus on acquisition of knowledge, especially on sea turtles and litter impacts. Some of them are in direct contact or have their rescue centre (e.g. IAMC-CNR in Sardinia, Italy, and PAU-DEKAMER in Turkey). INSTM also managed a stranding network and a rescue centre in Tunisia.
- **11 regional or national authorities** in charge of marine environment.
- **3 institutions** in charge of networking experts and of sharing information.

NOTE: Some stakeholders are referenced two times due to double activities developed or activities conducted in different areas. Moreover, all the stakeholders described before are involved on the indicator “Entanglement”, but some of them are also involved in the indicator “litter ingestion”.

The organisation of the networks is highly variable among countries, where sometimes a referent at the regional and/or national levels exists. In some countries, an official stranding network does not exist, but there are local organisations to be alerted to collect dead or live individuals on the field. Manipulations of sea turtles generally require an official authorisation and training (e.g., “carte verte” in France, delivered by the French Environment Ministry).

The way to sustain networks is an important topic that was intensely discussed within the consortium as with other projects and representatives during INDICIT and INDICIT II projects. Among the stakeholders contacted by the INDICIT I-II consortium, some either did not reply, or did not want to be involved in the project, and others need to fill some specific conditions. The co-signing 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.

Stakeholders had also other requirements, such as (i) to be trained to standard monitoring, (ii) to receive the summary of the results found locally, and (iii) to participate in the research projects as partners or be invited in workshops as experts. An amendment of 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 the support and its objectives.

#### V.2.4. Recommendations for networking

- In certain areas/countries, **a better development of the network** will be necessary, by e.g. creating/reinforcing stranding networks and rescue centres, ensuring constant means and training referents or coordinators at the local/regional levels.

- **Engaging stakeholders in certain zones** is 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 mainland Portugal and Spain.

- For entanglement specifically, **the inclusion of pictures of individuals on stranding protocols** is the better way to achieve accurate databases that could be reviews by experts on marine litter to harmonise and avoid confusion on litter classification.

- **Some tools may support data collection**, in particular when a turtle is observed at sea or found stranded or bycaught, for example phone applications or online platforms, such as RedPROMAR App, developed by the Canary islands government, allowing citizen or institutions (NGOs, rescue centers, stranding networks) to post pictures with GPS locations; or ObsEnMer which offers a collaborative platform, managed by Cybelle Planete (France).

- **Key-stakeholders, such as fishermen**, who are key to the recovery of sea turtles in certain areas, should be involved. Including them for the monitoring of litter instead of directly for recovering bycatch individuals, could support the collaboration.

- Arrange regional/national or international **workshops among stakeholders**, together with other experts and with representatives, could facilitate the involvement of new stakeholders. These workshops should propose training sessions and sharing experience on e.g. methodologies to monitor litter impacts, which could still be optimised.

- For a better reporting of information and data, **a diagram of the networks should be built** at the national and regional levels, considering specificities of each country. These diagrams should be disseminated to stakeholders in order to facilitate procedures and contacts.

## V.3. Standardised protocol for monitoring entanglement

### V.3.1. Development of a common methodology

Based on the results obtained on the pilot study conducted on INDICIT project (2017-2019), a common protocol or standardised methodology is required to collect data on litter entanglement of marine fauna in the European waters.

#### V.3.1.1 Main constraints

##### *a) Difficulties to distinguish entanglement from bycatch:*

The main constraint observed was the difficulty found by partners and stakeholders to distinguish between entanglement on fishing gears (ghost nets) and bycatch in active fishing gears (e.g., animals escaped with a part of the gear after an accidental capture). Many hours of discussions between partners and stakeholders in relation with this subject took place during the first months of the project.

To solve this problem, INDICIT II Consortium, decided to:

- Establish adequate definitions of entanglement and other concepts related (Table V.1),
- Establish different criteria to support distinguish entanglement from bycatch (Table V.2): Several criteria have been established for each type of cases (entanglement, bycatch, doubtful cases, and accidental catch in active structures), and one or more social media links and pictures has been disposed in the protocol to facilitate distinction.
- Verify criteria and definitions on images obtained from opportunistic platforms: An important source of data has been found on social media, where citizens, NGOs, associations, or institutions share videos and images from marine fauna found at sea or stranded. A deep review of images obtained from social media and opportunistic platforms has been conducted to verify the utility of criteria to solve the difficulties to distinguish entanglement from bycatch. The important results obtained in this review are described below.

**Table V.1.** List of definitions related to entanglement established by INDICIT Consortium.

**Marine litter (European Commission):** Items that have been deliberately discarded, unintentionally lost or transported by winds and rivers, into the sea and on beaches.

**Ghost gear:** any fishing gear that has been abandoned, lost, or discarded in the sea. There are many reasons why fishing gear can be lost or abandoned, including severe weather, snags beneath the surface, conflict with other gear, interaction with other vessels and, rarely, intentional discard when no other options are available.

**Entanglement (INDICIT II):** The process of being wrapped, trapped, or stuck in marine litter.

**Bycatch (European Commission):** The inadvertent catch of organisms that were not specifically targeted by a fishing operation (for example, non-target fish species, marine mammals, seabirds) that are either discarded or landed for commercial sale (Delgado et al., 2003).

**Doubtful cases:** when the item trapping the animal is not present or is not possible to ensure the distinction between entanglement in marine litter and bycatch in active fishing gears. (These cases should be also registered and included in the databases).

**Accidental catch in active structures:** The process of being wrapped, trapped, or stuck in anthropogenic structures disposed at sea for any other uses than fishing activities (E.g., anchoring structures, signalling structures, etc.).

**Table V.2.** Criteria established by INDICIT consortium to support distinguish entanglement from bycatch.

<p><b>1 - Criteria to identify Entanglement in marine litter:</b></p> <ul style="list-style-type: none"><li>– <u>Litter from land-based sources</u>: e.g. Packing straps, plastic bags, synthetic raffia sacks, etc.</li><li>– <u>Degradation of materials</u>: Degraded material indicates that the item is not suitable for use or has not been used for a long time. Therefore, should be considered as litter.</li><li>– <u>Biofouling attached</u>: Presence of biota attached indicates that the item has not been used for a considerable time. For this reason, active fishing gears rarely present biota attached, except in aquaculture gears.</li><li>– <u>Medium/small animals (turtles, seabirds, seals, small cetaceans) trapped on large fishing gear</u>: fishers are unlikely to discard a whole large gear due to the bycatch of medium/small animals, and medium/small animals are not strong enough to pull a whole large fishing gear.</li><li>– <u>Mix of different fishing gears or/and other marine litter</u>: Several materials mixed indicate that they have been circulating for long time on the surface and are therefore considered as litter.</li><li>– <u>Morphology distortion observed on the animal</u>: caused by long term entanglement.</li></ul> <p><b>2 - Criteria to identify Bycatch on active fishing gear:</b></p> <ul style="list-style-type: none"><li>– <u>Animals clearly bycaught by the fishing gear</u>: animals accidentally caught during active commercial or recreational fisheries, or directly send/delivered by fishers due to bycatch in their own gears.</li><li>– <u>Ingested hook</u>: Animal bycaught and released after cutting the line.</li><li>– <u>Heavy animals (whales) trapped on large fishing gears</u>: fishers could discard a whole gear if a large / heavy animal is caught. Also, large / heavy animals are strong enough to pull a whole fishing gear.</li><li>– <u>Accessory structures of fishing gears</u> (ex. ropes and buoys attached to pots): Animals could be trapped when the gear is working, or not, but it is a direct interaction with active fishing gears.</li></ul> <p><b>3 – Criteria to identify Doubtful cases:</b></p> <ul style="list-style-type: none"><li>– <u>Animal with typical injuries (flipper lacerations, throttle, etc.) but no material present</u>: Injuries could be caused by an active fishing gear or by entanglement on marine litter. In these cases, local scientific expertise could support the identification, or could be included as doubtful if distinction cannot be assured.</li><li>– <u>When the item trapping the animal is difficult to identify as fishing gear</u>.</li><li>– <u>Any other doubtful case that could not be solved by the rest of criteria</u>: For example, animal trapped on clean and non-degraded net.</li></ul> <p><b>4 - Criteria to identify Accidental catch in active structures (not related to fishing activity):</b></p> <ul style="list-style-type: none"><li>– <u>Animals entangled on any other structure disposed at sea but not related with fisheries</u>: For example: anchoring structures, nets to keep algae blooms, jellyfish protection nets, shark protection nets, etc.</li></ul>
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*b) Standardise the list of litter typologies involved on entanglement:*

One of the main constraints found was the great variety of “names” given to litter typologies by partners and stakeholders, not only based on the lingual differences between countries, but also between stakeholders from the same country.

Many difficulties were found in finalising the list of litter typologies for entanglement. The Activity 3 coordinators generated the list of litter typologies based on the MSFD litter classification, extracting the items susceptible to be involved on entanglement. The first draft has been shared with stakeholders, which asked to reduce the list of litter typologies to do it more practical and useful to be used on the field.

At the same time, first proposal of litter classification has been shared with the Technical Group on Marine Litter (TGML) from the MSFD, was trying to homogenise the litter classifications established by different projects, programs, and conventions from the European Union (OSPAR, Barcelona, etc.). The work conducted by the TGML to homogenise litter typologies was very complex and delayed the approval of the final list of litter typologies for entanglement to finalise the Standard protocol to monitor entanglement.

To solve this problem, the INDICIT II Consortium, decided to:

- Use the litter typologies established by the MSFD and updated by TGML group: Based on the last versions of litter typologies developed by the TGML group, the INDICIT Consortium has extracted the specific typologies susceptible to be involved on entanglement (Table V.3),
- Reduce the list of litter typologies in collaboration with stakeholders to facilitate data collection on the field: Many stakeholders need to be involved to monitor entanglement (Stranding Networks and Rescue Centres) in the European waters, where many different people are collecting data from each stranding event (stranding or found at sea). Reducing the list of litter typologies is important to support stakeholders on data collection, achieving a more practical use to obtain a larger amount of data.
- Include codes from different programs in the list of litter typologies involved on entanglement: to facilitate comparisons with other projects or programs (floating litter, beach litter, etc.).
- Verify the list of litter typologies for entanglement proposed by INDICIT II Consortium with images obtained from social media and opportunistic platforms: An important source of data has been found on social media, where citizens, NGOs, associations, or institutions share videos and images from marine fauna found at sea or stranded. A deep review of images obtained from opportunistic platforms has been conducted to verify the litter typologies established. The important results obtained in this review are described below.

**Table V.3.** Final list of litter typologies for entanglement established by INDICIT II.

LITTER CATEGORIES (entanglement)		DESCRIPTION	TGML CODES
Fisheries, aquaculture and maritime sources	Fishing nets	nets and pieces of nets	G52, G53, G54, G56
	Fishing lines	monofilaments used on fishing gears (nylon)	G59
	Ropes, string, cords	maritime ropes, strings, cords	G50, G49
	Floats, buoys, fenders	usually associated with nets, ropes, lines	G62, G63, G64
	Pots, tops and traps	for different species; different materials	G42, G44, G163, G184, G207
	Aquaculture nets	equipment for holding or protecting shellfish	G45, G46, G47
	Other fishing related	fish box, baits, lures, weights, etc.	G57, G58, G60, G61, G92, G164, G170, G182
Packing related	Plastic bags	shopping/grocery, dog faeces, fruit bags, etc.	G2, G3, G4, G5
	Mesh bags	plastic mesh bags for vegetable, fruit, etc.	G2, G37
	Heavy-duty sacks	animal feed, fertilizers, garden rubbish, etc.	G2, G36, G85
	Packing-rings	4/6-pack yokes & six-pack rings	G1
	Strappings bands	packing strappings bands	G66

	<b>Plastic greenhouse sheeting</b>	plastic sheeting used to cover greenhouses	
	<b>Food packing</b>	food packets / wrappers, caps and lids, etc.	<b>G20</b> , G30, G31, G21, G22, G23, G24, G18
	<b>Other packing</b>	corks, crates, boxes, baskets, pallets, etc.	<b>G170</b> , G67, G159, G160, G162
<b>Other land-based source</b>	<b>Textile</b>	clothing, footwear, hessian sacks, etc.	<b>G135</b> , G136, G137, G138, G139, G140, G143, G145
	<b>Medical and hygienic care</b>	gloves, bandaging, sanitary towels, etc.	<b>G135</b> , G40, G41, G144, G211, G133
	<b>Recreational related</b>	toys, balloons, balls, fireworks remain, etc.	G32, G125, G126, G155, G167
	<b>Other land-based</b>	cable ties, rubber bands, foams, etc.	G93, G87, G43, G131, G73, G194
<b>Unknown / not identified</b>		no data available about litter	

c) *Measure the impact of entanglement on the animal health*: Entanglement in marine litter is a global problem that results in the death or severe welfare problems of thousands of marine animals worldwide every year (Werner et al. 2016). Immediate death can be caused by direct asphyxiation, preventing animal resurfacing (mainly on small animals trapped on large or heavy items); ligatures around the neck or occlusion of the cetacean's blowhole (Cassoff et al., 2011); or reducing ability to escape from predators or ship strikes (Beck and Barros, 1991; Butterworth et al., 2012). Also, severance of the carotid artery by ingrown ligatures is known particularly for seals (DeLong et al., 1990) and haemorrhaging and debilitation due to severe damage of tissues, including laceration of large blood vessels, has been observed in whales (Cassoff et al., 2011).

Furthermore, entanglement may cause chronic or sub lethal effects that alter the biological and ecological performance of an individual over time, including reduced mobility, agility, or ability to ingest or digest food. All of which lead to reduced fitness, reproductive success and mobility. Tissue damage, such as skin lesions with ulcerations, or death of muscle tissue (necrotising myositis) is a widespread result of entanglement (CMS, 2014; Oros et al., 2005). Rope and line ligatures can cause amputation or wounds that leave sites open to infection, further reducing the likelihood of survival. For example, in turtles, entanglement usually results in flipper loss, where one flipper loss appears not to reduce their geographical range, whereas two flipper losses severely limit diving, feeding and nesting abilities. In addition, flipper stumps are vulnerable to further attacks by predators (Carrington, 2013). As the animal grows, entanglement loops cut into the skin, muscle and sometimes even bone. The loss of fins and tails on whales, dolphins, porpoises, or sharks is unlikely, but tail damage and deformation have been observed, and if it affects the bilateral axis of symmetry along the spine (midline), it is considered a very serious injury (Andersen et al., 2008).

To solve this important constraint and try to measure the impact of entanglement on individual health, the INDICIT II consortium decided to:

- Create a new parameter, "Impact severity" based on the effect of injuries caused by entanglement on the animal viability, with a scale of 5 degrees:

1. **No injuries**: When the animal does not present external injuries.



2. **Minor:** Minor injuries that could heal quickly on their own. For example, mild skin abrasions or slightly cuts affecting only the epidermis (scratch).
3. **Medium:** Deeper injuries that will take longer to heal. For example, deep cuts crossing epidermis and dermis, throttle on the neck causing suffocation of the animal (no death), or dermatitis affecting a large area of animal body.
4. **Severe:** Serious injuries that alter the natural body condition of the animal. For example, amputations, bone fracture (flippers or carapace), eye loss or body deformations caused by the affection.
5. **Extreme:** Injuries that kill the animal (natural death or euthanasia in the rescue centre) or do not allow to reintroduction of the animal on their natural habitat (unrecoverable animals).
6. **Unknown:** No data available about injuries

- Verify the utility of this new parameter with images obtained from social media and opportunistic platforms. Results obtained are described below.

#### V.3.1.2 Review of images obtained on social media and opportunistic platforms:

A specific protocol (Social media Protocol for entanglement) has been developed to conduct an in-depth search of images related to entanglement on social media and opportunistic platforms. This protocol (deliverable D3.9) has been shared with partners to conduct the search in their respective regions and countries.

At the same time, and due to the COVID-19 situation, the INDICIT II consortium launched an online campaign on social media, the INDICIT CHALLENGE, for any citizen, who was confined at home, to help us obtain images related to entanglement. The campaign is described in the section II.1, 28, and 97 images from marine fauna entangled on marine litter were obtained.

Finally, an important amount of data has been obtained, with more than 800 links with images from 743 individuals of marine fauna (538 sea turtles, 144 cetaceans, 46 seabirds, 10 seals and 5 from other taxa), where 415 individuals (55,9%) were entangled animals (Table V.4).

**Table V.4.** Number of total and entangled individuals obtained from social media and opportunistic platforms, arranged by country and taxa.

	SEA TURTLES		CETACEANS		SEABIRDS		SEALS		OTHER TAXA		
	<i>N</i>	<i>n entang (%)</i>	<i>N</i>	<i>n entang (%)</i>	<i>N</i>	<i>n entang (%)</i>	<i>N</i>	<i>n entang (%)</i>	<i>N</i>	<i>n entang (%)</i>	
<b>ATL</b>	<b>France</b>	7	4 (57,1%)								
	<b>Portugal</b>	29	18 (62,1%)	73	11 (15,1%)	36	22 (61,1%)	1	1 (100%)	5	3 (60,0%)
	<b>Spain</b>	288	219 (76,0%)	59	6 (10,2%)	3	2 (66,7%)				
<b>MED</b>	<b>France</b>	2	0 (0,0%)								
	<b>Spain</b>	38	28 (73,7%)	1	1 (100%)						
	<b>Italy</b>	28	24 (85,7%)	5	3 (60,0%)	1	1 (100%)				
	<b>Greece</b>	101	33 (32,7%)	3	1 (33,3%)	6	6 (100%)	8	1 (12,5%)		
	<b>Tunisia</b>	1	1 (100%)								
	<b>Cyprus</b>	6	6 (100%)								
	<b>Turkey</b>	38	23 (60,5%)	3	0 (0,0%)			1	1 (100%)		
<b>TOTAL</b>	<b>538</b>	<b>356 (66,2%)</b>	<b>144</b>	<b>22 (15,3%)</b>	<b>46</b>	<b>31 (67,4%)</b>	<b>10</b>	<b>3 (30,0%)</b>	<b>5</b>	<b>3 (60,0%)</b>	

Clearly a larger number of images from sea turtles were found on social media and opportunistic platforms, with high percentages of entangled animals (66,2%). The percentage of entanglement on seabirds was also high (67,4%), contrary to the low percentage of entangled cetaceans found (15,3%), despite the high number of images (144 individuals), probably caused by the large number of animals affected by bycatch (Table V.5). The number of images from seals and other taxa were very low.

On other hand, on some countries very few images were found in social networks, such us France (7 in the Atlantic and 2 in the Mediterranean basins) and Tunisia (only 1), probably because people from these countries are less likely to use social networks. The low percentages of entanglement observed on Greece (32,7% on sea turtles, 33,3% on cetaceans, and 12,5% on seals) was caused because there were found large number of animals affected by direct human interaction (severe strikes on the head on sea turtles and deep mortal cuts on the neck on dolphins and seals).

In general, the highest number of entangled animals were found on sea turtles in the Canary Islands (Atlantic Spain) (288 individuals, 76% entangled). Entanglement on sea turtles from the Spanish Mediterranean and Italy was also high (73,7% and 85,7% respectively). Despite the low number of images found on Cyprus, all the animals were entangled.

**Table V.5.** Number (n) and frequency (%) of individuals from different taxa obtained from social media and opportunistic platforms, classified by type of event (entanglement, bycatch, doubtful and other cases) based on definitions and criteria proposed by INDICIT II.

	<b>Entangled</b>	<b>Bycatch</b>	<b>Doubtful</b>	<b>Other</b>	<b>No Data</b>
	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>
SEA TURTLES (N = 538)	346 (64,3%)	79 (14,7%)	9 (1,7%)	81 (15,1%)	23 (4,3%)
CETACEANS (N = 144)	16 (11,1%)	66 (45,8%)	6 (4,2%)	6 (4,2%)	50 (34,7%)
SEABIRDS (N = 46)	18 (39,1%)	1 (2,2%)	13 (28,3%)	11 (23,9%)	3 (6,5%)
SEALS (N = 10)	3 (30,0%)	0 (0,0%)	0 (0,0%)	4 (40,0%)	3 (30,0%)
OTHER - Shark (N = 2)	0 (0,0%)	0 (0,0%)	2 (100%)	0 (0,0%)	0 (0,0%)
<b>Total (N = 740)</b>	<b>383 (51,8%)</b>	<b>146 (19,7%)</b>	<b>30 (4,05%)</b>	<b>102 (13,8%)</b>	<b>79 (10,7%)</b>

*a) Verifying the difficulty to distinguish entanglement from bycatch:*

The availability of large number of images found on social media and opportunistic platforms has allowed the verification of the utility of the definitions and criteria proposed by the INDICIT consortium to distinguish entanglement from bycatch.

A different degree of difficulty was observed depending on the animal taxa. For example, the higher number of doubtful cases observed on seabirds (28,3%) has been caused because many of the animals were “entangled” on fishing lines with hooks externally embedded in the body of the animals (not ingested). In these cases, the animal could be caught when the fishing gear was actively working, or when the fishing line with their hooks has been lost by fishermen and stranded in the shores as litter. On other hand, there is a clear large number of cetaceans affected by bycatch (45,8%), that were easily differentiated on the images reviewed.

In general, the criteria disposed by the INDICIT consortium were very useful to distinguish entanglement from bycatch when images are available, where in general only 4% of the cases were classified as doubtful.

Within the different species of sea turtles (Table V.6), the greatest difficulty was found with the leatherback turtle (*Dermochelys coriacea*), presenting the higher percentage of doubtful cases (18,2%). This difficulty was based on:

1. All the animals were only affected by materials derived from fisheries and maritime sources (no land-based litter).
2. The large size of the animals makes them strong enough to trawl an active fishing gear.
3. The animals are so heavy (around 300-500kg), that fishermen could cut the fishing gear to take them out of the gear.

However, no doubtful cases have been found on green turtles and few doubtful cases has been found on loggerhead turtles (1%).

In conclusion, we could confirm that the definitions and criteria established by INDICIT II consortium are useful to distinguish entanglement from bycatch, mainly when images from each event are available.

**Table V.6:** Number (n) and frequency (%) of sea turtles from different species obtained from social media and opportunistic platforms, classified by type of event (entanglement, bycatch, doubtful and other cases) based on definitions and criteria proposed by INDICIT II.

	Entanglement n (%)	Bycatch n (%)	Doubtful n (%)	Other n (%)	No Data n (%)
<i>Caretta caretta</i> (N = 484)	327 (67,6%)	67 (13,8%)	5 (1,0%)	70 (14,5%)	15 (3,1%)
<i>Chelonia mydas</i> (N = 26)	11 (42,3%)	6 (23,1%)	0 (0,0%)	8 (30,8%)	1 (3,8%)
<i>Dermochelys coriacea</i> (N = 22)	6 (27,3%)	3 (13,6%)	4 (18,2%)	3 (13,6%)	6 (27,3%)

*b) Verify the list of litter typologies for entanglement proposed by INDICIT II Consortium:*

The availability of a large number of images of entangled animals (N = 415) has allowed to check whether the list of litter typologies proposed by the INDICIT II consortium include all the real litter typologies that are impacting marine fauna on the European waters (Table V.7 and fig. V.1).

**Table V.7:** Number (n) and frequency (%) of animals from each taxon entangled by litter typologies established by the INDICIT II consortium. Data from images obtained on social media and opportunistic platforms. (Red squares = litter typologies not found or only 1 individual).

	ALL TAXA (N = 415)	TURTLES (N = 356)	CETACEANS (N = 22)	SEABIRDS (N = 31)	SEALS (N = 3)	OTHER (N = 3)
Fisheries & maritime sources	259 (62,4%)	209 (58,7%)	16 (72,7%)	28 (90,3%)	3 (100%)	3 (100%)
Fishing nets	121 (29,2%)	105 (29,5%)	9 (40,9%)	3 (9,7%)	2 (66,7%)	2 (66,7%)
Fishing lines	89 (21,4%)	62 (17,4%)	5 (22,7%)	20 (64,5%)	1 (33,3%)	1 (33,3%)
Rope, string, cord	118 (28,4%)	104 (29,2%)	7 (31,8%)	7 (22,6%)	0 (0,0%)	0 (0,0%)
Floats, buoys, fenders	46 (11,1%)	42 (11,8%)	4 (18,2%)	0 (0,0%)	0 (0,0%)	0 (0,0%)
Pots, tops, traps	1 (0,2%)	1 (0,3%)	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)
Aquaculture related	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)
Other fishing related	34 (8,2%)	14 (3,9%)	1 (4,5%)	18 (58,1%)	0 (0,0%)	1 (33,3%)
Land-based sources	128 (33,3%)	132 (37,1%)	5 (22,7%)	1 (3,2%)	0 (0,0%)	0 (0,0%)
Plastic bag	11 (2,7%)	8 (2,2%)	2 (9,1%)	1 (3,2%)		
Mesh bags	2 (0,5%)	2 (0,6%)	0 (0,0%)	0 (0,0%)		

Heavy-duty sacks	113 (27,2%)	113 (31,7%)	0 (0,0%)	0 (0,0%)		
Packing rings	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)		
Packing strapping bands	7 (1,7%)	7 (2,0%)	0 (0,0%)	0 (0,0%)		
Plastic sheeting greenhouse	1 (0,2%)	1 (0,3%)	0 (0,0%)	0 (0,0%)		
Food packing	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)		
Other packing	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)		
Textile	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)		
Medical and hygienic care	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)		
Recreational related	3 (0,7%)	3 (0,8%)	0 (0,0%)	0 (0,0%)		
Other land-based	18 (4,3%)	14 (3,9%)	3 (13,6%)	1 (3,2%)		
Unknown / not identified	32 (7,7%)	31 (8,7%)	0 (0,0%)	1 (3,2%)	0 (0,0%)	0 (0,0%)

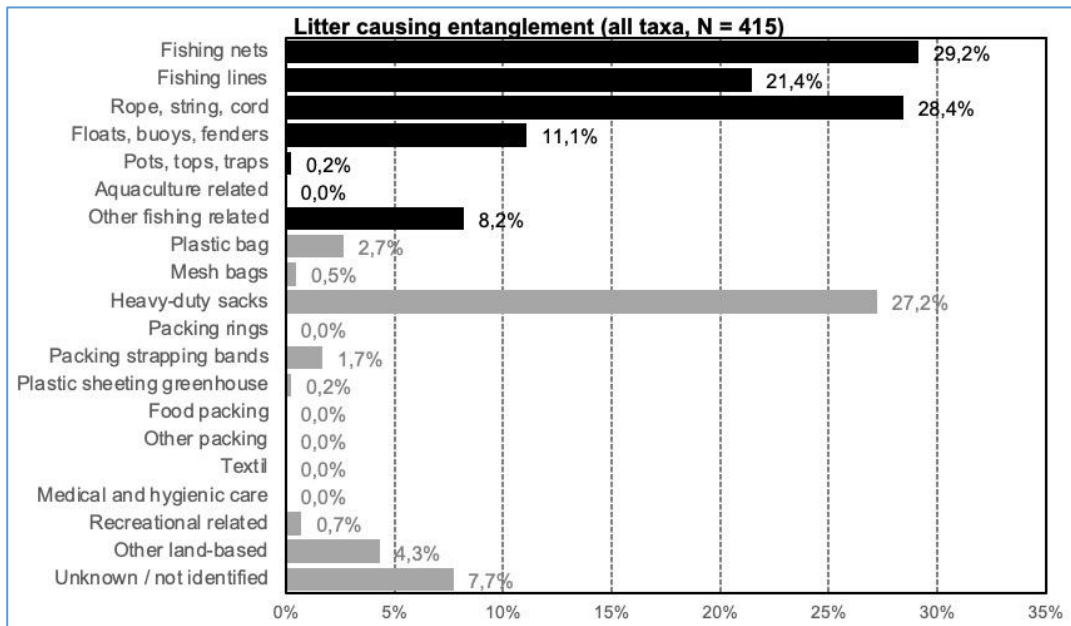
The list of litter categories proposed by INDICIT II consortium covered the main litter typologies observed in the images found and reviewed, where the most frequent typologies were: “fishing nets” (29,2%); “ropes, strings and cords” (28,4%) and “fishing lines” (21,4%) on the categories derived from fisheries and maritime uses, and “heavy-duty sacks” (27,2%) in land-based sources categories.

Furthermore, several typologies have never been observed (or only in one case, red squares, Table V.7), such as: “Pots, tops and traps” and “Aquaculture related” on categories derived from fisheries and maritime sources, and “Packing rings”, “Food packing”, “Plastic sheeting greenhouse”, “Textile” and “Medical and hygienic care”, in land-based sources categories.

Clearly, when images are available, the “Unknown” cases decrease (only 7,2% of the cases were not possible to identify the litter category).

No new typologies of those proposed by INDICIT II consortium has been considered, because individuals included on “other fishing related” or “other land-based” were:

- Specific materials frequently observed but always linked to other typology described on the list, such as hooks, lures and weight, usually attached to “fishing lines”.
- Isolated cases (only 1 case) of very specific materials, such as balloon, deck chair or bike wheel (included on “recreational related”).
- Cases described as “plastics” by people discovering the animals and the images do not show the material entangling the animal (included on “other land-based” typology).

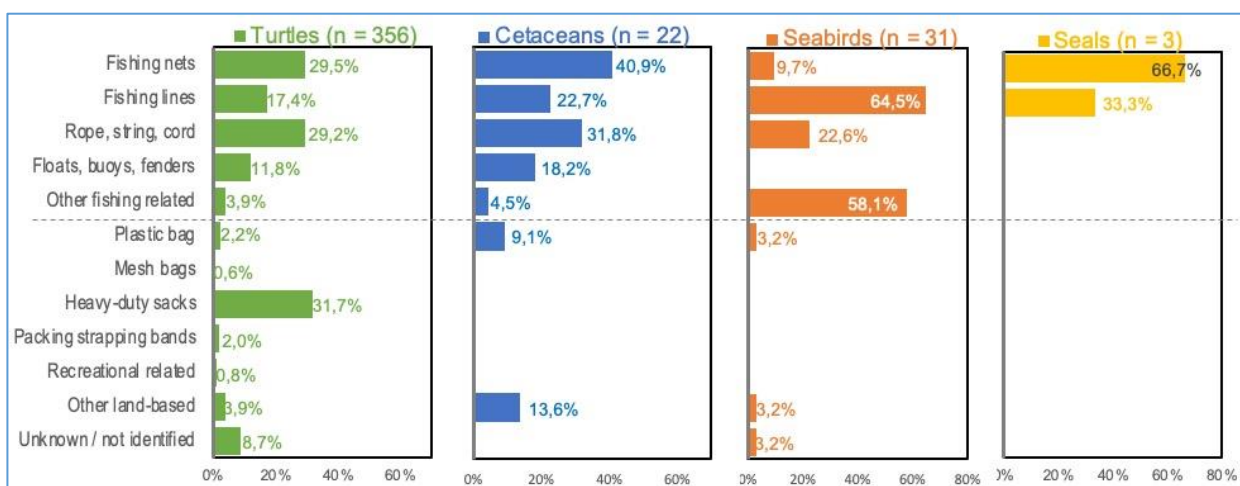


**Figure V.1.** Frequency of individuals (all taxa) entangled on litter typologies proposed by INDICIT II consortium. (Data from images obtained on social media and opportunistic platforms).

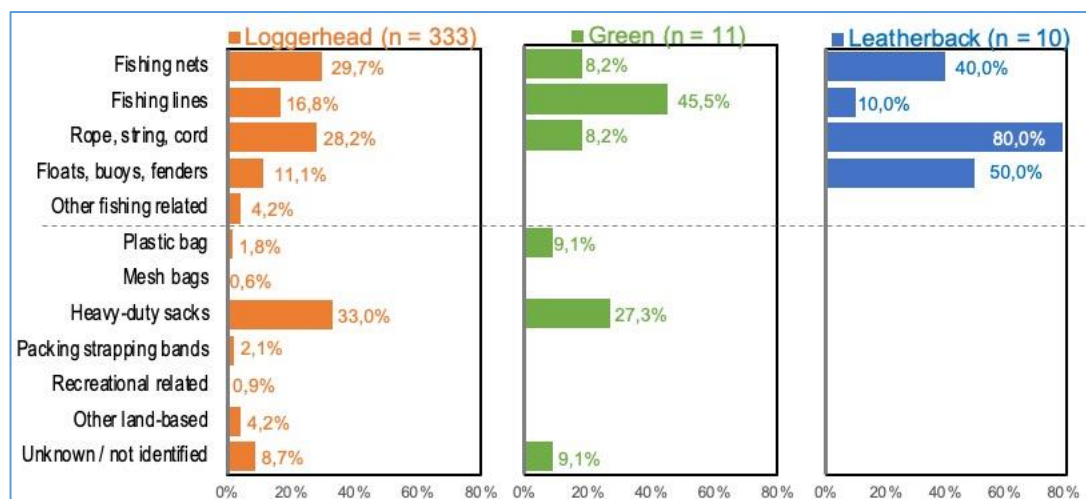
Figure V.2 shows the differences observed among taxa. The greatest variety of litter typologies causing entanglement has been found in sea turtles, considering that the greatest number of individuals has also been found (N = 356). This is the only taxon where “heavy-duty sacks” present a very important impact, showing the higher frequency (31,7%), followed by “fishing nets” (29,5%), “fishing lines” (17,1%), and “ropes, strings and cords” (29,2%). Other litter categories have only been observed entangling sea turtles, such as, “mesh bags”, “strapping bands” and “recreational related”.

The rest of taxa (cetaceans, seabirds and seals) have been clearly affected by litter derived from fisheries and maritime sources (72,7%, 90,3% and 100% respectively), with the exception of “plastic bags” in cetaceans (9,1%) and to a lesser degree on seabirds (3,2%).

In general, cetaceans are more impacted by “fishing nets” (40,9%), “fishing lines” (22,7%), “ropes, strings, cords” (31,8%) and “floats, buoys and fenders” (18,2%). The 13,6% of cetaceans affected by “Other land-based” include many cases described as “plastics” by the people founding the animals. In seabirds, most part of the cases were affected by “fishing lines” with “other fishing related” (hooks, lures and weight attached to fishing lines), and few cases of entanglement on “fishing nets” has been found. The low number of images from seals found on social media (N = 3) prevents a proper analysis, even though all of them were affected by “fishing nets” or “fishing lines”.



**Figure V.2.** Frequency of individuals from each taxon entangled on litter typologies proposed by INDICIT consortium. Data from images obtained on social media & opportunistic platforms.



**Figure V.3.** Frequency of litter typologies entangling individuals by sea turtle species. (Data from images obtained on social media & opportunistic platforms).

Within the sea turtle group, differences were also found according to species (Fig. V.3).

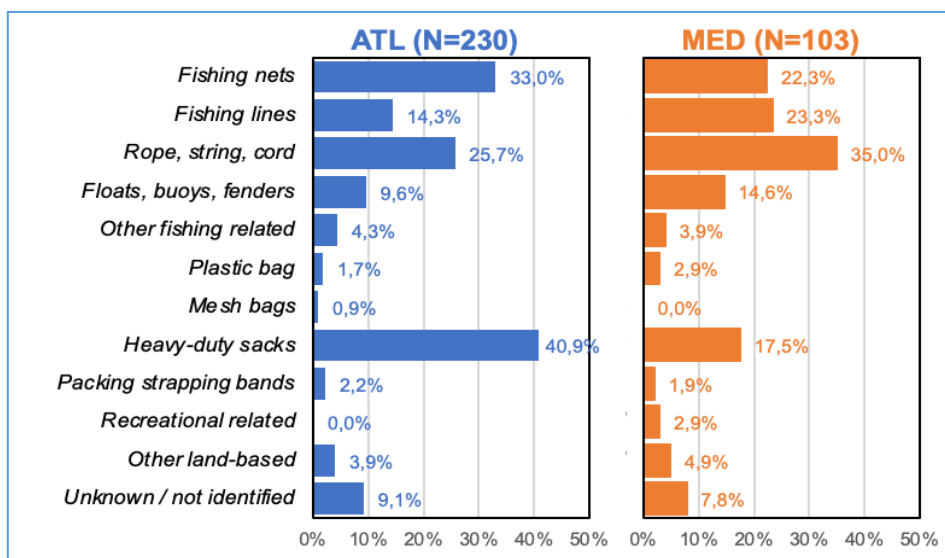
Loggerhead and green turtles were impacted by litter derived from fisheries and maritime sources but also from litter derived from land-based sources, mainly “heavy-duty sacks” on both species (33,0% and 27,3% respectively) and by “plastic bags” on green turtles (9,1%).

In relation with litter derived from fisheries and maritime sources, green turtle is clearly more impacted by “fishing lines” (45,5%), probably caused by their neritic behaviour, inhabiting close to the shores, instead of the oceanic behaviour of loggerhead and leatherback turtles, more impacted by “ropes, strings and cords” (28,2% and 80% respectively) and “fishing nets” (29,7% and 40,0% respectively).

### V.3.2. Analysis of litter typologies by regions (images obtained from social media and online platforms)

Important regional differences have been found in relation with litter typologies impacting marine fauna in the European waters. Only data from loggerhead turtle (N = 333) has been included on the analysis to avoid differences between taxa and species (Fig. V.4).

Based on data from images obtained from social media and opportunistic platforms, the main difference on litter typologies impacting loggerhead turtles between the Atlantic and Mediterranean basins was generated by the larger number of animals affected by “heavy-duty sacks” on the Atlantic (40,9%) compared to the Mediterranean (17,5%).



**Figure V.4.** Frequency of litter typologies entangling loggerhead turtle in MED and Atlantic areas (Data from images obtained on social media & opportunistic platforms).

Another difference observed between basins was a slightly higher percentage of “ropes, strings, cords” and “floats, buoys, fenders” observed in the Mediterranean (35% and 14,6%) compared to the Atlantic (25,6% and 9,6%), where the most frequent litter typology related to fisheries and maritime uses was “fishing nets” (33%).

Other small differences were that “mesh bags” were only observed in the Atlantic and “recreational related” only in the Mediterranean (balloon, deck chair, bike wheel, etc.).

To better understand differences between regions, both Med and Atlantic basins have been subdivided to obtain 4 different regions:

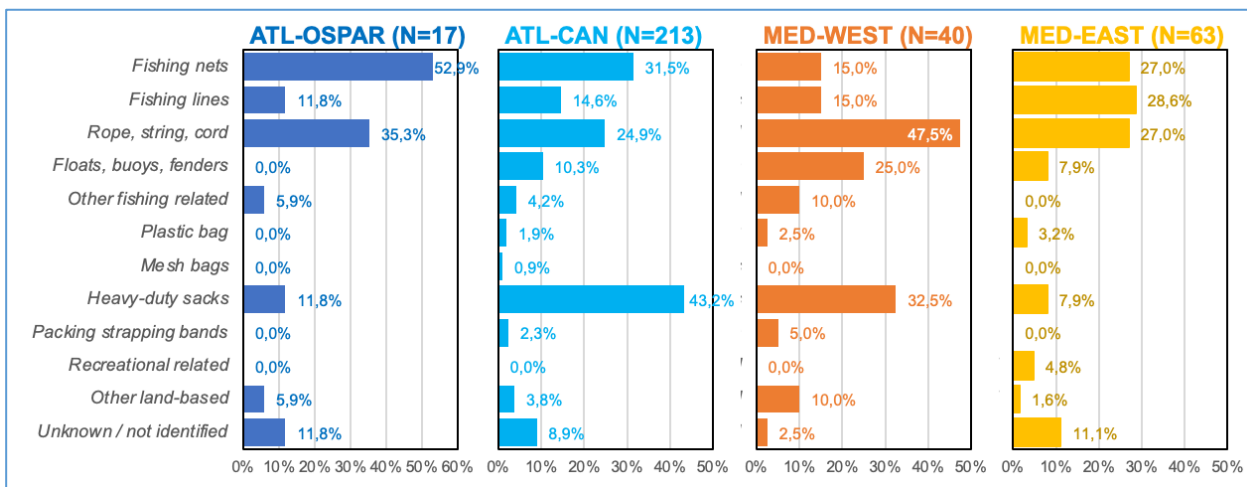
1. *Atlantic OSPAR region (ATL-OSPAR)*: including data from Atlantic France, Portugal (Azores, Madeira, mainland), and mainland Atlantic Spain.
2. *Atlantic Canary region (ATL-CAN)*: including data from Canary Islands
3. *Western Mediterranean (MED-WEST)*: including data from Spain (Mediterranean coast), France (Mediterranean coast) and Italy (Tyrrhenian and Ligurian seas).



4. *Eastern Mediterranean (MED-EAST)*: including data from Greece, Cyprus, Turkey, Tunisia and Italy (Adriatic and Ionic seas).

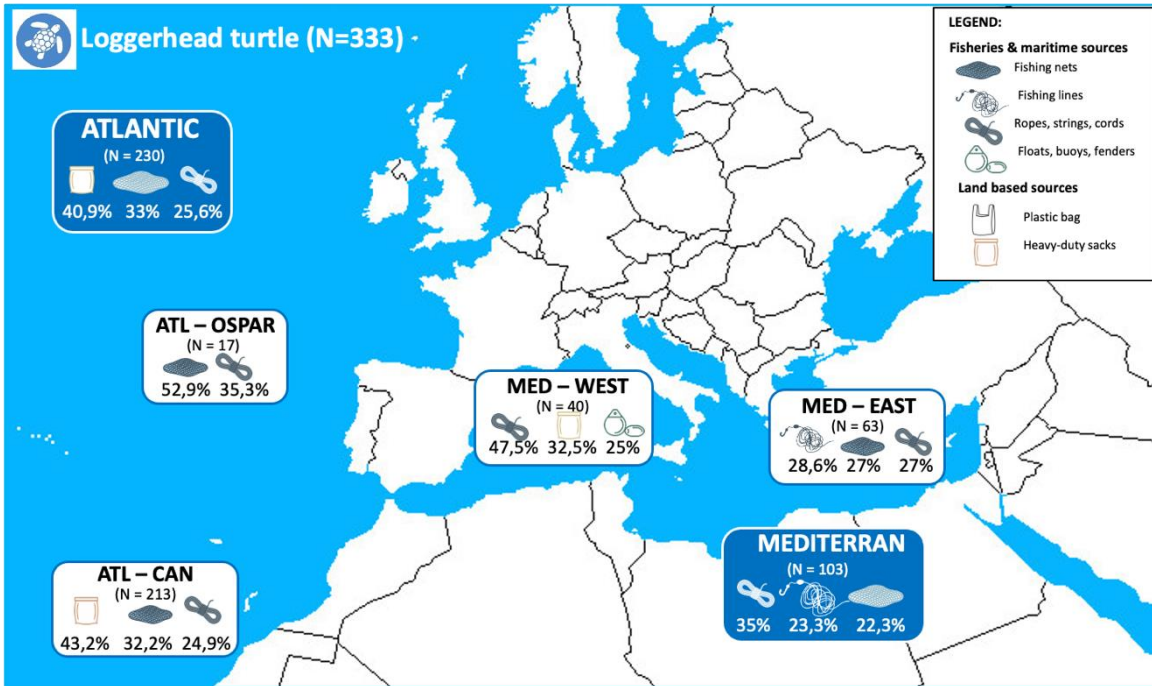
Based on data from images obtained from social media and opportunistic platforms, the main results observed on litter typologies impacting loggerhead turtles between the 4 regions established shown (Figs. V.5 and V.6):

- “Heavy-duty sacks” impact loggerhead turtles in all the 4 regions, moreover, is most frequent on Canary Islands (43,2% ATL-CAN) and western Mediterranean (35,5% MED-WEST), been the most important litter category impacting loggerhead in the Canary Islands.
- “Fishing nets”, “fishing lines” and “ropes, strings, cords” are frequently observed impacting loggerhead turtles in all the 4 regions, with differences in frequency.
- The most important litter typologies observed by regions are:
  - ATL-OSPAR: “fishing nets” (52,9%,) and “ropes, strings and cords” (35,3%)
  - ATL-CAN: “Heavy-duty sacks” (43,2%), “fishing nets” (31,5%) and “ropes, strings, cords” (24,9%).
  - MED-WEST: “ropes, strings, cords” (47,5%), “heavy-duty sacks” (32,5%) and “floats, buoys, fenders” (25,0%).
  - MED-EAST: “fishing lines” (28,6%), “fishing nets” (27%) and “ropes, stings, cords” (27%)
- There has been found an important source of litter in the Mediterranean Sea, originated by FADs (Fish Aggregation Devices), composed by “ropes, stings, cords” attached with artisanal “floats and buoys” (usually plastic bottles), mainly on the western Mediterranean.



**Figure V.5.** Frequency of litter typologies entangling loggerhead turtle per basin (Data from images obtained on social media & opportunistic platforms).

## SOCIAL MEDIA REVIEW



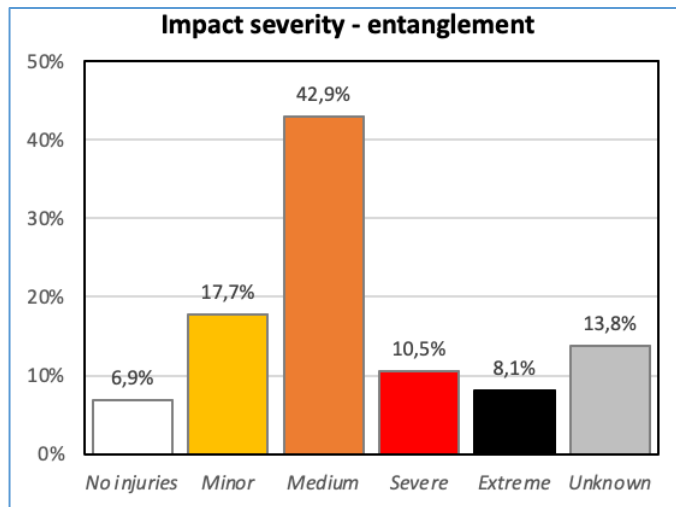
**Figure V.6.** Frequency of most important litter typologies entangling loggerhead turtles in each basin and regions. Data from images obtained on social media & opportunistic platforms.

### V.3.3. Impact of entanglement on animal health (impact severity, images from social media):

The “impact severity” established by INDICIT II consortium (based on the effect of injuries / lesions caused by entanglement on animal viability), has been analysed (Fig. V.7).

In general, the higher percentages (42,9%) of animals affected by entanglement presented a “medium” degree of impact, with typical injuries of deep cuts affecting epidermis and dermis, with different degree of infection, but not arriving to produce important alterations on the body condition or the viability of the animals.

The body condition or viability of 18,6% of the animals analysed were affected, where 10,5% suffered flipper amputation or body deformations (severe), and 8,1% dead due to entanglement (extreme).



**Figure V.7.** Impact severity of entanglement on loggerhead turtle. (Data from images obtained on social media and opportunistic platforms).

The “Impact severity” in relation with litter typologies were also analysed (Table V.8). Litter typologies derived from fisheries and maritime sources e.g. animals entangled on “fishing lines” presented the greater impact: 15,5% of these animals presented severe effects (amputations, bone fracture, eye loss) and 19% died due to the impact of the entanglement (extreme severity).

Litter typologies derived from land-based sources, e.g. “plastic bags” presented the higher mortality (50% extreme severity), followed by “other land-based” (28,6%) and “packing strapping bands” (14,3%).

“Fishing lines” and “heavy-duty sacks” presented the highest percentages of animals with severe impacts (15,5% and 10% respectively), where the body condition and the viability of the animals were altered (amputations, bone fractures, eye loss, etc.), because these materials are most thin and very resistant, presenting a greater capacity to cut the skin, producing deeper wounds in a very short time.

**Table V.8.** Frequency of Impact severity caused by litter typologies on entangled loggerheads. Data from images obtained on social media and opportunistic platforms.

<b>Loggerhead turtles (only)</b>	<b>no injuries</b> (N = 23)	<b>minor</b> (N = 59)	<b>medium</b> (N = 143)	<b>severe</b> (N = 35)	<b>extreme</b> (N = 27)	<b>unknown</b> (N = 46)
<i>Fisheries &amp; maritime sources (N=191)</i>	17 (8,9%)	38 (19,9%)	72 (37,7%)	14 (7,3%)	22 (11,5%)	28 (14,7%)
Fishing nets (N=89)	1 (1,1%)	25 (28,1%)	37 (41,6%)	2 (2,2%)	9 (10,1%)	15 (16,9%)
Fishing lines (N=58)	1 (1,7%)	7 (12,1%)	24 (41,4%)	9 (15,5%)	11 (19,0%)	6 (10,3%)
Rope, string, cord (N=94)	6 (6,4%)	16 (17,0%)	42 (44,7%)	5 (5,3%)	6 (6,4%)	19 (20,2%)
Floats, buoys, fenders (N=37)	2 (5,4%)	6 (16,2%)	18 (48,6%)	2 (5,4%)	2 (5,4%)	7 (18,9%)
Other fishing related (N=14)	0 (0,0%)	3 (21,4%)	6 (42,9%)	1 (7,1%)	0 (0,0%)	4 (28,6%)
<i>Land-based sources (N=130)</i>	6 (4,6%)	21 (16,2%)	73 (56,2%)	11 (8,5%)	7 (5,4%)	12 (9,2%)
Plastic bag (N=6)	0 (0,0%)	0 (0,0%)	3 (50,0%)	0 (0,0%)	3 (50,0%)	0 (0,0%)
Mesh bags (N=2)	0 (0,0%)	0 (0,0%)	2 (100%)	0 (0,0%)	0 (0,0%)	0 (0,0%)
Heavy-duty sacks (N=110)	4 (3,6%)	20 (18,2%)	65 (59,1%)	11 (10,0%)	1 (0,9%)	9 (8,2%)
Packing strapping bands (N=7)	0 (0,0%)	1 (14,3%)	5 (71,4%)	0 (0,0%)	1 (14,3%)	0 (0,0%)
Recreational related (N=3)	1 (33,3%)	0 (0,0%)	2 (66,7%)	0 (0,0%)	0 (0,0%)	0 (0,0%)
Other land-based (N=13)	1 (7,1%)	2 (14,3%)	6 (42,9%)	0 (0,0%)	4 (28,6%)	1 (7,1%)
Unknown / not identified (N=27)	0 (0,0%)	2 (6,9%)	10 (34,5%)	9 (31,0%)	0 (0,0%)	8 (27,6%)

#### V.3.4. Main conclusion (images review)

The review of images obtained on social media and opportunistic platforms showed:

- Definitions and criteria developed by INDICIT II consortium are very useful** to distinguish entanglement from bycatch in most of the cases, mainly when images are collected. However, larger animals, such as leatherback turtle and cetaceans, present more difficulties to distinguish entanglement from bycatch.
- The list of litter typologies established by INDICIT II consortium is appropriate** to monitor litter entanglement on marine fauna. Although, the list could be reduced to facilitate data collection by stakeholders, according to the taxon and the region.
- An important source of data about entanglement has been found on social media and online platforms** (415 entangled individuals were found).
- Important differences have been found on litter entanglement in relation with taxon** and sea turtle species, probably caused by different behaviours and habitat uses.
- Entanglement was more frequently observed in sea turtles**, the loggerhead turtle being the most abundant (N = 333). Therefore, loggerhead turtle could be proposed to monitor entanglement on oceanic habitats in the Atlantic and Mediterranean basins.

6. Few data about green turtle were found on social media (N = 11), but, this species could be proposed to monitor neritic / coastal habitats.
7. There is a lack of data from loggerhead turtle on the Atlantic OSPAR region (only 17 cases were found), in relation with the rest of regions.
8. **The parameter “Impact severity” could be used to identify the impact of entanglement** on individual viability and to identify specific litter typologies that could induce greater impacts on the animals.

#### V.4. Standardised Protocol to monitor entanglement for stakeholders

The external examination of live and dead specimens of marine mega-fauna (sea turtles, seabirds and marine mammals) will be used to describe the impact of marine litter on biota, particularly caused by entanglement, in the perspective of defining an indicator for the D10C4 criteria. INDICIT II consortium has developed a Standard Protocol to homogenise data collection to monitor entanglement in the European waters (Deliverable D.3.10).

All European countries have arranged organisational structures or systems to manage stranded or injured animals founded at sea or in the shores (stranding networks). Generally, stranded or injured animal founded alive are carried to adequate facilities for rehabilitation (rescue centres), and dead animals are brought to authorized facilities (rescue centres, veterinarian facilities, etc.) to conduct complete necropsies.

In general, stranding networks conduct a continuous effort in specific areas / regions / countries, achieving an important source of data to monitor marine fauna threats and impacts along the time. A great variety of experts are involved in the stranding networks (veterinarians, biologist, environmental authorities, etc.) and different parameters are collected to describe each stranding event. In most cases, rescue centres are coordinated with stranding networks, where more data about the animals are collected.

INDICIT II project has involved these key stakeholders (stranding networks and rescue centres) to monitor entanglement in the European waters, through standardised data collection based on the Standard Protocol developed by INDICIT II consortium. The implementation of this protocol on the official stranding protocols used by European countries will allow to monitor entanglement in the future.

A common database has been created to be updated with standard data collected by stakeholders and conduct different analyses to calculate GES in the European waters.

##### V.4.1. Key stakeholders for data collection on entanglement

Organisations or institutions in charge of the stranding network in a region/country, under environmental authorities permits, which conduct continuous effort along the time and included trained staff for data collection (veterinarians, biologists, public environmental staff, experts, trained volunteers, etc.). Main stakeholders involved:

- Local / regional / national stranding networks coordinated by environmental authorities.
- Public / private Rescue Centres in charge of, or associated with, stranding networks.
- Public / private Research Institutions in charge of stranding networks (under official permits), or involved in marine colonies monitoring (seabirds, seals).
- NGO managing stranding networks (under official permits) or involved in marine colonies monitoring (seabirds, seals).
- Other organizations involved or collaborating with stranding networks and rescue centres, or involved in marine colonies monitoring (seabirds, seals).

The list of Stakeholders engaged on this activity and networking conducted during the project has been described in section 3.2.

#### V.4.2. Data proposed to be collected, taxonomic groups & species

This protocol focused mainly on most frequent sea turtles in the European waters: The Loggerhead (*Caretta caretta*), the Leatherback (*Dermochelys coriacea*), and the Green turtle (*Chelonia mydas*). Data on other taxonomic groups has been also proposed: cetaceans, seabirds and seals.

On an experimental basis, the main data useful to monitor entanglement are, the frequency of occurrence, the type of litter involved in the entanglement and the severity of impact caused by the interaction. Other information is useful during the development stage of D10C4 criteria, and to evaluate the biological constraints of the indicator.

Two kinds of data have been included in the Standard Protocol:

*General data (FO%)*: To obtain Frequency of occurrence (FO %) of entanglement per region and per year. Data to be collected are:

- Area covered by the Stakeholder
- Number of total animal stranded / registered by the stakeholder per year
- Number of animals affected by entanglement per year

*Individual data*: To obtain accurate data to deep on the impact of entanglement on marine fauna. Specific data from each litter typology could be obtained to identify main litter involved on entanglement per region, spatial and temporal variations, taxa and species affected and impact generated.

Several parameters from each entangled animal have been included in the Protocol, included in 4 different sections:

1. Stranding event characterisation: date, location, circumstance, etc.
2. Individual characteristics: size, sex, conservation status (if dead), etc.
3. Litter characterisation: to classify and characterize litter involved.
4. Litter impact: Parameter "*Impact severity*" developed by INDICIT II consortium, based on the effect of injuries / lesions caused by entanglement on animal viability.

All data are described in the Standard protocol (Deliverable D3.10).

*Common Database*: an Excel file has been developed and distributed within INDICIT II Partners to be shared with key stakeholders involved in the project for data collection.

The review of historical databases from key stakeholders has been proposed to all Partners to better understand and identify the impact of entanglement in each specific region in the precedent years. In general, stranding networks and rescue centres target on the recovery of stranded animals, so, they usually focus on the health status and lesions observed in the animal, and little attention is paid on other relevant data, such as litter involved on entanglement events. For example, many cases of entanglement are classified by stakeholders as "fisheries interaction", where both, bycatch animals on active fishing gears and individuals entangled on marine litter (usually ghost nets) are included. In many cases, extra data included in the datasheets and databases could give indices to better identify and classify each stranding event. For this reason, the review of historical databases from key stakeholders with new and homogeneous criteria was highly recommended.

*Litter typologies for entanglement*: Based on the results obtained on the review of images collected from social media and opportunistic platforms, the list of litter typologies established by INDICIT II consortium has been included in the Standard Protocol, keeping the complete list (even the typologies that has not been observed in the review) (Table V.3).

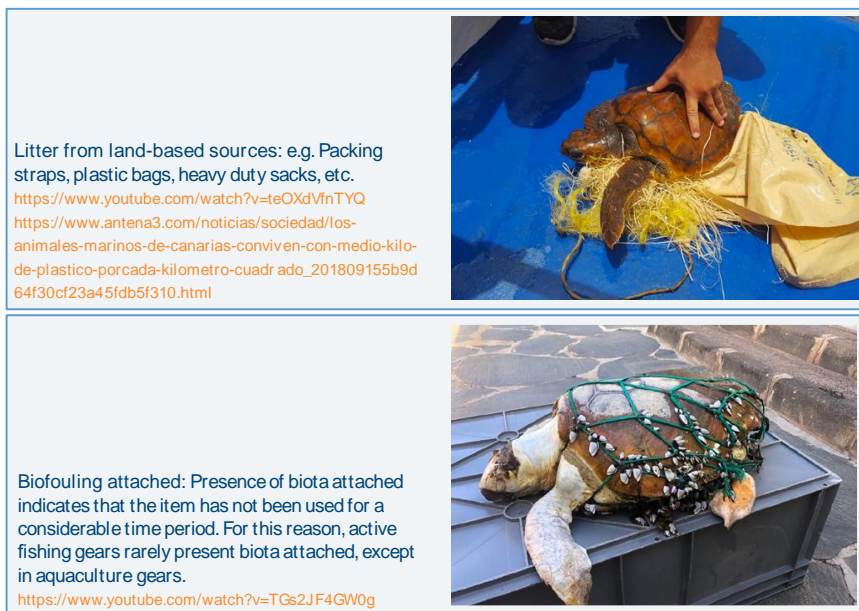
#### V.4.3. Supporting materials

To support and improve data collection on entanglement, some materials (figures and schemes) have been created to obtain more detailed data from each entanglement event.

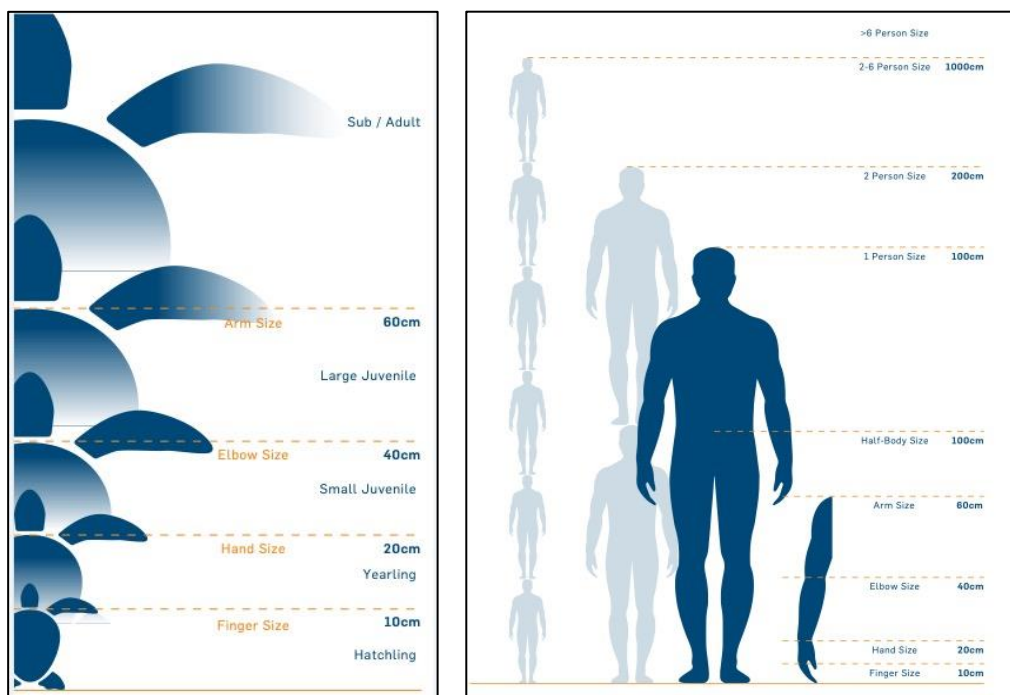
Concepts and criteria to distinguish entanglement from bycatch: All the criteria have been supported with pictures, descriptions and 1-2 web links (Fig. V.8), to facilitate event classification (entanglement, bycatch, doubtful).

*Size reference*: Schemes to facilitate size reference has been created. The scheme could be used to refer size of the animals (Fig. V.9 left) or for the litter (Fig.V.9 right).

*Litter typologies*: Schemas to facilitate litter typology identification established by INDICIT II (Fig. V.10).



**Figure V.8.** Example of supporting material related to criteria to distinguish entanglement from bycatch.

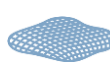






**Figure V.9.** Supporting material to collect data on individual size (left) and litter size (right).







## LITTER CATEGORIES

## DESCRIPCIÓN (TG-ML CODE)





### Fisheries, aquaculture, and Maritime uses

-  **Nets:** nets and pieces of nets (G52, G53, G54, G56)
-  **Fishing lines:** monofilaments used on fishing gears (e.i. nylon)
-  **Ropes and cords:** maritime ropes, strings, cords (G50, G49)
-  **Floats, buoys, fenders:** associated with nets, ropes, fishing lines) (G62, G63, G64)
-  **Pots, tops, traps:** for different species; different materials (G42, G44, G163, G184, G207)
- Aquaculture related:** equipment for holding or protecting shellfish (G45, G46, G47)
- Other fishing related:** fish box, baits, lures, weights (G57, G58, G60, G61, G92, G164, G170, G182)

### Packing related

-  **Plastic bags:** shopping/grocery bags, dog faeces bags, fruit bags, etc. (G2, G3, G4, G5)
-  **Mesh bags:** plastic mesh bags for vegetable, fruit and other (G2, G37)
-  **Heavy-duty sacks:** animal feed, fertilizers, garden rubbish, salt, etc. (G2, G36, G85)
-  **Packing-rings:** 4/6-pack yokes & six-pack rings (G1)
-  **Strapping bands:** packing strappings bands (G66)
-  **Plastic sheeting greenhouse:** plastic sheeting used to cover greenhouses (no code)
- Food packing:** food packets and wrappers, caps and lids, etc. (G20, G30, G31, G21, G22, G23, G24, G18)
- Other packing** corks, crates, boxes, baskets, pallets, etc. (G170, G159, G160, G162)

### Other land-based sources

-  **Textile:** clothing, footwear, hessian sacks, backpacks, etc. (G135, G136, G137, G138, G139, G140, G143, G145)
-  **Medical - hygienic care:** gloves, bandaging, sanitary towels, etc. (G135, G40, G41, G144, G211, G133)
-  **Recreational related:** toys, balloons, balls, fireworks remain, etc. (G32, G125, G126, G155, G167)
-  **Other land-based:** cable ties, rubber bands, foams, etc. (G93, G87, G43, G131, G73, G194)

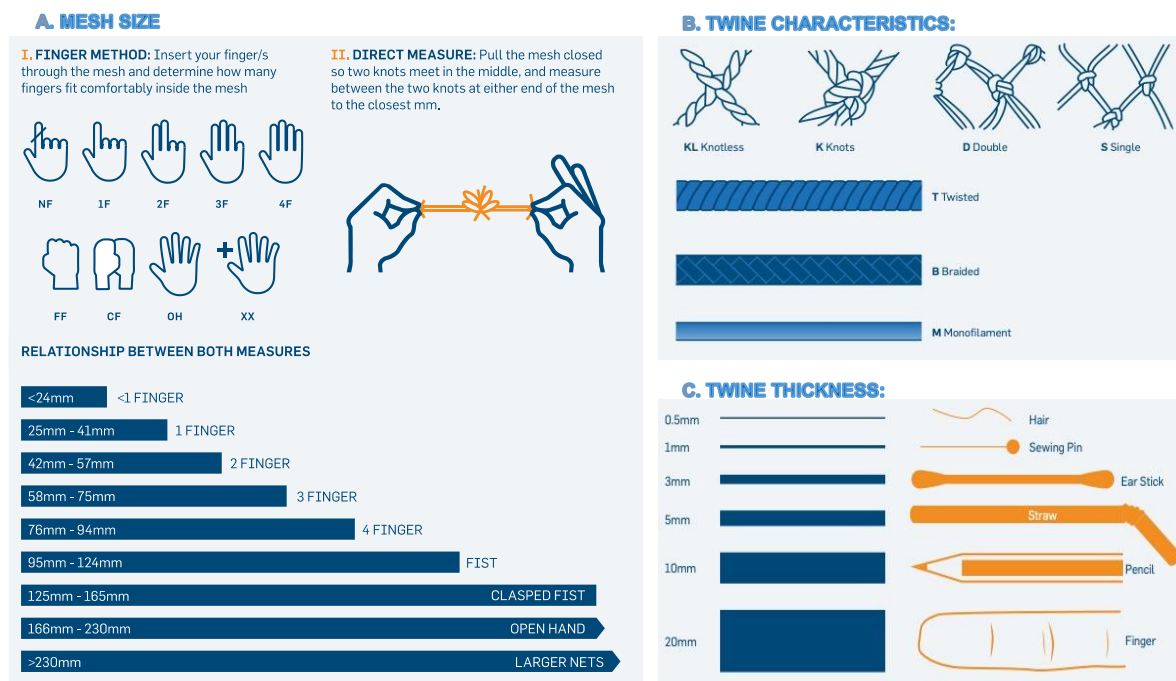
### Unknown

No data available about litter

Figure V.10. List of litter typologies established by INDICIT II consortium.



Characterisation of litter derived from fishing gears: Based on *Ghost Net ID Guide, Australia (Gunn, 2015)*, several schemas to characterize nets, fishing lines and maritime ropes has been included on the protocol (Fig. V.11).



**Figure V.11.** Supporting material to collect data on litter characterization: a) Mesh size; b) Twine characteristics; c) Twine thickness. (Based on Gunn, 2015).

#### V.4.4. Include pictures in the stranding network protocols

Taking pictures of each stranding event is highly recommended to improve data collection. Each image is an important source of data that could be used to improve the description of the event, or in the future to collect relevant information that was not registered in the stranding moment. Include pictures in the general stranding protocols of each organisation is the first step to improve data collection.

In the specific case of entanglement, pictures are essential to better identify and classify litter typologies involved on entanglement, where experts on marine litter could go deeper, reference size between litter and individual, and even the impact of the entanglement (main injuries, animal status, etc.).

The INDICIT II consortium has include several recommendations in the Standard Protocol to collect pictures from each stranding event:

- The pictures should be as clear as possible and taken from several angles:
  - General picture of the stranded animal and all the elements involved
  - Details of litter entangling the animal
  - Detail of main external injuries caused by the entanglement
- Include elements to refer size (rule, metric tape measure, pen, etc.).
- Request pictures to the people founding the animal: because in many cases the first instinctive act of people is to remove debris from an entangled animal, so, when the official staff arrives, the material has already been removed.
- The pictures should be carefully codified and stored: for this reason, the use of **“Image storage applications”** (Image App) is highly recommended as an important tool on the stranding networks to better

identify each stranding event. Generally, these Apps collect: i. Date; ii. Location; iii. Images, which are the most relevant data to describe each stranding event (other data could be extracted later from the image: specie, relative size, animal status, main injuries, etc.).

To monitor entanglement, the inclusion of “Image App” in the official stranding protocols could be very relevant to facilitate data collection and homogenization.

## V.5. Dissemination of the standardised methodology for monitoring litter impacts, costs and recommendations

*Dissemination:* Important difficulties were experienced to conduct training sessions with stakeholders during INDICIT II project due to COVID-19 pandemic situation. INDICIT II Partners disseminated the Standard protocol to stakeholders by email and conducting several regional online meetings (along 2021) and 1 regional face-to-face meeting (see section V.2.2).

### *Costs and recommendations:*

- In certain areas/countries, a better development of the network will be necessary, by e.g. creating/reinforcing stranding networks and rescue centres, ensuring constant means and training referents or coordinators at the local/regional levels.
- Engaging stakeholders in certain areas is necessary to provide accurate assessments of litter impacts and GES in some regions. This is especially the case in the Atlantic OSPAR area where some stakeholders failed to be involved, particularly in mainland Portugal and mainland Atlantic Spain.

*NOTE:* Stakeholders from mainland Atlantic Spain were involved at the end of the project thanks to the Spanish Ministry.

- For entanglement, the inclusion of **pictures** of individuals on stranding protocols is the better way to achieve accurate databases that could be reviews by experts on marine litter to harmonise and avoid confusion on litter classification. Some tools may support data collection, in particular when a turtle is observed at sea or found stranded or bycaught, for example phone applications or online platforms, such as *RedPROMAR App*, developed by the Canary islands government, allowing citizen or institutions (NGOs, rescue centres, stranding networks) to post pictures with GPS locations; or *ObsEnMer* which offers a collaborative platform, managed by *Cybelle Planete* (France).
- Key-stakeholders, such as fishermen, who are keys to recover sea turtles in certain areas, should be involved. Including them for the monitoring of litter instead of directly for recovering bycatch individuals, could support the collaboration.
- Arrange regional / national or international workshops among stakeholders, together with other experts and with representatives, could facilitate the involvement of new stakeholders. These workshops should propose training sessions and sharing experience on e.g. methodologies to monitor litter impacts, which could still be optimised.
- For a better reporting of information and data, a diagram of the networks should be built at the national and regional levels, considering specificities of each country. These diagrams should be disseminated to stakeholders in order to facilitate procedures and contacts.
- 1-2 experts on marine litter and marine fauna could be involved as focal points in each country / region to coordinate data collection by stakeholders and harmonize litter classification involved on entanglement (review pictures, identify new litter typologies, etc.).

## V.6. Collection of standard data and data banking

### V.6.1. Main constraints on data collection from stakeholders

#### *a) Harmonisation of data collection*

The main issues found was the big differences in data collected from stakeholders. In many cases, databases were not updated and not homogenised, so many stakeholders not arrived to share their databases with INDICIT II project, such us France (Atlantic), Tunisia and Turkey. Moreover, many stakeholders worked hard in their databases and are including the standard data proposed to monitor entanglement in their protocols.

Other stakeholders required a great deal of time and personal effort to update and homogenise their databases, such us the deep review of the 4429 data (2055 entangled animals) collected from sea turtles stranded in the Canary Islands (Spain) from 1989 to 2019 (9 stakeholders), or the 1413 individual data (56 entangled animals) of sea turtles stranded in the Valencia region (Spain) from 1995 to 2020.

To solve this relevant constraint, the INDICIT II consortium had shared with stakeholders the Standard protocol (Deliverable 3.10) described in the precedent section, which includes many supporting materials (images and schemas) to homogenize data collection. Many stakeholders started to collect data described in the Standard protocol in 2020. INDICIT II consortium has conducted several regional trainings (described in section 3.2), even if COVID-19 pandemic generated important alterations (see below).

#### *b) COVID-19 pandemic situation*

A large constraint has been produced by the COVID-19 pandemic situation, where no face-to-face training could be conducted, or even the visits to stakeholders to support and encourage them to homogenise and collect standardised data to monitor entanglement.

To solve this constraint, the INDICIT consortium conducted online trainings (described in section IV.2.2), and organised an INDICIT challenge (online campaign described in section II, p. 28).

#### *c) Litter classification*

Many difficulties were experienced in finalising the list of litter typologies for entanglement. The Activity 3 coordinators generated the list of litter typologies based on the MSFD litter classification, extracting the items susceptible to be involved on entanglement. The first draft has been showed to stakeholders, which asked to reduce the list of litter typologies to do it more practical and useful to be used on the field.

At the same time, first proposal of litter typologies has been shared with the Technical Group on Marine Litter (TGML) from the MSFD, that were trying to homogenise the litter classifications established by different projects, programs, and conventions from the European Union. The difficulties found by the TGML to homogenise litter typologies, increased by the difficulties generated by COVID-19 pandemic, delayed the approval of the final list of litter typologies for entanglement to finalise the Standard protocol to monitor entanglement.

To solve this constraint, INDICIT II consortium decided to finalise the 1<sup>st</sup> Version of the Standard protocol to monitor entanglement. This version could be reviewed in the future when more standard data will be collected.

#### *d) Wide disparity in the number of data collected from different regions*

When most part of stakeholders shared their databases with INDICIT II project, the common database presented a great disparity in the amount of data collected from each region. The large amount of data collected from the Canary Islands (2055 entangled sea turtles) strongly influence the common database, which can introduce significant errors in the statistical analysis, showing only the results obtained in this region.

To solve this constraint, INDICIT II consortium has focus on data collected during the last Marine Strategy cycle (2013-2020), considering them as “current data”. The historical data has also been analysed looking for changes in the entanglement FO%, or even in the litter typologies involved on entanglement.

#### *e) Few data obtained from other taxa*

Even if many stakeholders (Stranding networks and Rescue Centres) work with different taxa, the databases from other taxa (cetaceans, seabirds, seals) are in a very poor state of updating and harmonisation, mainly

caused because sea turtles databases has been worked during the INDICIT project (2017-2019) to collect data about litter ingestion on sea turtles.

On the other hand, it seems that the impact of entanglement on other taxa, such us cetaceans and seabirds, was much lower than in sea turtles, as was observed in the review conducted on social media and opportunistic platforms. In addition, it should be noted that great difficulties have been found in distinguishing entanglement from bycatch in other taxa, such us cetaceans, mainly caused by the large size of the animals that makes them strong enough to trawl an active fishing gear; or seabirds, where many animals were entangled on “fishing lines” with hooks, lures, weights, etc., externally embedded in the body of the animals (not ingested). In these cases, the animal could be caught when the fishing gear was actively working, or when the fishing line was lost by fishermen and arrives to the shores as litter.

To solve this constraint, experts on other taxa were contacted and several recommendations were obtained to monitor marine litter on other taxa:

- Cetaceans: entanglement monitoring could be conducted directly on the field by whale watching recreational boats, or a deeper analysis needs to be conducted on data collected by stranding networks.
- Seabirds: monitoring the litter used by seabirds on nest construction.
- Seals: monitoring entangled animals directly on breeding colonies together with the animals recovered on rescue centres. Moreover, most part of the data are from the northern water of the European countries, where seals are more frequent.

*f) No standard data was obtained from some specific regions*

Main stakeholders from mainland Portugal were contacted several times, but no collaboration with INDICIT II project were obtained. In addition, on data published from this region no distinction is made between entanglement and bycatch, where all data are classified as “interaction with fishing gears”.

Stakeholders from mainland Atlantic Spain were contacted (CEMMA, Galicia; and CEGMA Algeciras), but databases were still not harmonised, and data could not be included in the INDICIT II common databases. Moreover, standard protocols for entanglement developed by INDICIT II consortium are being included in the national protocols for stranding marine turtles by the Spanish Minister, so standard data from these regions will be collected for the next cycle of the Marine Strategy.

Main stakeholders from Atlantic France, Tunisia and Turkey, had not arrived to share standard data about entanglement with INDICIT II consortium. Many of them have important difficulties to harmonize their databases, but they had working hard on their databases and had included the Standard protocol for entanglement in their protocols, so, standard data from these regions could be included in the next cycle of the Marine Strategy.

#### **V.6.2. Procedures used for data gathering and cleaning**

From the beginning of the project, all Partners contacted key regional stakeholders to review their data collected on entanglement and collect these ones that were available (historical and current data). Once the Standard Protocol was ready (April 2020), there was shared with key stakeholders to collect standardized data on entanglement.

During the last months of the project, stakeholders shared their databases with Partners that sent it to the Activity 3 coordinators to update the common standard database (Standard DB). All data had been reviewed one by one by Activity 3 Coordinators to homogenise and cleaning the Standard DB.

Furthermore, all data disposed in the Standard DB were compared with the database obtained from social media and opportunistic platforms (Social media DB), to identify those animals arranged in both databases, and to complete the information in the Standard DB when required, thanks to the availability of images. In many cases litter entangling the animals was not described in the Standard data, and the images solved this lack of information.

At the end of the project the Standard DB presented individual data from 2450 animals, with **2391 entangled animals** (Table V.9).

**Table V.9.** Resume of standard data collected from each country.

COUNTRY (region)	Period covered	ALL TAXA			SEA TURTLES			CETACEANS			
		<i>N total</i>	<i>Historic data</i>	<i>Current data</i>	<i>N total</i>	<i>Historic data</i>	<i>Current data</i>	<i>N total</i>	<i>Historic data</i>	<i>Current data</i>	
<b>ATL</b>	<b>France</b>	No data	NoData	NoData	NoData	NoData	NoData	NoData	NoData	NoData	
	<b>Portugal (Azores)</b>	(2010 - 2020)	17	5	12	13	2	11	4	3	1
	<b>Spain (Canary Is.)</b>	(1989 - 2019)	2190	1724	464	2190	1724	464	NoData	NoData	NoData
<b>MED</b>	<b>France (Gulf of Lion)</b>	(2016 - 2020)	8	NoData	8	8	NoData	8	NoData	NoData	NoData
	<b>Spain (Valencia)</b>	(1995 - 2020)	59	27	32	59	27	32	NoData	NoData	NoData
	<b>Italy</b>	(2016 - 2019)	56	NoData	56	33	NoData	33	23	NoData	23
	<b>Greece</b>	(2013- 2017)	54	NoData	54	54	NoData	54	NoData	NoData	NoData
	<b>Tunisia</b>	No data	NoData	NoData	NoData	NoData	NoData	NoData	NoData	NoData	NoData
	<b>Cyprus</b>	(2019)	7	NoData	7	7	NoData	7	NoData	NoData	NoData
	<b>Turkey</b>	No data	NoData	NoData	NoData	NoData	NoData	NoData	NoData	NoData	NoData
<b>TOTAL</b>	(1989 - 2020)	2391	1756	633	2364	1753	609	27	3	24	

*Historical data: before 2013; Current data: from 2013 to date*

In general, the greatest majority of data collected was from sea turtles (98,9%, 2364 sea turtles), and only 27 were entangled cetaceans, from Italy (23) and Azores (4). However, the database was clearly influenced by the huge quantity of data obtained from the Canary Islands (91,6% of total data), which can introduce significant errors in the data analysis, showing only the results obtained in this region.

From a temporal point of view, 73,4% of data were historical data collected by stakeholders before 2013 in Portugal and Spain (mainly from Canary Islands, 1724 individuals). It should be noted that historical are less accurate data, due to the time elapsed since there were collected. However, still in the current data (from 2013 to 2020), the database is strongly influenced by data obtained from Canary Islands (73,3% of total current data collected).

Looking for sea turtle species (Table V.10), the loggerhead turtle presented the great majority of data (98,64%), followed far behind by the green turtle (1%) and finally leatherback turtle (0,3%). For this reason, main analysis on entanglement focusses on loggerhead turtle.

**Table V.10.** Resume of standard data collected from sea turtles per country and species. (Historical data: before 2013; Current data: from 2013 to 2020).

COUNTRY (region)	Period covered	<i>Loggerhead</i>			<i>Green</i>			<i>Leatherback</i>			
		<i>N total</i>	<i>Historic data</i>	<i>Current data</i>	<i>N total</i>	<i>Historical data</i>	<i>Current data</i>	<i>N total</i>	<i>Historic data</i>	<i>Current data</i>	
ATL	France	No data	NoData	NoData	NoData	NoData	NoData	NoData	NoData	NoData	
	Portugal (Azores)	(2010 - 2020)	10	2	8	3	0	3	0	0	0
	Spain (Canary Is.)	(1989 - 2019)	2174	1717	457	10	4	6	4	3	1
MED	France (Gulf of Lion)	(2016 - 2020)	7	NoData	7	1	NoData	1	NoData	NoData	NoData
	Spain (Valencia C.)	(1995 - 2020)	57	26	31	NoData	NoData	NoData	2	1	1
	Italy	(2016 - 2019)	33	NoData	33	0	NoData	0	NoData	NoData	NoData
	Greece	(2013- 2017)	50	NoData	50	3	NoData	3	1	NoData	1
	Tunisia	No data	NoData	NoData	NoData	NoData	NoData	NoData	NoData	NoData	NoData
	Cyprus	(2019)	1	NoData	1	6	NoData	6	NoData	NoData	NoData
	Turkey	No data	NoData	NoData	NoData	NoData	NoData	NoData	NoData	NoData	NoData
<b>TOTAL</b>	(1989 - 2020)	2332	1745	587	23	4	19	7	4	3	

A common Standard DB is stored in an Excel file with many other biological data collected from each individual, such us: size, weight, status (alive/dead), conservation status, etc.; and other data related with the litter involved on the entanglement (litter size, litter characteristics, etc.).

## V.7. Evaluation of GES and indicator's characteristics at the RCS scale

### V.7.1. Results obtained on entanglement on sea turtles

#### V.7.1.1. Frequency of occurrence (FO %) of entanglement

The entanglement FO % is the number of entangled individuals in relation with the total number of animals registered per year in one specific area. Each stakeholder shared the number of total individuals (Ntot) and entangled individuals (Nent) per year, and the Table V.11 presents the total data collected on sea turtles by all the stakeholders from the European waters.

**Table V.11.** Entanglement FO % on sea turtle species from the European waters.

	Loggerhead		Green		Leatherback	
	Ntot	Nent (FO%)	Ntot	Nent (FO%)	Ntot	Nent (FO%)
1990	6	1 (16,7%)				
1991	30	8 (26,7%)				
1992	39	16 (41,0%)				
1993	90	38 (42,2%)				
1994	17	10 (58,8%)				
1995	43	9 (20,9%)				
1996	12	4 (33,3%)				
1997	53	30 (56,6%)	1	0 (0,0%)	1	0 (0,0%)
1998	109	45 (41,3%)	3	1 (33,3%)	4	0 (0,0%)
1999	151	74 (49,0%)	1	0 (0,0%)	0	
2000	257	113 (44,0%)	2	0 (0,0%)	0	
2001	409	96 (23,5%)	7	0 (0,0%)	1	0 (0,0%)
2002	248	111 (44,8%)	5	1 (20,0%)	0	
2003	296	103 (34,8%)	1	0 (0,0%)	2	0 (0,0%)
2004	250	102 (40,8%)	2	0 (0,0%)	2	1 (50,0%)
2005	208	127 (61,1%)	2	0 (0,0%)	2	1 (50,0%)
2006	329	155 (47,1%)	1	0 (0,0%)	3	0 (0,0%)
2007	246	123 (50,0%)	5	0 (0,0%)	1	1 (100%)
2008	277	122 (44,0%)	6	1 (16,7%)	1	0 (0,0%)
2009	219	117 (53,4%)	3	0 (0,0%)	2	0 (0,0%)
2010	202	68 (33,7%)	5	1 (20,0%)	0	
2011	136	75 (55,1%)	3	0 (0,0%)	4	0 (0,0%)
2012	247	94 (38,1%)	4	0 (0,0%)	7	1 (14,3%)
<i>Historical data</i>	<b>3876</b>	<b>1642 (42,4%)</b>	<b>51</b>	<b>4 (7,8%)</b>	<b>30</b>	<b>4 (13,3%)</b>
2013	257	95 (37,0%)	5	2 (40,0%)	1	0 (0,0%)
2014	242	61 (25,2%)	10	0 (0,0%)	1	1 (100%)
2015	276	79 (28,6%)	5	1 (20,0%)	2	0 (0,0%)
2016	385	77 (20,0%)	10	2 (20,0%)	5	0 (0,0%)
2017	297	54 (18,2%)	5	2 (40,0%)	0	
2018	321	60 (18,7%)	5	0 (0,0%)	0	
2019	378	72 (19,0%)	133	8 (6,0%) <sup>2</sup>	4	0 (0,0%)
2020	140	54 (38,6%) <sup>1</sup>				
<i>Current data*</i>	<b>2296</b>	<b>552 (24,0%)</b>	<b>173</b>	<b>15 (8,7%)</b>	<b>13</b>	<b>1 (7,7%)</b>
<b>Total</b>	<b>6170</b>	<b>2193 (35,5%)</b>	<b>224</b>	<b>19 (8,5%)</b>	<b>50</b>	<b>6 (12,0%)</b>

\* Data from Italy and Greece on loggerhead and from Greece on green turtle has been not included due to the lack of total number of individuals registered (Ntot); <sup>1</sup> There is no data from 2020 from Canary Islands; <sup>2</sup> Include data from Cyprus (only from 2019).

The Loggerhead turtle is clearly the species more impacted by entanglement in the European waters, with 35,5% of the animals registered from 1990 to 2020 impacted by entanglement, instead of the 8,5% observed on Green turtle and 12,0% on Leatherbacks.

An important decrease in FO% is observed in the loggerhead turtle between historical and current data, because historical data are mainly from Spain (Canary Islands in the Atlantic and Valencia region in the Mediterranean) and the rest of the stakeholders, with lower impact of entanglement (FO%), collected only current data (from 2013 to 2020).

Important differences are shown on the impact of entanglement on loggerheads from Atlantic and Mediterranean basins (Table V.12), where almost half of the loggerheads registered in the Atlantic (47,7%) were impacted by entanglement, instead of the 7,4% of the loggerheads observed in the Mediterranean waters.

Even if the impact of entanglement in the Mediterranean waters was lower, there was found an important increase between historical and current data (4,5% and 9,0% respectively), mainly because stakeholders from Italy, France, Greece, Cyprus and several new regions in the Spanish Mediterranean (Balearic Islands, Murcia, Catalunya, Almería) collected only current data, and more accurate data are showed.

**Table V.12.** Entanglement FO % on Loggerheads from the European basins.

Loggerhead	ATL		MED	
	Ntot	Nent (FO%)	Ntot	Nent (FO%)
1990	6	1 (16,7%)		
1991	30	8 (26,7%)		
1992	39	16 (41,0%)		
1993	90	38 (42,2%)		
1994	17	10 (58,8%)		
1995	18	8 (44,4%)	25	1 (4,0%)
1996	12	4 (33,3%)	0	
1997	53	30 (56,6%)	0	
1998	70	44 (62,9%)	39	1 (2,6%)
1999	151	74 (49,0%)	0	
2000	205	111 (54,1%)	52	2 (3,8%)
2001	285	88 (30,9%)	124	8 (6,5%)
2002	248	111 (44,8%)	0	
2003	236	100 (42,4%)	60	3 (5,0%)
2004	191	101 (52,9%)	59	1 (1,7%)
2005	208	127 (61,1%)	0	
2006	281	153 (54,4%)	48	2 (4,2%)
2007	245	122 (49,8%)	1	1 (100%)
2008	193	118 (61,1%)	84	4 (4,8%)
2009	182	115 (63,2%)	37	2 (5,4%)
2010	166	65 (39,2%)	36	3 (8,3%)
2011	136	75 (55,1%)	0	
2012	163	93 (57,1%)	84	1 (1,2%)
<b>Historical data</b>	<b>3227</b>	<b>1613 (50,0%)</b>	<b>649</b>	<b>29 (4,5%)</b>
2013	215	92 (42,8%)	42	3 (7,1%)
2014	153	59 (38,6%)	89	2 (2,2%)
2015	185	77 (41,6%)	91	2 (2,2%)
2016	170	71 (41,8%)	215	6 (2,8%)
2017	107	42 (39,3%)	190	12 (6,3%)
2018	147	51 (34,7%)	174	9 (5,2%)
2019	105	49 (46,7%)	273	23 (8,4%)
2020	4	2 (50,0%)	136	52 (38,2%)
<b>Current data</b>	<b>1086</b>	<b>443 (40,8%)</b>	<b>1210</b>	<b>109 (9,0%)</b>
<b>TOTAL</b>	<b>4313</b>	<b>2056 (47,7%)</b>	<b>1859</b>	<b>138 (7,4%)</b>

Dividing each basin, 4 regions were established to compare entanglement in Loggerheads (Table V.13 and fig. V.12):

- ATL-OSPAR: data from Portugal (Azores) and France Atlantic (no data collected)
- ATL-CAN: data from Canary Islands
- MED-WEST: data from Spanish Mediterranean and France (Gulf of Lion)



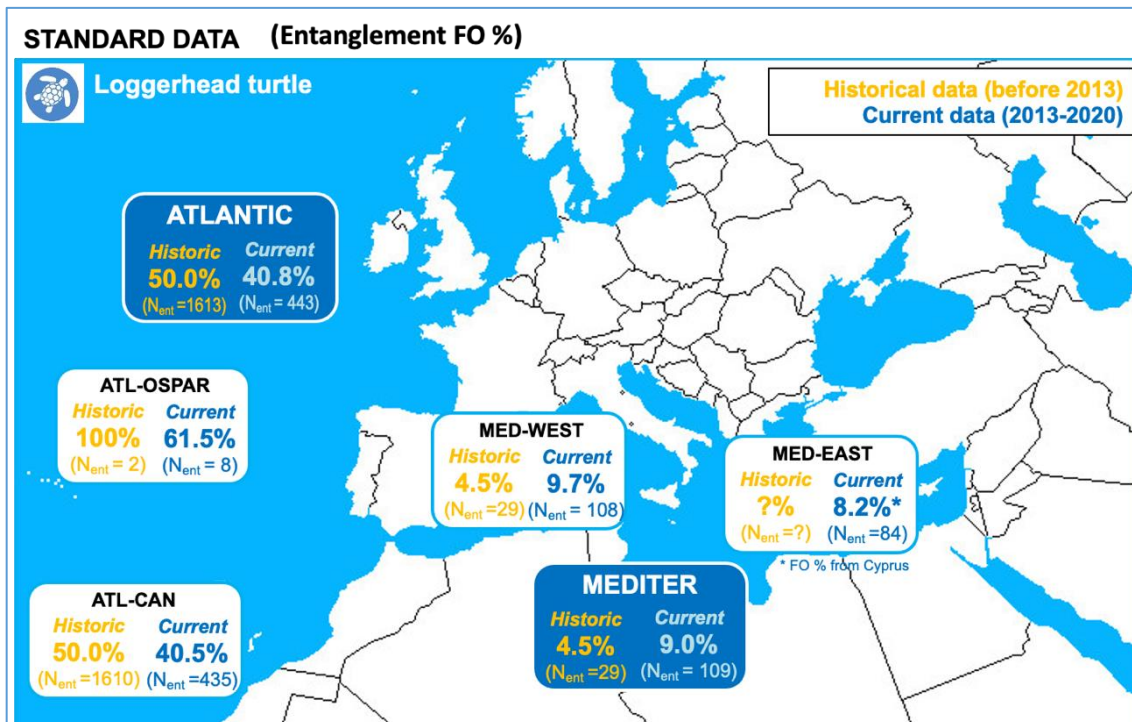
- MED-EAST: data from Italy, Greece, Turkey (no data collected), Tunisia (no data collected) and Cyprus (only data from 2019).

In the Atlantic basin, the impact of entanglement is very high (50% and 40,8% of historical and current data). Moreover, highest FO% were observed in the OSPAR area (100% and 61,5% respectively), even if the total number of individuals impacted by entanglement is much higher the Canary Island region (Nent = 2046). In general, there are many more Sea turtle stranding in the Canary Islands and the deep review conducted were very important to characterise different threats impacting sea turtles in the region, including the relevance of entanglement.

In the Mediterranean basin, very few data were collected from the MED-EAST, where only data from Cyprus could be used to calculate FO%. The number of entangled loggerheads found in Italy and Greece is not negligible (33 and 50 respectively), but more data are required to better understand the impact of entanglement in this region.

**Table V.13.** Frequency of entanglement in loggerhead turtles in each basin and regions.

Loggerhead	ATL-OSPAR		ATL-CAN		MED-WEST		MED-EAST	
	Ntot	Nent (FO%)	Ntot	Nent (FO%)	Ntot	Nent (FO%)	Ntot	Nent (FO%)
1990			6	1 (16,7%)				
1991			30	8 (26,7%)				
1992			39	16 (41,0%)				
1993			90	38 (42,2%)				
1994			17	10 (58,8%)				
1995			18	8 (44,4%)	25	1 (4,0%)		
1996			12	4 (33,3%)	0			
1997			53	30 (56,6%)	0			
1998			70	44 (62,9%)	39	1 (2,6%)		
1999			151	74 (49,0%)	0			
2000			205	111 (54,1%)	52	2 (3,8%)		
2001			285	88 (30,9%)	124	8 (6,5%)		
2002			248	111 (44,8%)	0			
2003			236	100 (42,4%)	60	3 (5,0%)		
2004			191	101 (52,9%)	59	1 (1,7%)		
2005			208	127 (61,1%)	0			
2006			281	153 (54,4%)	48	2 (4,2%)		
2007			245	122 (49,8%)	1	1 (100%)		
2008			193	118 (61,1%)	84	4 (4,8%)		
2009			182	115 (63,2%)	37	2 (5,4%)		
2010	1	1 (100%)	165	64 (38,8%)	36	3 (8,3%)		
2011	0		136	75 (55,1%)	0			
2012	1	1 (100%)	162	92 (56,8%)	84	1 (1,2%)		
<i>Historical data</i>	<i>2</i>	<i>2 (100%)</i>	<i>3223</i>	<i>1610 (50,0%)</i>	<i>649</i>	<i>29 (4,5%)</i>		
2013	0		215	92 (42,8%)	42	3 (7,1%)		14 (?%)
2014	0		153	59 (38,6%)	89	2 (2,2%)		10 (?%)
2015	1	0 (0,0%)	184	77 (41,8%)	91	2 (2,2%)		10 (?%)
2016	0		170	71 (41,8%)	215	6 (2,8%)		14 (?%)
2017	4	3 (75,0%)	103	39 (37,9%)	190	12 (6,3%)		22 (?%)
2018	2	2 (100%)	145	49 (33,8%)	174	9 (5,2%)		6 (?%)
2019	2	1 (50,0%)	103	48 (46,6%)	176	22 (12,5%)	97	8 (8,2%)
2020	4	2 (50,0%)			136	52 (38,2%)		
<i>Current data</i>	<i>13</i>	<i>8 (61,5%)</i>	<i>1073</i>	<i>435 (40,5%)</i>	<i>111</i>	<i>108 (9,7%)</i>	<i>unknown</i>	<i>84 (?%)</i>
<b>TOTAL</b>	<b>15</b>	<b>10 (66,7%)</b>	<b>4298</b>	<b>2046 (47,6%)</b>	<b>176</b>	<b>137 (7,8%)</b>	<b>unknown</b>	<b>84 (?%)</b>



**Figure V.12.** Frequency of entanglement in loggerhead turtles in each basin and region (historical and current data).

In the MED-WEST, the Spanish coasts showed the higher impact of entanglement in all the Mediterranean Sea (12,1% FO). In 2019 and mainly in 2020 an important increase was observed (13,4% and 38,2%), where data from new regions of the Spanish Mediterranean were included, such as Murcia and Balearic Islands. Both regions showed important impact of entanglement on loggerheads. This increase is also observed in France Mediterranean waters, where 8,5% and 5,3% of the animals registered in 2018 and 2019 were impacted by entanglement. In general, more data are required to better understand the impact of entanglement on a sub-regional level.

#### V.7.1.2. Type of litter involved on entanglement

Considering all data collected from all stakeholders (historical and current data), the type of litter involved on entanglement was not identified in many cases (67,8%, table V.14). Instead of this, the main litter typologies involved on entanglement in the European waters were “fishing nets” (10,07%), “fishing lines” (5,03%) and “ropes, strings and cords” (3,76%), in litter derived from fisheries and maritime sources, and “heavy-duty sacks” on litter from land-based sources.

**Table V.14.** FO % of the type of litter involved on entanglement on sea turtle species.

	<b>All turtles</b> (N = 2364)	<b>Loggerhead</b> (N = 2334)	<b>Green</b> (N = 23)	<b>Leatherback</b> (N = 7)
<b>Fisheries &amp; maritime sources</b>	447 (18,9%)	435 (18,6%)	8 (34,8%)	4 (57,1%)
Fishing nets	238 (10,1%)	232 (9,9%)	5 (21,7%)	1 (14,3%)
Fishing lines	119 (5,0%)	116 (5,0%)	3 (13,0%)	0 (0,0%)
Rope, string, cord	89 (3,8%)	88 (3,8%)	0 (0,0%)	1 (14,3%)
Floats, buoys, fenders	10 (0,4%)	10 (0,4%)	0 (0,0%)	0 (0,0%)
Pots, tops, traps	7 (0,3%)	6 (0,3%)	0 (0,0%)	1 (14,3%)
Aquaculture related	1 (0,0%)	1 (0,0%)	0 (0,0%)	0 (0,0%)
Other fishing related	16 (0,7%)	15 (0,6%)	0 (0,0%)	1 (14,3%)
<b>Land-based sources</b>	294 (12,4%)	288 (12,3%)	6 (26,1%)	0 (0,0%)
Plastic bag	8 (0,3%)	6 (0,3%)	2 (8,7%)	0 (0,0%)
Mesh bags	6 (0,3%)	6 (0,3%)	0 (0,0%)	0 (0,0%)
Heavy-duty sacks	217 (9,2%)	215 (9,2%)	2 (8,7%)	0 (0,0%)
Packing rings	2 (0,1%)	2 (0,1%)	0 (0,0%)	0 (0,0%)
Packing strapping bands	5 (0,2%)	5 (0,2%)	0 (0,0%)	0 (0,0%)
Plastic sheeting greenhouse	6 (0,3%)	6 (0,3%)	0 (0,0%)	0 (0,0%)
Food packing	1 (0,0%)	1 (0,0%)	0 (0,0%)	0 (0,0%)
Other packing	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)
Textile	1 (0,0%)	1 (0,0%)	0 (0,0%)	0 (0,0%)
Medical and hygienic care	1 (0,0%)	0 (0,0%)	1 (4,3%)	0 (0,0%)
Recreational related	1 (0,0%)	1 (0,0%)	0 (0,0%)	0 (0,0%)
Other land-based	53 (2,2%)	52 (2,2%)	1 (4,3%)	0 (0,0%)
<b>Unknown / not identified</b>	1602 (67,8%)	1590 (68,1%)	9 (39,1%)	3 (42,9%)

Regarding Sea turtle species, “plastic bags” (8,7%) and “medical & hygienic care” (4,3%) presented a more specific impact on green turtle, probably caused by the neritic behaviour of this species (the sample size is however small in this species and % have to be interpreted carefully), in comparison with the oceanic behaviour of loggerhead and leatherback turtles. Green turtle is also impacted by “fishing nets” (21,7%), “fishing lines” (13,0%) and “heavy-duty sacks” (8,7%).

Leatherback turtle presented few numbers of cases (N = 7), but all the individuals were impacted by litter derived from fisheries and maritime sources (“fishing nets”, “ropes, strings, cords”, “floats, buoys and fenders” and “other fishing related”). Surprisingly, no impact by “fishing lines” were registered.

In general, the loggerhead turtle presented most of the data collected, for this reason a deeper analysis was conducted on this species.

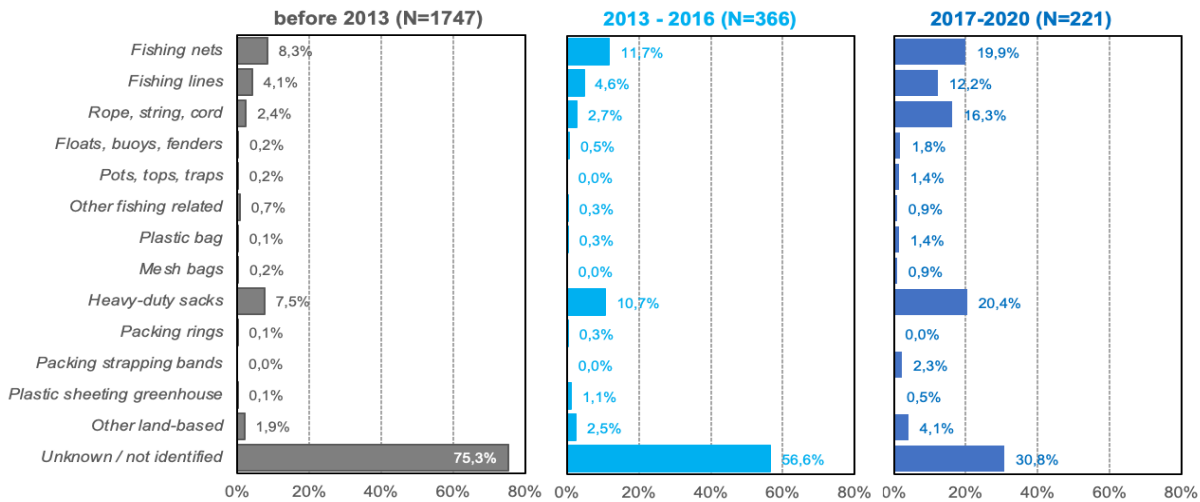
#### V.7.1.3. Temporal analysis

The most important difference observed between historical and current data focus on the high percentage of historical cases with not identified litter typology (75,3%). An important decrease was observed on data collected from 2013 to 2020 (current data), where 46,8% of the cases the litter typology was not identified (Fig. V.13). Even more, during the years where INDICIT projects were in progress (2017-2020), a greater attention was paid by stakeholders on litter typologies involved on entanglement, because the percentage of not identified cases decreased until 30,8%. So, INDICIT project has improved data collection on entanglement between European stakeholders.

In any case, in both historical and current data, litter typologies derived from fisheries and maritime sources presented the greatest impact on loggerhead turtle (15,3% and 28,6% in historical and current data respectively), in comparison with litter derived from land-based sources (9,8% and 19,8% in historical and current data respectively).

Looking for the specific litter typologies involved on entanglement on loggerhead turtle, “fishing nets” and “heavy duty-sacks” were the litter typologies more frequently observed entangling the animals.

**NOTE:** The overall analysis is strongly influenced by the high amount of data collected from Canary Islands. The regional analysis will help to better analyse the impact of litter typologies.



**Figure V.13.** FO % of types of litter involved on entanglement on loggerheads by periods.

#### V.7.1.4. Regional analysis (only current data)

Standard data collected from Greece have no info about litter typologies. However, most part of the images collected from social media from Greece were animals registered by the rescue centre in charge of sea turtles stranded in Greece (i.e. the ONG Archelon). For this reason, we have included the data obtained from Archelon’ social media in the analysis of the Standard data from Greece. Similarly, data from social media from Turkey has been included in the analysis of Standard data.

Important differences were observed on litter typologies involved on entanglement of loggerheads found in the Atlantic and the Mediterranean basins (Table V.15). A high percentage of cases remains with litter not identified (56,1%) in the Atlantic, instead of the 16,9% found in the Mediterranean.

In the Atlantic, FO % of litter derived from fisheries and maritime sources (26,7%) are very close to the FO% of litter from land-based sources (20,9%), instead of the big difference observed in the Mediterranean where FO% of litter derived from fisheries and maritime sources were more than three times higher than FO% of litter derived from land-based sources.

**Table V.15.** FO % of types of litter involved on entanglement on loggerheads by basins.

<b>Loggerhead only</b> (Current data 2013-2020)	<b>ATL</b> (N = 465)	<b>MED</b> (N=118)
<b>Fisheries &amp; maritime sources</b>	124 (26,7%)	79 (66,9%)
Fishing nets	63 (13,5%)	37 (31,4%)
Fishing lines	36 (7,7%)	22 (18,6%)
Rope, string, cord	33 (7,1%)	24 (20,3%)
Floats, buoys, fenders	5 (1,1%)	4 (3,4%)
Pots, tops, traps	3 (0,6%)	0 (0,0%)
Aquaculture related	0 (0,0%)	1 (0,8%)
Other fishing related	3 (0,6%)	0 (0,0%)
<b>Land-based sources</b>	97 (20,9%)	24 (20,3%)
Plastic bag	2 (0,4%)	4 (3,4%)
Mesh bags	2 (0,4%)	0 (0,0%)
Heavy-duty sacks	75 (16,1%)	10 (8,5%)
Packing rings	1 (0,2%)	0 (0,0%)
Packing strapping bands	3 (0,6%)	2 (1,7%)
Plastic sheeting greenhouse	5 (1,1%)	0 (0,0%)
Food packing	1 (0,2%)	0 (0,0%)
Other packing	0 (0,0%)	0 (0,0%)
Textile	0 (0,0%)	0 (0,0%)
Medical and hygienic care	0 (0,0%)	0 (0,0%)
Recreational related	0 (0,0%)	3 (2,5%)
Other land-based	12 (2,6%)	6 (5,1%)
<b>Unknown / not identified</b>	261 (56,1)	20 (16,9%)

Considering the 4 regions established dividing each basin in two regions (Fig. V.14), important differences were observed mainly on the Atlantic regions.

In the Atlantic, several typologies were only observed in the ATL-CAN (“floats, buoys and fenders”; “pots, tops and traps”, “other fishing related”, “plastic bags”, “mesh bags”, “packing strapping bands”, “plastic sheeting greenhouse”), although it could be caused by the low number of entangled animals observed in the ATL-OSPAR (n=8) in relation with the ATL-CAN (n=457).

In the ATL-OSPAR entanglement is clearly dominated by “fishing nets” (87,5%), followed by far by “ropes, strings, cords” (25,0%). On the contrary, in the ATL-CAN region the litter typology most frequently observed entangling loggerheads is “heavy-duty sacks” (16,4%), followed by “fishing nets” (12,3%).

In the Mediterranean, differences between regions are smaller, mainly found on the higher FO% of “heavy-duty sacks” observed in the MED-WEST (15,6%), instead of the 4,1% observed in the MED-EAST. This difference, together with the FO% of “other land-based” (11,1%) observed in the MED-WEST, constituted the greater FO% from litter derived from land-based sources observed in the MED-WEST (33,3%).

In both Mediterranean regions, “fishing nets” and “ropes, strings and cords” are the litter typologies most frequently observed (33,3% - 30,1% and 20,0% - 20,5% respectively), followed by “fishing lines”, where a slightly higher frequency was found in the MED-EAST (21,9%), than in the MED-WEST (13,3%).

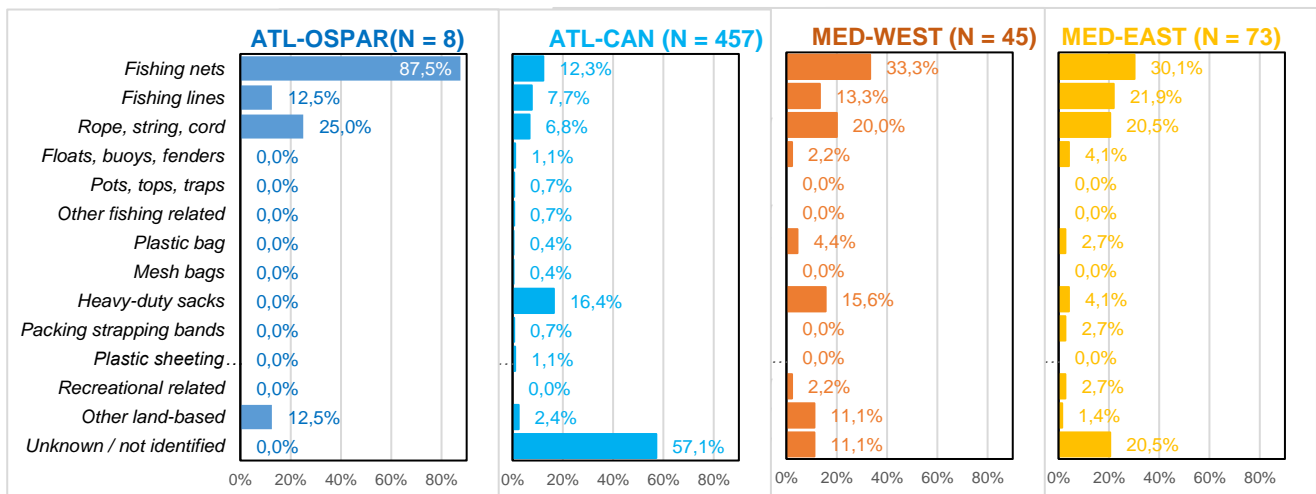


Figure IV.14. FO % of types of litter involved on entanglement on loggerhead turtles by regions.

Figure V.15 provides a global view of the frequencies of types of litter observed in the Med and Atlantic basin, with several regional differences in the Med basin. The most important differences were observed in France (highest impact of “ropes, stings, cords”; 57.1%), Spain (highest impact of “heavy-duty sacks”, 22.6%), and Turkey (highest impact of “fishing lines”). In Cyprus, only 1 loggerhead was registered entangled on “heavy-duty sacks”, which was also observed in Green turtles on this region.

### STANDARD DATA

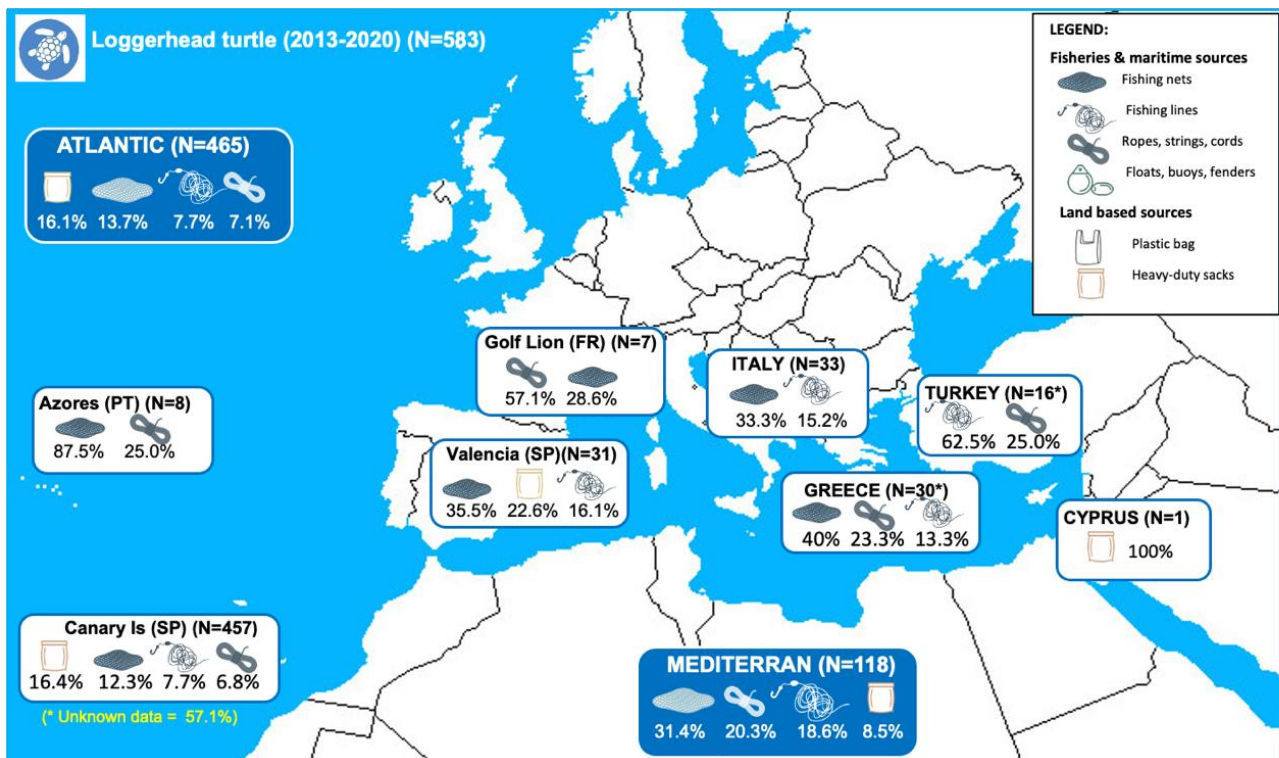


Figure V.15. Distribution of litter involved in Loggerhead turtle in the European countries/regions. (Standard data from loggerhead turtle, 2013 - 2020).

### V.7.1.5. Impact severity

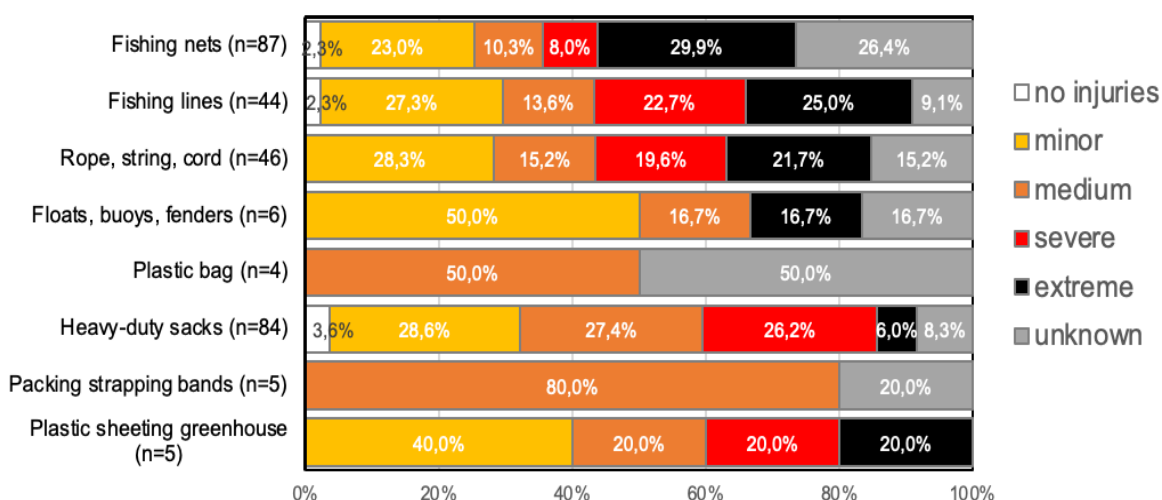
Another important factor to consider is the impact of entanglement on animal health. It is measured by the parameter “Impact severity”, which is based on the animal viability after the lesions/injuries caused by the entanglement.

Greater mortality was generated by litter typologies derived from fisheries and maritime sources (46,2% extreme severity), caused by the larger size and weight presented by this litter typologies (“fishing nets”, “ropes, strings, cords”), which usually drown the entangled animals (Table V.16). On the contrary, litter typologies derived from land-based sources are usually lightweight plastics (“heavy-duty sack”, “plastic bags”) that float on the surface, so the entangled animals are more likely to survive.

**Table V.16.** Impact severity observed on entangled loggerheads classified by litter source (Standard data on loggerhead turtle, 2013-2020).

	No injuries (N = 9)	Minor (N = 119)	Medium (N = 108)	Severe (N = 129)	Extreme (N = 104)	Unknown (N = 90)
Fisheries & maritime sources (N=168)	3 (33,3%)	37 (31,1%)	22 (20,4%)	22 (17,1%)	48 (46,2%)	36 (40,0%)
Land-based sources (N=116)	3 (33,3%)	27 (22,7%)	30 (27,8%)	29 (22,5%)	11 (10,6%)	16 (17,8%)
Unknown / not identified (N=275)	3 (33,3%)	55 (46,2%)	56 (51,9%)	78 (60,5%)	45 (43,3%)	38 (42,2%)

Looking in detail the impact severity of main litter typologies (Fig. V.16), extreme impact (*injuries kill the animal or do not allow to recover*) is more frequent on “fishing nets” (29,9%), “Fishing lines” (25,0%) and “ropes, strings, cords” (21,7%). In other hand, severe impact (*alter the natural body condition of the animal*) is more frequent on animals entangled on “heavy-duty sacks” (26,2%) and “fishing lines” (22,7%), because they have higher cutting capacity, causing most part of flipper amputations. “Ropes, strings and cords” also showed a high frequency of severe impact (19,6%), although is highly dependent on the rope thickness (Again, note that the overall analysis is strongly influenced by the high amount of data collected from Canary Islands, where a great majority of the animals are found alive).



**Figure V.16.** Impact severity (%) generated by main litter types on entangled Sea turtles (Standard data on Loggerhead turtle, 2013-2020).

### V.7.2. Results obtained on entanglement on other taxa

Very few Standard data were collected from cetaceans by stakeholders. Only data from Azores (4 cases) and Italy (N = 23) were included in the database. Remember that more difficulties were found to distinguish between entanglement and bycatch in cetaceans (Table V.17).

Most part of cetaceans registered were impacted by “fishing nets” (74,1%). Only one case was entangled on a “plastic” (included on category “other land-based”), that was also attached on a “fishing net”. The unknown cases were the 4 entangled animals registered in Azores, where types of litter were not identified.

**Table V.17.** FO% of types of litter involved on entanglement on cetaceans in European waters (Standard data, 2013-2020).

	<b>CETACEANS</b> (N = 27)
<b>Fisheries &amp; maritime sources</b>	23 (85,2%)
Fishing nets	20 (74,07%)
Fishing lines	1 (3,7%)
Rope, string, cord	3 (11,1%)
Floats, buoys, fenders	0 (0,0%)
Pots, tops, traps	0 (0,0%)
Aquaculture related	0 (0,0%)
Other fishing related	1 (3,7%)
<b>Land-based sources</b>	1 (3,7%)
Plastic bag	0 (0,0%)
Mesh bags	0 (0,0%)
Heavy-duty sacks	0 (0,0%)
Packing rings	0 (0,0%)
Packing strapping bands	0 (0,0%)
Plastic sheeting greenhouse	0 (0,0%)
Food packing	0 (0,0%)
Other packing	0 (0,0%)
Textile	0 (0,0%)
Medical and hygienic care	0 (0,0%)
Recreational related	0 (0,0%)
Other land-based	1 (3,7%)
<b>Unknown / not identified</b>	4 (14,81%)

### V.7.3. Indicator’s constraints (Loggerhead turtle)

Regional differences have been found in litter typologies involved on entanglement but other biological factors must be considered in this important impact.

*Animal size/stage:* This factor needs to be considered because some differences were found according to size/life stages of Sea turtles (Table V.18).



**Table IV.18.** FO % of types of litter involved on entanglement on standard data from loggerheads (2013-2020) in relation with size/stage of individuals.

	<b>Hand size</b> <i>yearling</i> ( <i>&lt;20cm</i> )  ( <i>N=18</i> )	<b>Elbow size</b> <i>small juvenile</i> ( <i>20-40cm</i> )  ( <i>N = 285</i> )	<b>Arm size</b> <i>large juvenile</i> ( <i>40-60cm</i> )  ( <i>N = 89</i> )	<b>Half-body size</b> <i>Subadult /</i> <i>Adult (&gt;60cm)</i>  ( <i>N=21</i> )
<b><i>Fisheries &amp; maritime sources</i></b>	2 (11,1%)	71 (24,9%)	38 (42,7%)	14 (66,7%)
Fishing nets	1 (5,6%)	35 (12,3%)	24 (27,0%)	8 (38,1%)
Fishing lines	0 (0,0%)	18 (6,3%)	8 (9,0%)	5 (23,8%)
Rope, string, cord	0 (0,0%)	27 (9,5%)	7 (7,9%)	4 (19,0%)
Floats, buoys, fenders	0 (0,0%)	4 (1,4%)	0 (0,0%)	1 (4,8%)
Other fishing related	0 (0,0%)	2 (0,7%)	1 (1,1%)	0 (0,0%)
<b><i>Land-based sources</i></b>	9 (50,0%)	76 (26,7%)	13 (14,6%)	2 (9,5%)
Plastic bag	0 (0,0%)	3 (1,1%)	0 (0,0%)	1 (4,8%)
Mesh bags	0 (0,0%)	1 (0,4%)	1 (1,1%)	0 (0,0%)
Heavy-duty sacks	7 (38,9%)	57 (20,0%)	7 (7,9%)	1 (4,8%)
Packing rings	0 (0,0%)	1 (0,4%)	0 (0,0%)	0 (0,0%)
Packing strapping bands	0 (0,0%)	4 (1,4%)	1 (1,1%)	0 (0,0%)
Plastic sheeting greenhouse	0 (0,0%)	4 (1,4%)	1 (1,1%)	0 (0,0%)
Other land-based	2 (11,1%)	9 (3,2%)	3 (3,4%)	0 (0,0%)
<b><i>Unknown / not identified</i></b>	8 (44,4%)	153 (53,7%)	40 (44,9%)	5 (23,8%)

Litter typologies derived from land-based sources (heavy-duty sacks) are more frequently observed impacting smaller individuals (38,9% in yearlings, 20,0% in small juveniles), than larger animals (7,9% in large juveniles, 4,8% in subadults/adults). The frequency of occurrence of “heavy-duty sacks” varies inversely to the size of the individuals. On the contrary, frequency of litter typologies derived from fisheries and maritime sources increase with animal size.

This clear difference could be caused by the different behaviours observed on loggerhead turtle related to their size/stage, where smaller animals remain on the open oceans linked to drift currents, which aggregate a great variety of floating litter, instead of larger animals which are more actively looking for food, approaching high productivity areas where more fishery activities are located. Adults and subadults, mainly found in the Mediterranean, usually present specific feeding areas, many times linked to high productivity zones, where fisheries are also working.

#### V.7.4. GES scenarios

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*”.

**Data collected on entanglement clearly focused on the loggerhead turtle**, in which most data has been collected (N = 2332, 97,53%). So, baselines and GES can only be proposed for this species. Data on other species,

such as the green and leatherback turtle, or other taxa (cetaceans, seals and seabirds), are very interesting and could now be collected during the next years with the Standard protocol developed by INDICIT-II consortium.

Globally, data collected from different regions (stakeholders) are very diverse, with important differences in time periods included, where most accurate data were collected from 2017 to date. Importantly, new databases have been created and many stakeholders updated and homogenised their existing databases thanks to the Standard protocol for entanglement developed by INDICIT-II consortium.

In relation with GES scenarios, **not enough data are currently available to establish GES** and baselines (with confident values and powerful trend). However, the collection of the available data can be used to propose several GES scenarios.

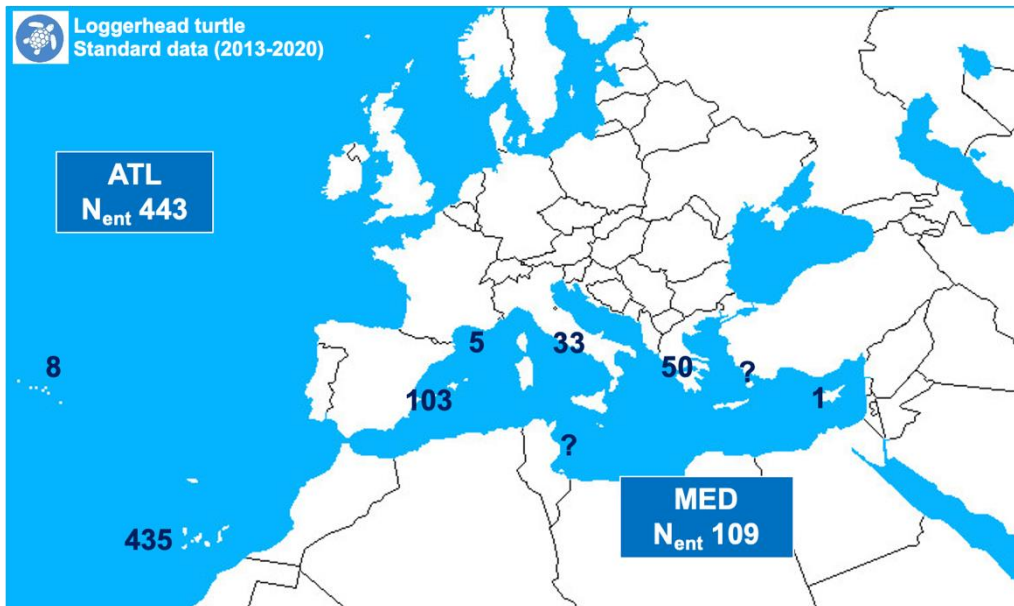
***Scenario 1 – based on “number of individuals”:***

*“The number of individuals impacted by entanglement in each region during a 6-year cycle will be reduced”*

**The baselines from each region** (entangled loggerheads from 2013 to 2020) can be obtained by the currently observed respective **numbers** (Fig. V.17).

Temporal and spatial variations in loggerhead turtle abundance needs to be considered, because the number of entangled individuals could be affected by the abundance of loggerhead turtles inhabiting each region during each period. For example, the high number registered on Canary Islands (N=435) could be caused by the high abundance of loggerhead turtle in this region, and not only by the pressure of entanglement.

Each region needs to be analysed separately, and the “number of entangled individuals” needs to be related with data from “Indicator Abundance” collected on Descriptor 1 of the MSFD for sea turtles.



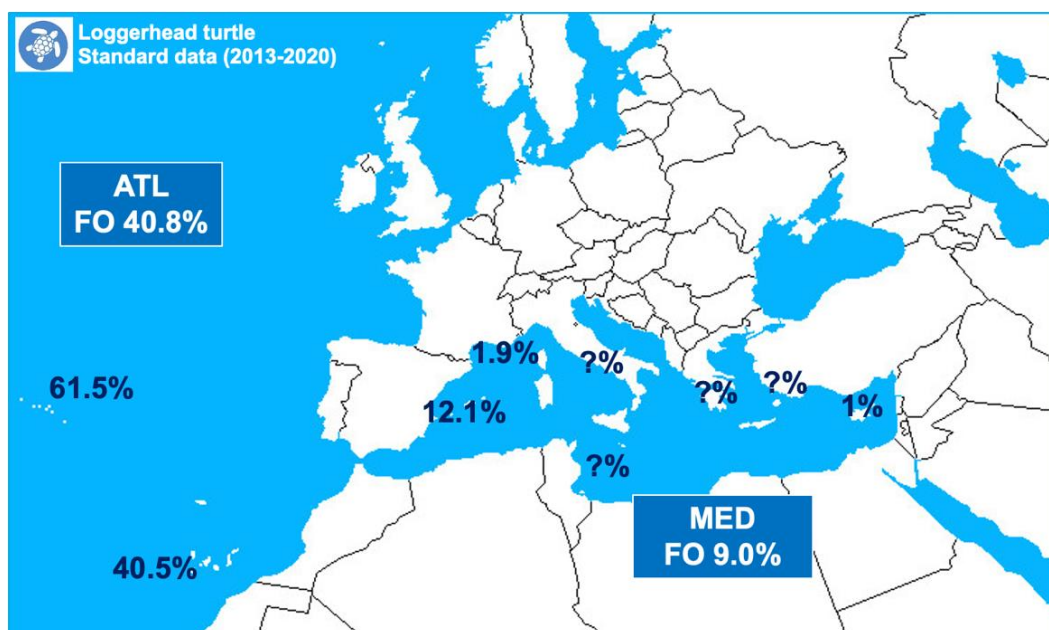
**Figure V.17.** Number of entangled loggerheads (standard data) registered between 2013 to 2020 on the European regions (baselines).

**Scenario 2 – based on “Entanglement FO%”:**

*“The FO% of entangled loggerheads in each region during a 6-year cycle will be reduced”*

**Baselines from each region** (entangled loggerhead turtle from 2013 to 2020) can be obtained by the currently observed **frequency of entanglement** (Fig. V.18). FO% of different threats impacting the loggerhead turtle in each region needs to be considered, because variations if the FO% of other impacts, such as bycatch, could affect the entanglement FO%. For example, the low FO% registered in Mediterranean France (1,9%) could be caused by the high FO% of bycatch generated by the important work developed with fishermen in this area (most part of the animals registered in this area is delivered by fishermen).

Each region needs to be analysed separately, and the variations on FO% from main threats (bycatch, entanglement, boat collisions, human interaction, etc.) needs to be considered.



**Figure V.18.** FO% of entangled Loggerheads (standard data) registered between 2013 to 2020 on the European regions (baselines).

**Scenario 3 – based on “Litter typologies FO%”:**

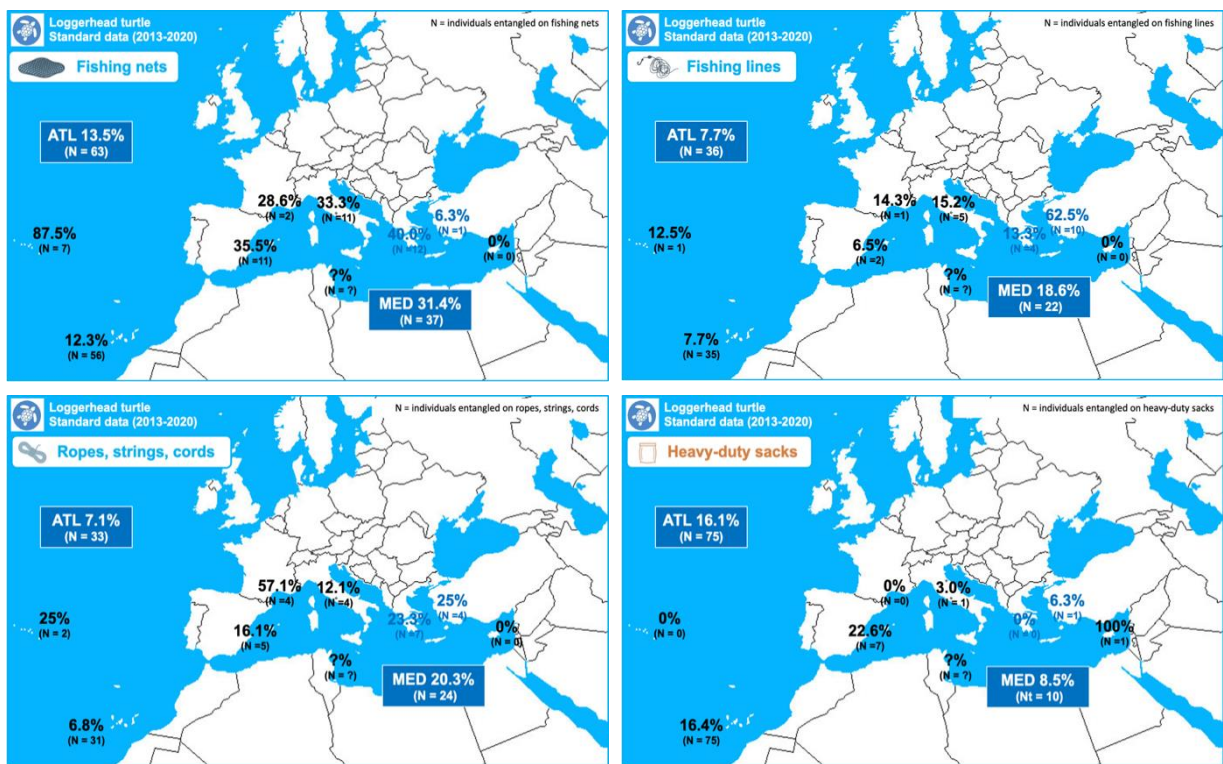
*“The FO% of specific litter typologies (fishing nets, fishing lines, ropes, stings, cords and heavy-duty sacks) entangling loggerhead turtles in each region during a 6-year cycle will be reduced”.*

The types of litter most frequently registered in the European waters are “fishing nets”, “fishing lines”, “ropes, strings, cords” and “heavy-duty sacks”. Other types could be also included in specific regions when required.

Each region needs to be analysed separately, and the variations on FO% from other types of litter needs to be considered, because decrease / increase in FO% of one litter typology could affect the FO% of the other types of litter.

This scenario could be directly related with POMs implemented in each region, for example, POMs focus on reducing litter derived from fisheries.

**Baselines for main types of litter on each region** (on entangled loggerheads from 2013 to 2020) can be obtained by the currently observed **frequency of the different types of litter** (Fig. V.19).



**Figure V.19.** Types of Litter (FO%) involved in entangling loggerhead turtles (standard data) registered between 2013 to 2020 on the European regions (baselines). (Blue letters: data obtained from images collected from social media and opportunistic platforms).

## V.8. Recommendations:

### Networking:

- In certain areas/countries, **a better development of the network will be necessary**, by e.g. creating/reinforcing stranding networks and rescue centres, ensuring constant means and training referents or coordinators at the local/regional levels.
- **Engaging stakeholders in certain zones is 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 mainland Portugal and Spain.
- **Key-stakeholders, such as fishermen**, who are keys to recovering sea turtles in certain areas, should be involved. Including them for the monitoring of litter instead of directly for recovering bycatch individuals, could support the collaboration.
- **Arrange regional / national or international workshops among stakeholders**, together with other experts and with representatives, could facilitate the involvement of new stakeholders. These workshops should propose training sessions and sharing experience on e.g. methodologies to monitor litter impacts, which could still be optimised.
- For a **better reporting of information and data**, a diagram of the networks should be built at the national and regional levels, considering specificities of each country. These diagrams should be disseminated to stakeholders in order to facilitate procedures and contacts.

### Harmonisation of data collection:

- For entanglement specifically, **the inclusion of pictures** of individuals on stranding protocols is the best way to achieve accurate databases that could be reviewed by experts on marine litter to harmonise and avoid confusion on litter classification. Some tools may support data collection, in particular when a turtle is observed at sea or found stranded or bycaught, for example phone applications or online platforms, such as *RedPROMAR App*, developed by the Canary islands government, allowing citizen or institutions (NGOs, rescue centres, stranding networks) to post pictures with GPS locations; or *ObsEnMer* which offers a collaborative platform, managed by *Cybelle Planete* (France).
- **1-2 experts on marine litter and marine fauna could be involved as “focal points”** in each country/region to coordinate data collection by stakeholders, harmonise litter classification involved on entanglement (review pictures, identify new litter typologies, etc.), and establish connections with national authorities to facilitate transfer of data for the Marine Strategy evaluation.

### Data banking and cleaning:

- **1-2 experts on marine litter and marine fauna could be involved as “focal points”** in each country/region to coordinate data collection by stakeholders, harmonise litter classification involved on entanglement (review pictures, identify new litter typologies, etc.), and establish connections with national authorities to facilitate transfer of data for the Marine Strategy evaluation.

### Need of additional data and further analyses to evaluate GES scenarios and indicator’s constraints:

- Most part of data collected on entanglement by INDICIT II consortium was on loggerhead turtle (N = 2332, 97,53%), so, GES evaluation and scenarios can only be generated with this species.

In general, data collected from different regions (stakeholders) during INDICIT II project are diverse and disperse, with important differences between species and time periods included, where most accurate data were collected from 2017 to date. Moreover, important bases have been created and most part of stakeholders updated and harmonized their databases and incorporated most important parameters described on the standard protocol for entanglement, so, evaluation of GES scenarios and indicator’s constraints could be established more accurately in the next marine strategy cycle.

**Data on other species**, such as green and leatherback turtle, or other taxa (cetaceans, seals and seabirds), are **very interesting** and could be collected during the next years following the standard protocol developed by INDICIT II consortium.

### Recommendations by taxa:

- *For sea turtles*: the large network trained to collect data on entanglement on loggerhead turtle could include data collection on other species, such as green and leatherback turtle. Many stakeholders are already including data collection on these species. In addition, data collected on the **green turtle** during INDICIT II project (standard and data collected from social media) show that **this species could be a very interesting indicator** for marine litter on neritic ecosystems.

- *For cetaceans*: **a deeper analysis on data collected** by stranding networks (most of them involved on sea turtles monitoring) is required, updating and harmonising databases. In addition, data collected on entanglement from recreational boats (mainly whale watching companies) could be also evaluated.

- *For seals*: harbour seal (*Phoca vitulina*) and grey seal (*Halichoerus grypus*) in the northern Atlantic, and monk seal (*Monachus monachus*) in the Mediterranean, **could be evaluated to see their relevance as indicator**, based on their abundance, the prevalence of entanglement and the existence of a network for their observation and their rescue. **New stakeholders need to be included** to monitor entanglement on seals: rescue centres and teams in charge of monitoring their colonies (researchers and NGOs).

- *For seabirds*: monitoring the litter used by seabirds from **nest construction** has been proposed by experts. Main species proposed are, European shag (*Phalacrocorax aristotelis*), the Scopoli's shearwater (*Calonectris Diomedea*) and the northern Gannet (*Morus bassanus*). Observations appeared most often during nest surveys or fisheries observer campaigns.

## VI. Indicator micro-litter ingested by fish

### VI.1. Introduction

Oceans and seas have represented main sinks for human waste for many years. To deal with the several threats posed by litter to the marine ecosystems, the Marine Strategy Framework Directive (2008/56/EC) with the new Commission Decision (2017/848/EU) replaced the INDICATOR 10.2.1 “Trends in the amount and composition of litter ingested by marine animals (e.g. stomach analysis)” (2010/477/EU), with the CRITERION D10C3 “*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 implies that EU Member States must develop monitoring programs for assessing litter ingestion by marine organisms.

Two bio-indicator species have already been chosen for monitoring macro-litter (>5 mm) ingestion: the seabird *Fulmarus glacialis* for the Northern European Sea (van Franeker, 2004; van Franeker et al., 2011), and the loggerhead sea turtle *Caretta caretta* for the Mediterranean basin (Matiddi et al., 2011; 2017; 2019).

On the contrary, no monitoring strategies have been yet developed for assessing the ingestion of micro-litter, which includes anthropogenic particles smaller than 5 mm in size, both of synthetic (*i.e.*, microplastics) and natural origin (*i.e.*, some micro-fibers from textiles).

Expert researchers of the MSFD TG-ML (Galgani *et al.*, 2013) established several basic requirements for the selection of bioindicators of litter ingestion, such as:

- **Sample availability:** “Samples of a monitoring species should be available with adequate numbers of individuals over a wider span of time and space”;
- **Regular litter consumption:** “Frequency of occurrence and amounts of plastic found in stomachs should be high enough to allow detection of trends over time and geographical patterns”;
- **Marine feeding habits:** “Stomach contents should reflect only the marine environment”.

Fish, including teleost and elasmobranchs (Bray et al., 2019; Valente *et al.*; 2019; Tsangaris *et al.*, 2020) seem to be the most suitable target organisms for monitoring micro-litter ingestion.

Recent studies have demonstrated that micro-litter ingestion occurs in various fish species, including species of commercial importance (Foekema *et al.*, 2013; Güven et al; 2017 Herrera et al., 2019; Pereira et al., 2020). However, none of the proposed species have been selected to plan a common monitoring strategy (UNEP/MAP WG.439/Inf.12.2017; Fossi *et al.*, 2018; Bray *et al.*, 2019). Moreover, the different methods, procedures of sampling and analysis, are still not harmonised (Silvestri *et al.*, 2018). The heterogeneity of applied methods for the collection and analysis of fish samples does not allow a reliable comparison of results from different studies (Cowger *et al.*, 2020). For this reason, the development of a standard protocol for evaluating micro-litter ingestion by fish is a primary need, in order to collect homogeneous data and elaborating proposal of scenarios to define and achieve the Good Environmental Status.

This report shows the INDICIT protocol (<https://indicit-europa.eu>) which can be used **for a standard collection of data in a monitoring program on micro-litter ingestion in fish**. Also, this report provides the results obtained through the collected data according to this standard protocol by some partners and stakeholders in a dedicated pilot action. Moreover, it also proposes a **possible scenario to achieve the Good Environmental Status** using the ingestion of micro-litter by fish.

## VI.2. Development of common analytical approaches

Monitoring activities must be based on common analytical approaches to ensure data comparability over time and space. However, since the study of micro-litter pollution is in its infancy, there are no widely shared methodological guidelines (Miller *et al.*, 2021). A continuous interaction with the MSFD TG-ML was performed during all the Fish protocol elaboration. A questionnaire was shared with all the Member States delegates and their experts on fish, in order to collect the common methods "microplastic ingestion by fish" procedures. Then, the first draft of the protocol was shared among partners and posted on the MSFD TG-ML platform (Wiki), where it received different inputs. All the inputs were included and the new version was posted again on Wiki. This draft is now included in the new MSFD TG-ML Guidance: Marine Litter Impact on Biota, waiting for approval by the experts and publication by JRC. The INDICIT II consortium addressed this issue by developing the Deliverable 4.9. "Monitoring Marine Micro-Litter Ingestion in Fish: A harmonized protocol for MFSD and RSCs areas" (<https://indicit-europa.eu>). The following subsections summarize the main results, while technical details are available in the above-mentioned deliverable of the project.

The first step in implementing the protocol was to identify a set of analytical steps that are crucial for monitoring purposes. To reach this goal, a questionnaire was produced and sent to a large group of researchers all around Europe. The aim of this action was to obtain an overview of what researchers with different expertise considered important for the development of a micro-litter ingestion monitoring strategy.

The received answers together with further discussions among partners, some stakeholders involved in the project, and also within the experts of the MSFD Technical Group on Marine Litter, were considered and examined for defining analytical best practices. Then, the harmonised protocol for monitoring micro-litter ingestion by fish in the MSFD and RSCs areas was produced by identifying a set of mandatory activities during all the analytical process.

### a) Target species:

Recent studies highlighted that feeding habits of different fish species determine differences in micro-litter ingestion rates (Lopes *et al.*, 2020) and in the analytical methods of analysis to be used for identification (Bianchi *et al.*, 2020). Moreover, the distribution of micro-litter items in the marine environment varies according to their different shape, size, and chemical composition (Palazzo *et al.*, 2020). Even several environmental factors (such as waves, tides, and currents) at different geographical scales contribute to define different accumulation pathways for different marine litter types (Angiolillo *et al.*, 2021).

In this view, more than one fish species must be selected for describing micro-litter contamination of the marine environment. In particular, the target species must have a different feeding behavior in order to reflect the contamination status of the three main environmental marine compartments (*i.e.*, pelagic, demersal, and benthic).

In order to reduce possible variability in micro-litter ingestion due to the variation of feeding behaviors of fish during life stages (*e.g.*, juveniles *vs.* adults), comparable individuals must be chosen. Sample sizes, measured as the total length of the animal, should be fixed around the size of first maturity, defined according to the FishBase dataset (Froese and Pauly, 2019), including a variability of 10% as tolerance.

### b) Sample sizes:

The number of collected specimens must not be lower than 30 individuals per species, to combine right effort and statistical analysis (Di Giacomo and Koespell, 1986). For very clean areas (*i.e.* scarce microplastic source of pollution) it is necessary to increase the number of fish to 50 individuals. Considering that for each monitoring site should be investigated three environmental compartments (*i.e.*, benthic, demersal, and pelagic), a total of 90 individuals (30 individuals x 3 species) per site must be collected.

### c) Fishing gear:

A trawling net seems to be the most reliable way of sampling, including opportunistic approaches (fish stock assessments cruises, including demersal and pelagic fish stocks assessment, and stomach content analysis performed on regular basis). Other fishing methods can be applied ensuring that no potential biases are



introduced. Sampling duration must be kept as short as practically realistic to reduce as much as possible the exposure of the animals to the fishing gear. It is also necessary to avoid long hauls as the net can re-suspend microplastic particles, scraping on the seabed.

Samples must be collected directly on board, checking each fish for any disease and ensuring that all the individuals showing signs of net feeding or regurgitation are rejected by checking in the mouth (Lusher *et al.*, 2017).

Individuals must be rinsed with ultrapure water and frozen upon collection. To minimize spatio-temporal variability, all samples must be collected from the same location and at the same time (ideally from the same haul, or at least the same season).

#### d) Laboratory activities:

The procedure to analyse micro-litter ingestion by fish includes four distinct steps:

1. extraction and digestion of the gastrointestinal tract (GI) with its content;
2. filtration on a membrane;
3. identification of micro-litter items using a dissecting microscope;
4. characterisation of the collected micro-litter items through morphometric shape, size and Fourier Transform Infrared Spectroscopy (FT-IR) or Raman Spectroscopy.

Since micro-litter extraction procedures hide several pitfalls that can affect their accuracy (cfr. Dehaut *et al.*, 2016; Enders *et al.*, 2017; Hermsen *et al.*, 2017; Song *et al.*, 2015; Renner *et al.*, 2017), a quality assurance/quality control (QA/QC) framework must be defined.

#### e) Sample processing in brief: Extraction, digestion, and filtration:

The laboratory analysis starts with the dissection of each animal and the digestion of the gastrointestinal tract (GI), to facilitate the extraction of the ingested micro-litter items. Each fish must be weighed (grams up to the first decimal), and the total length measured (up to the nearest mm).

Fish must be dissected in laboratory to extract the GI, from the mouth to the cloacae. Entire GI tracts must be weighed (grams up to the first decimal) before tissue digestion.

The GI must be digested including the wall using hydrogen peroxide H<sub>2</sub>O<sub>2</sub> 15% (MEDSEALITTER project modified) or potassium hydroxide KOH 10% (Rochman *et al.*, 2015 modified).

After the digestion phase, micro-litter items must be extracted through filtration on membranes with small pore sizes using a vacuum pump system.

#### f) Micro-litter characterization:

All the micro-litter items, recovered through the processing of the samples, must be counted and identified (see also "Data reporting").

Each item must be assigned to one of seven shape categories (*i.e.*, fiber, filament, film, fragment, granule, pellet, and foam), and to one of three size classes (100-330 µm; 330-1000 µm; 1-5 mm) (Valente *et al.*, 2019).

It is important to distinguish fibers from non-fibrous particles: fibers are only from textiles and must be noted in a separate category from other filaments (*i.e.* fishing lines), as they generally are composed by natural or semi-natural material (Avio *et al.*, 2020).

According to the new Commission Decision (2017/848/EU), in order to distinguish between artificial polymer and other, the spectroscopic analyses are mandatory to identify the chemical composition of the micro-litter items found ingested by each species for each location.

#### *g) Quality assurance/quality control (QA/QC):*

Background contamination is one of the major threats to the reliability of the quantification of ingested micro-litter (Prata *et al.*, 2021). Therefore, it is necessary to reduce airborne contamination with some specific procedures. For instance, samples must be processed under a laminar flow cabinet or glove box (Torre *et al.*, 2016). Similarly, during stereo-microscopy observation of the membrane, petri dishes must be covered by a glass dish. Whenever possible, only glass and metal labware must be used. A blank control must be performed at every step.

Following Avio *et al.* (2020), field results should be adjusted according to a blank-subtraction approach.

#### *h) Data reporting:*

A specific template for data collection is proposed in the INDICIT protocol (Deliverable 4.9) with basic and optional information required. It is divided in two sheets, which specify information on fish and on micro-litter items. Data include the following information:

1. Total number of fish,
2. Total FO% (Frequency of Occurrence = Fish with ingested Plastic/Total samples),
3. Total number of items for size class,
4. Total number of items for shape category,
5. Total number of items for colour: white (include yellow), black (include brown), green (all the tonalities), blue (from sky blue to light blue), red (including orange and pink), and other (including multi-colours), each one as transparent or opaque.

### **VI.3. Pilot action**

#### **VI.3.1. Objectives**

The pilot action was planned to support the implementation of the protocol developed under the framework of INDICIT II project (see Section 2). During this action, the monitoring protocol was tested by all the partners in order to evaluate potential criticalities. In particular, each partner tested:

- i) every step of the laboratory analysis, to verify its applicability;
- ii) the feasibility of the procedures, according to their own National regulations and laws;
- iii) the local fishing methods and the availability of the target species, according to seasonally or spatially distribution of the species and/or life stages.

#### **VI.3.2. Outcomes on the development of common analytical methods**

##### *a) Sampling activities*

Most of specimens were collected using fishing nets. Trawling nets were the most used fishing gear. However, this type of fishing gear showed some complications: i) the trawling net can resuspend microplastic particles by scraping on the seabed, then long hauls should be avoided; ii) it is the most impacting fishing technique for the benthic community and it cannot be used in some areas according to the local laws; iii) some regions do not use this fishing gear due to the seabed depth (*e.g.* Azores); iv) fish regurgitation may increase with sampling depth or the speed of net raise.

The collection of fish on board ships is the best method to select the samples and avoid bias, but in some cases, it is very time consuming and aggravated by local regulations. Moreover, the cost for boat hiring to carry out on board sampling is very expensive and needs a dedicated budget. An alternative proposed solution would be to collect the fish at the landing point, and to offer a training for fishermen (for instance in the framework of a collaborative project).

## b) Selection of target species

Many target species have been proposed for the Mediterranean Sea (UNEP/MAP WG.439/Inf.12.2017; Fossi *et al.*, 2018; Bray *et al.*, 2019), and also in deep-water habitat (Alomar and Deudero, 2017; Valente *et al.*, 2019), Atlantic Ocean (Herrera *et al.*, 2019; Pereira *et al.* 2020), and North Sea (Kühn *et al.*, 2020). In order to investigate contrasting marine compartments within a specific area, the protocol proposed three different species as target for monitoring. The pilot action led to the choice of three species to investigate micro-litter ingestion, namely *Scomber colias* (pelagic), *Merluccius merluccius* (demersal), and *Mullus barbatus* (benthic), which have very different feeding behaviors and habitat uses. Since in some partners countries these species are not available or not caught by local fisheries, the congeneric species *Scomber scombrus* and *Mullus surmuletus* were suggested as alternative species for assessing micro-litter ingestion in the pelagic and demersal compartments, respectively.

Another problem that emerged during the pilot action was the environmental variability to which the regions in the project are subject. In fact, it was not possible to collect all the samples from the different countries at the same time, due to differences in the period of availability of the species in different regions.

The genus *Merluccius* showed different critical aspects in functioning as target species, since it presents a high degree of regurgitation in samples from bottom trawling. Furthermore, it has a wide length range, which affects its feeding behavior. Finally, it is of very high commercial value in some countries, and in the Mediterranean areas the species have been considered as Vulnerable according to IUCN Red list (Di Natale *et al.*, 2011). Then, different benthopelagic fish species were suggested as candidates by some partners. For instance, the bogue *Boops boops* is a commercial fish with low cost and is already validated as an indicator by different authors in the Mediterranean Sea (Garcia-Garin *et al.*, 2019; Sbrana *et al.*, 2020; Tsangaris *et al.*, 2020). However, the bogue is not present in the North Sea/Baltic Sea areas and it has no feeding habits comparable with other species with a wider distribution.

Aiming at reducing possible variability in micro-litter ingestion due to the variation of feeding behaviors of fish during the different life stages (e.g., juveniles vs. adults), it is suggested to choose comparable individuals, fixing the fish size around the size of first maturity, but more studies are needed to investigate the relationship between micro-litter ingestion and the ontogenetic stages of different species. Many partners collected fish bigger than proposed, due to the available target size from local fisheries, suggesting the collection of only complete mature fish to harmonise samples.

Nevertheless, the pilot action showed that the size of the fish samples and the season of sampling cannot be the same from all the countries, therefore an accurate planning should be assessed before a wide monitoring, considering all the biological constraints of the target species.

It could also allow to compare males with females as micro-litter ingestion rates could be affected by the sexual behaviors (Sbrana *et al.*, 2020).

All the INDICIT partners agreed on fix the minimum sample size at 30 individuals for each species, but for very clean areas a minimum of 50 individuals could be necessary to ensure the collection of an appropriate number of micro-litter items for statistical analysis. The data elaboration on the collected samples during the pilot action, suggests the collection of 50 samples per species.

## c) Laboratory analyses

In laboratory all precaution measures should be taken to avoid secondary contamination as proposed by the protocol, mainly in the use of laminar flow cabinet and blank controls in every processing step for each batch of samples. Micro-litter type found in the blank control, should be subtracted from the same specific micro-litter type value in the samples of the same batch. FTIR or RAMAN spectroscopy should be used to identify the composition of all the collected micro-litter items.

Different opinions have been discussed regarding the bath temperature with oxidative (e.g., H<sub>2</sub>O<sub>2</sub>) or alkaline reagents (e.g., KOH). In the protocol, the suggested temperature of 40 °C was not considered by some partners as sufficient, resulting in an incomplete digestion of the organic material (food and GI walls), especially for *Merluccius*. An incomplete organic digestion can lead into the clogging of the membrane, resulting in the risk of

losing micro-litter items masked into the remaining tissue. In contrast, an extension of the digestion time can increase the exposure to the risk of airborne contamination, while a higher temperature could lead to polymers degradation and items melting. Fixing the lower limit of micro-litter items at 100 µm for monitoring purposes can help this step allowing a pre-filtration onto a 100 µm mesh sieve, reducing the risk of membrane clogging.

The three chosen sub-classes for items size (100 µm < x < 330µm; 330 µm < x < 1mm; 1 mm < x < 5mm) were used by the majority, but some partners were not able to go lower than 1 mm. A training course was needed, but the Covid Pandemic, did not allow it to take place.

To divide the sub-classes, some partners used three different sieves (1mm; 330 µm; 100 µm) stacked one on the others, and some others filtered all the material directly on the membrane, measuring the items at the end of the process. It can create a bias due to a different interpretation on the size of the items. In order to harmonise the procedure and the results, we determined a better definition for micro-plastic: **“All sorts of small particles of plastic, less than 5 mm in two of their three dimension or diameter that pass through a 5 mm mesh sieve but are retained by a lower one”**.

The polymer identification is a very important step to achieve non-biased results on microplastic ingestion. This step is fundamental to distinguish synthetic polymers from items of natural origin (e.g., organic fibers), as it is requested by the new Commission Decision (2017). Moreover, organic particles derived from natural diet (fish scales or bones crustacean exoskeletons, etc.) are very often confused with plastics. However, spectroscopy requires very expensive equipment and a time-consuming activity that requests a high level of expertise by the staff.

### VI.3.3. Field results

During the pilot action, data was collected for *Scomber* spp., *Merluccius merluccius* and *Mullus* spp. The total number of samples was 386 (60 *Scomber scombrus*, 163 *Scomber colias*, 153 *Merluccius merluccius*, 50 *Mullus surmuletus*, and 260 *Mullus barbatus*). Some partners were not able to do more detailed analysis, analysing only the upper size class (1 mm < x < 5mm) and data were not comparable with others. Then, a subsample of entire dataset was analysed in order to have the widest and more accurate data elaboration (Subsection 3.3.1)

Since few countries collected data on *Merluccius merluccius*, it has been evaluated separately from the other species (see subsection 3.3.2).

#### VI.3.3.1 Results from pelagic and benthic species

Table VI.1 reports the number of samples examined by the different partners. All partners collected at least 30 individuals per species, according to the Standard Protocol (Deliverable 4.9).

**Table VI.1.** Number of samples for different fish species analyzed for micro-litter ingestion by the different partners.

No. of samples			
Institution	<i>Mullus sp.</i>	<i>Scomber sp.</i>	All species
DEKAMER	73	39	112
FRCT-IMAR	34	34	68
IAMC-CNR	30	30	60
ISPRA	30	30	60
ULPGC	30	30	60
<b>All Institutions</b>	197	163	360

Table VI. 2 reports the local and total Frequency of Occurrence - FO (100 x number of fish with at least one ingested item / total number of examined samples).

**Table VI.2.** Frequency of Occurrence of micro-litter ingestion across the different partners.

Frequency of Occurrence (%)			
Institution	<i>Mullus sp.</i>	<i>Scomber sp.</i>	All species
DEKAMER	46.58	76.92	57.14
FRCT-IMAR	44.12	50.00	47.06
IAMC-CNR	16.67	46.67	31.67
ISPRA	56.67	83.33	70.00
ULPGC	26.67	56.67	41.67
<b>All Institutions</b>	<b>40.10</b>	<b>63.19</b>	<b>50.56</b>

Both species show a FO near to 50%, and it means that one sample per every two fish have ingested micro-litter.

The pelagic species (*i.e. Scomber spp.*) are generally more affected than the benthic species (*i.e. Mullus spp.*).

It is interesting to note that the maximum and the minimum FO's are referred to samples from Italian institutes. In particular, the maximum FO's reported for both species are referred to the samples collected by ISPRA, while the minimum values were recorded for samples collected by IAMC-CNR. To explain this difference, it should be noted that ISPRA collected the fish in marine areas that are adjacent to the mainland, and in particular near the city of Rome, where there is the mouth of one of the biggest Mediterranean rivers (Tiber river). On the contrary, IAMC-CNR collected fish near the Sardinian Island, far from the mainland and without strong local anthropogenic pressures (Sbrana *et al.*, 2020; Tsangaris *et al.*, 2020).

Tables VI.3 and VI.4, report the average number of ingested micro-litter items, both considering all the examined samples and only the individuals that ingested micro-litter.

**Table VI. 3.** Number of Ingested marine micro-litter in all samples.

Average $\pm$ se No. of Ingested MPs (considering all the examined samples)			
Institution	<i>Mullus</i>	<i>Scomber</i>	All species
DEKAMER	0.79 $\pm$ 0.12	3.05 $\pm$ 0.53	1.58 $\pm$ 0.22
FRCT-IMAR	1.15 $\pm$ 0.29	1.24 $\pm$ 0.29	1.19 $\pm$ 0.21
IAMC-CNR	0.30 $\pm$ 0.15	0.73 $\pm$ 0.18	0.52 $\pm$ 0.12
ISPRA	0.83 $\pm$ 0.17	3.17 $\pm$ 0.47	2.00 $\pm$ 0.29
ULPGC	0.37 $\pm$ 0.13	1.00 $\pm$ 0.27	0.68 $\pm$ 0.15
<b>All Institutions</b>	<b>0.72 <math>\pm</math> 0.08</b>	<b>1.89 <math>\pm</math> 0.19</b>	<b>1.25 <math>\pm</math> 0.10</b>

**Table VI.4.** Number of Ingested marine micro-litter considered only affected individuals.

<b>Average <math>\pm</math> se No. of Ingested MPs</b> (considering only individuals with ingested MP)			
<b>Institution</b>	<b><i>Mullus</i></b>	<b><i>Scomber</i></b>	<b>All species</b>
<b>DEKAMER</b>	1.71 $\pm$ 0.16	3.97 $\pm$ 0.59	2.77 $\pm$ 0.32
<b>FRCT-IMAR</b>	2.60 $\pm$ 0.42	2.47 $\pm$ 0.41	2.53 $\pm$ 0.29
<b>IAMC-CNR</b>	1.80 $\pm$ 0.58	1.57 $\pm$ 0.23	1.63 $\pm$ 0.22
<b>ISPRA</b>	1.47 $\pm$ 0.17	3.80 $\pm$ 0.48	2.86 $\pm$ 0.34
<b>ULPGC</b>	1.38 $\pm$ 0.26	1.76 $\pm$ 0.39	1.64 $\pm$ 0.28
<b>All Institutions</b>	<b>1.80 <math>\pm</math> 0.12</b>	<b>2.99 <math>\pm</math> 0.25</b>	<b>2.47 <math>\pm</math> 0.16</b>

It is important to notice that the average value for all the species is approximately one ingested item per fish. Moreover, all the reported values reflect the FO% values. In fact, according to this data (Tables 2 and 3), ISPRA shows the most polluted area followed by respectively DEKAMER, FRCT-IMAR, ULPGC, and IAMC-CNR. Considering only individuals with ingested micro-litter, the number of ingested items is not so high, showing an average value less than three items per fish.

#### VI.3.3.2. Results from the demersal species (*M. merluccius*)

Table VI.5 reports a summary of the results of the sampling activities carried out to collect samples of the demersal species *M. merluccius*. Total length, Total weight and GI weight are expressed as mean  $\pm$  se.

**Table VI.5.** No. of samples and biological parameters of *M. merluccius*.

<b>Institution</b>	<b>No. of samples</b>	<b>Total length (cm)</b>	<b>Total weight (g)</b>	<b>GI weight (g)</b>
<b>DEKAMER</b>	39	28.5 $\pm$ 4.6	208.1 $\pm$ 33.3	10.6 $\pm$ 1.7
<b>IAMC-CNR</b>	30	23.0 $\pm$ 4.2	112.3 $\pm$ 20.5	5.8 $\pm$ 1.1
<b>ISPRA</b>	30	28.9 $\pm$ 5.3	172.5 $\pm$ 31.5	6.3 $\pm$ 1.2
<b>ULPGC</b>	30	68.3 $\pm$ 12.5	2360.9 $\pm$ 431.1	65.0 $\pm$ 11.9
<b>UNIVPM</b>	24	30.0 $\pm$ 6.1	198.9 $\pm$ 40.6	7.1 $\pm$ 1.5
<b>All Institutions</b>	<b>153</b>	<b>35.5 <math>\pm</math> 2.9</b>	<b>603.0 <math>\pm</math> 48.8</b>	<b>18.9 <math>\pm</math> 1.5</b>

Results highlight a high variability of biological parameters per fish from different countries. In particular, samples from ULPGC were very large compared to fish from the other regions.

Table IV.6 reports FO and the average number of ingested micro-litter items, both considering all the examined samples and only the individuals that ingested micro-litter.

**Table VI.6.** proportion of FO and average number ( $\pm$  se) of ingested micro-litter items.

Institution	FO (%)	Items abundance (considering all the examined samples)	Items abundance (considering only individuals with ingested MP)
DEKAMER	64.1	1.41 $\pm$ 0.24	2.20 $\pm$ 0.26
IAMC-CNR	40.0	0.50 $\pm$ 0.12	1.25 $\pm$ 0.13
ISPRA	66.7	1.43 $\pm$ 0.27	2.15 $\pm$ 0.30
ULPGC	76.7	1.73 $\pm$ 0.30	2.26 $\pm$ 0.32
UNIVPM	33.3	0.38 $\pm$ 0.12	1.12 $\pm$ 0.12
<i>All Institutions</i>	57.5	1.14 $\pm$ 0.11	1.98 $\pm$ 0.14

In contrast with results obtained for *Scomber* spp. and *Mullus* spp., the highest FO and number of ingested micro-litter items were recorded in samples from ULPGC. As highlighted above, this difference could be due to the larger sizes of the samples from the Canary Island compared to the samples from all the explored Mediterranean Sea sub-regions.

More information is needed to better understand the comparability of samples with size so difference. Also in this case the species show a FO near to 50%, and it means that one sample per every two fish have ingested micro-litter.

#### VI.4. Possible GES scenario

The results of the pilot action demonstrate that the harmonised protocol, agreed among partners, is a good tool to use in the new monitoring programs within the next cycle for the implementation of MSFD. Fish represent a good indicator for micro-litter ingestion, since they can highlight differences among differently polluted areas. Moreover, the results underline that a minimum of two species in different marine compartment (*i.e.*, pelagic, and benthic) are needed to assess the investigated areas. Even demersal species should be chosen to improve information on micro-litter pollution.

The results of the pilot action suggest the use of the Frequency of Occurrence (FO%) as an effective tool to highlight differences among areas, while the number of micro-litter items appears to be less variable. Indeed, the average number of micro-litter items ingested by fishes is less than three items per individual (considering only the individuals that ingested micro-litter), and near 1 item per fish considering all samples. For this reason, the number of items or their corresponding weight, requested in the new Commission Decision (2017), cannot be considered as a reliable indicator, considering the need of analyzing a relatively low number of samples (N=30).

To obtain a representative baseline and propose a threshold value, more data is needed. Nevertheless, following the GES scenario proposed for the Northern Fulmar and the Loggerhead Sea Turtle, the possible scenario for micro-litter ingestion by fish can be the use of samples collected in a pristine area as a reference, or the minimum detected value in the dataset as a proxy of pristine area.

After these considerations, the possible GES scenario could be:

*“There should be less than X % of fish having ingested micro-litter per assessment area (GSA, Country, MSFD Subregion)”*

The number of sampling stations must be representative of the entire area assessed. In each station a minimum of 30 (50) individuals per habitat compartment must be collected (30 (50) pelagic, 30 (50) demersal, 30 (50) benthic).

X % is the average value of the three different FO% (pelagic, demersal, benthic) in the pristine area.

The other information (items size classes, shapes, colors and chemical compositions) are useful to distinguish different sources, and to address the programs of measures. For example, the fragmentation of high-density polyethylene (HDPE) films from single-use supermarket plastic bags and then the ingestion rate in fish, should be reduced after the adoption of the Directive 2019/904 on single use plastic.

Similar to the use of bioindicators for macro-litter ingestion (*i.e.*, *Caretta caretta* and *Fulmarus glacialis*), this scenario is not directly linked to the health status of the animals, as it is requested by the criterion D10C3 - “*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*” (Commission Decision (2017/848/EU).

Different authors did not find a relationship between micro-plastic ingestion and fish health (*e.g.* Compa *et al.*, 2018; Tsangaris *et al.*, 2020), while this relationship was found by others (*e.g.*, Sbrana *et al.*, 2020). More studies are needed to better understand the phenomena, but up to now, this scenario should be considered the best to be used for monitoring purposes.

The Commission Decision (2017/848/EU) requires Member States to establish threshold values for the level of micro-litter ingested by marine animals through regional or sub-regional cooperation. This project shows the strength of fish as bioindicators for this kind of pollution, both globally and at local scales. The harmonised protocol permits a standard comparison of data among different countries, as well as the possibility to reach GES.

## VI.5. Recommendations

- The INDICIT project shows that **fish are good target indicator** for the requirement of the MSFD at the criterion D10C3 “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”.
- Analysing data from literature exhibits the incomparability of the results among different studies. The recommendation from the INDICIT experiments (analysing micro-litter ingested by fish in different lab with the same protocol) aimed to **highlight the main step to perform for monitoring purposes**:
  - In order to have comparable data, it is **fundamental to establish a lower limit for the collected micro-items**, as done in the pilot action (100 µm).
  - The new definition of microplastic “**All sorts of small particles of plastic, less than 5 mm in two of their three dimension or diameter that pass through a 5 mm mesh sieve but are retained by a lower one**” seems to satisfy all the possible variables and reduce bias among different stakeholders.
- **Secondary contamination must be reduced** as much as possible and blank control should be performed at each batch.
- **People in charge of the monitoring must follow all the steps** of the monitoring process, including fishes catch, in order to verify the goodness of the samples.
- **Research studies are still determinant** to understand how biological constraints (*e.g.* sex, age, etc.) can influence micro-litter ingestion rate, being a source of possible bias in monitoring.
- In order to propose GES values, **a wider data collection will be needed**, especially to cover the different MSFD areas. At the same time, the investigation on the impact of micro-litter ingestion should be developed through addressed research.



## VII. Synergies with other (inter)national programs

### VII. 1. Introduction

The main objective of this activity was to foster the synergy between INDICIT II and other projects as well as the interaction with expert groups and the authorities aiming at exchanging data and expertise in order to provide more accurate assessments of the indicators in line with the European Commission and the RSCs. Activity 5 also tried to facilitate the flow of the gained expertise (networks, knowledge, data, tools) within INDICIT II, to other projects, mainly those which needed information on marine fauna's behavior, spatial distribution and exposure to anthropogenic debris. The work of this activity was divided into three tasks, namely, firstly the identification of international projects suitable for cooperation, as well as the appropriate meetings of projects or groups of experts to be attended by the coordinator or the leaders of the activities, secondly the formation of the best way of exchange data with ongoing projects, and thirdly the best utilization of the results with the help of a GIS tool for the automatic calculation of the distance from GES.

### VII. 2. Identification and collaboration with international/national projects - Data sharing

The partners were involved in complete and/or ongoing programs (such as MEDSEALITTER (MED-INTERREG), MEDCIS (DG ENV), DeFishGear (IPA ADRIATIC), MISTIC SEAS III (DG ENV), Plastic Busters MPAs (MED-INTERREG)), and projects funded under this call (e.g. MEDREGION, QUIETMED). As experts in marine litter monitoring and/or sea turtle and marine sciences, the partners were also involved in other types of projects such as LIFE, or national implementation of e.g. action plans, or more specific topics (e.g. sea turtle and by catch, etc.). The list and types of collaborations are presented in Appendix 4. In summary, we can highlight the following collaborations:

- Plastic Busters MPAs (2018-2022). The data on the health status collected during necropsy of stranded sea turtles are shared with the experts of Plastic Buster MPAs. A protocol to collected samples for ecotoxicological analysis has been discussed (in order to be feasible for stakeholders outside the field of research), allowing to send to the University of Siena (sub-contractor) a number of samples of tissues and organs.

- LIFE EUROTURTLES (2016-2021) and LIFE MEDTURTLES (2020- ) have relative activities with INDICIT-II and so MoUs have been signed with both projects describing the areas of collaboration. Guidelines and protocols produced by INDICIT and INDICIT II have been shared, and LIFE EUROTURTLES and LIFE MEDTURTLES have been of strong support to identify and mobilize the stakeholders (rescue centers, stranding networks) for implementing the monitoring of the indicators. Experience and knowledge have been shared on the methodologies for assessing turtles' health (body condition, potential effect of litter) and the way to collaborate with fishermen (who can provide samples of sea turtles in certain areas as well as important information on the entanglement of marine animals in nets or plastic litter).

- Two additional MoUs have been signed with MISTIC SEAS III (2019-2021) and Blue Circular Economy and Circular Ocean (2015-2021) for sharing of the INDICIT data base (Body Condition Index for healthy turtles) and data collected on entanglement of sea turtles, marine birds and mammals in order to be uploaded on the web site developed by the former project.

- In addition to the scientific collaborations that have been established, a more strategic collaboration should be noted with the CleanAtlantic (2017-2020) project regarding the specific task of data storing. This program uses a platform Data Litter (DALI, developed by IFREMER, France) for collecting data on litter (on beach, sea floor, surface, ingested and causing entanglement) at the OSPAR scale. The INDICIT partners have been involved in order to test the web interface for data uploading and INDICIT II agreed to be included in the marine litter knowledge database.

During the whole duration of INDICIT II, the project's partners have been in close collaboration with expert groups (e.g. MSFD TG ML), Regional Sea Conventions and Member States representatives (including members of the External Advisory Board) in order to refine the products of the project for the concrete implementation

of the second cycle of the MSFD. Appendix 5 summarises these meetings. It should be highlighted, though, that the effort of the INDICIT-II project to harmonize the protocols for D10C3 and D10C4 monitoring has been used:

- by MED POL and SPA/RAC (The Mediterranean Biodiversity Centre of UN Environment/Mediterranean Action Plan) in developing and preparing the report “Defining the Most Representative Species for IMAP Candidate Indicator24”
- for the Regional operational strategy for monitoring IMAP Candidate Indicator 24 (indicator ingestion of macro-litter by sea turtles),
- by SPA RAC in order to elaborate a specific protocol for monitoring interactions between marine litter and marine turtles, mainly focusing on ingestion and entanglement,
- HELCOM has validated both protocols for microplastics in fish and for entanglement,
- by TG ML in the revised guidance on marine litter monitoring, where the INDICIT protocols for plastic ingestion by sea turtles, microplastics ingestion by fish and entanglement will be part of the impact chapter.

Furthermore, the GES scenarios for the Indicator “Litter ingested by sea turtles”, proposed by INDICIT II, were discussed during the Meeting of the Ecosystem Approach Correspondence Group on Marine Litter Monitoring (30 April 2021), while, based on the INDICIT II outputs, the impacts of litter ingestion and entanglement were discussed during the MSFD TG ML meeting (22-23 June 2021), where potential proposal of baseline and threshold were also discussed.

### VII. 3. A tool for automatic calculation of the distance to GES in the Mediterranean Sea

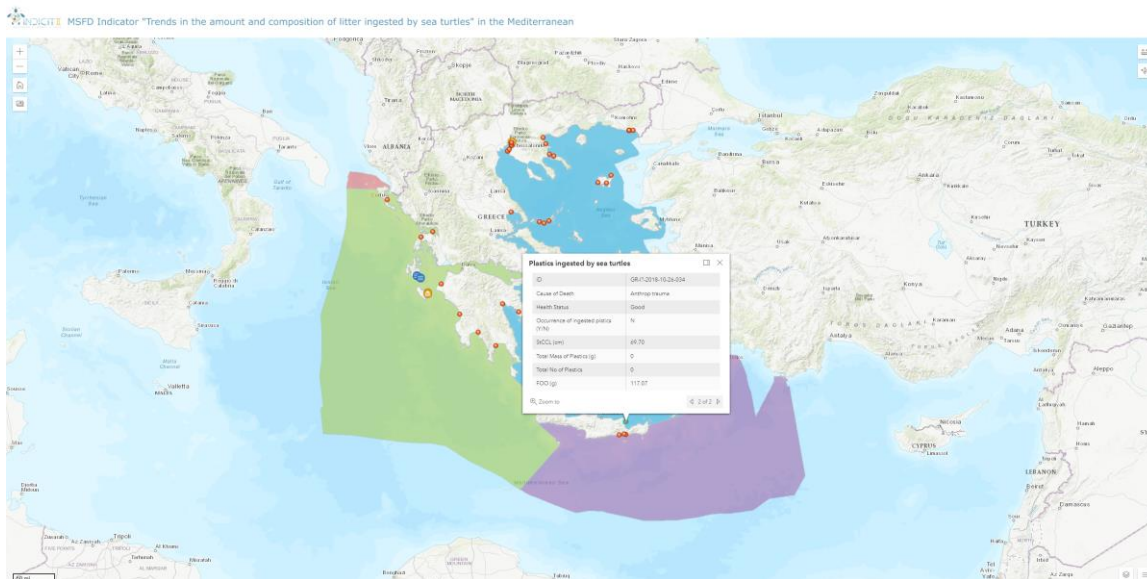
The initial proposal of INDICIT II was to integrate, in collaboration with QuietMED, marine litter (D10) into the GIS tool developed for noise (D11). However, further examination showed that this was not possible due to the different type of data produced for the two descriptors. For the visualisation of the INDICIT II outputs, the HCMR team has developed, based on the QuietMED product, two distinct software solutions: an **ArcGIS Online web application** and an **ArcGIS desktop Toolbox**, both aiming at collecting and visualising available data on litter ingested by sea turtles as well as collectively estimating GES/no GES Status in the corresponding Marine Reporting Unit (MRU), as proposed by INDICIT II experts. In case of no GES, the tool calculates the distance from GES of the Mediterranean sub regions or the MSFD MRUs.

The **ArcGIS Online web application** comprises of three spatial layers:

1. a point layer representing the location of each stranded sea turtle on the map. By clicking on each point, a pop-up box appears with the relevant information for each specimen (e.g. species name, date, coordinates, length of the carapace, the number and mass of ingested plastics etc.),
2. a polygon layer representing the Marine Reporting Units marked in different colours. By clicking on each MRU, a pop-up box appears showing collective information on the indicator “litter ingestion by sea turtles” (e.g. number of analysed specimens, descriptive statistics such as size distribution, frequency of occurrence, type and volume of litter, etc.) as well as the average abundance of ingested litter by sea turtles in the area and the calculated distance from GES (based on the GES calculated in the less impacted areas).
3. a point layer representing project stakeholders (currently available for Greece as an indicative visualization). By clicking on each stakeholder, a pop-up box with the relevant information (country of origin, partner, TAXA, Institution name, collaboration type etc.) is shown.

The application can be accessed publicly through the following link:

<https://hcmrathens.maps.arcgis.com/apps/instant/media/index.html?appid=404dd2ed6f564e7997a27c782827909f>



**Figure VII. 1.** Screenshot of the online web application with the pop-up functionality (turtle layer enabled).

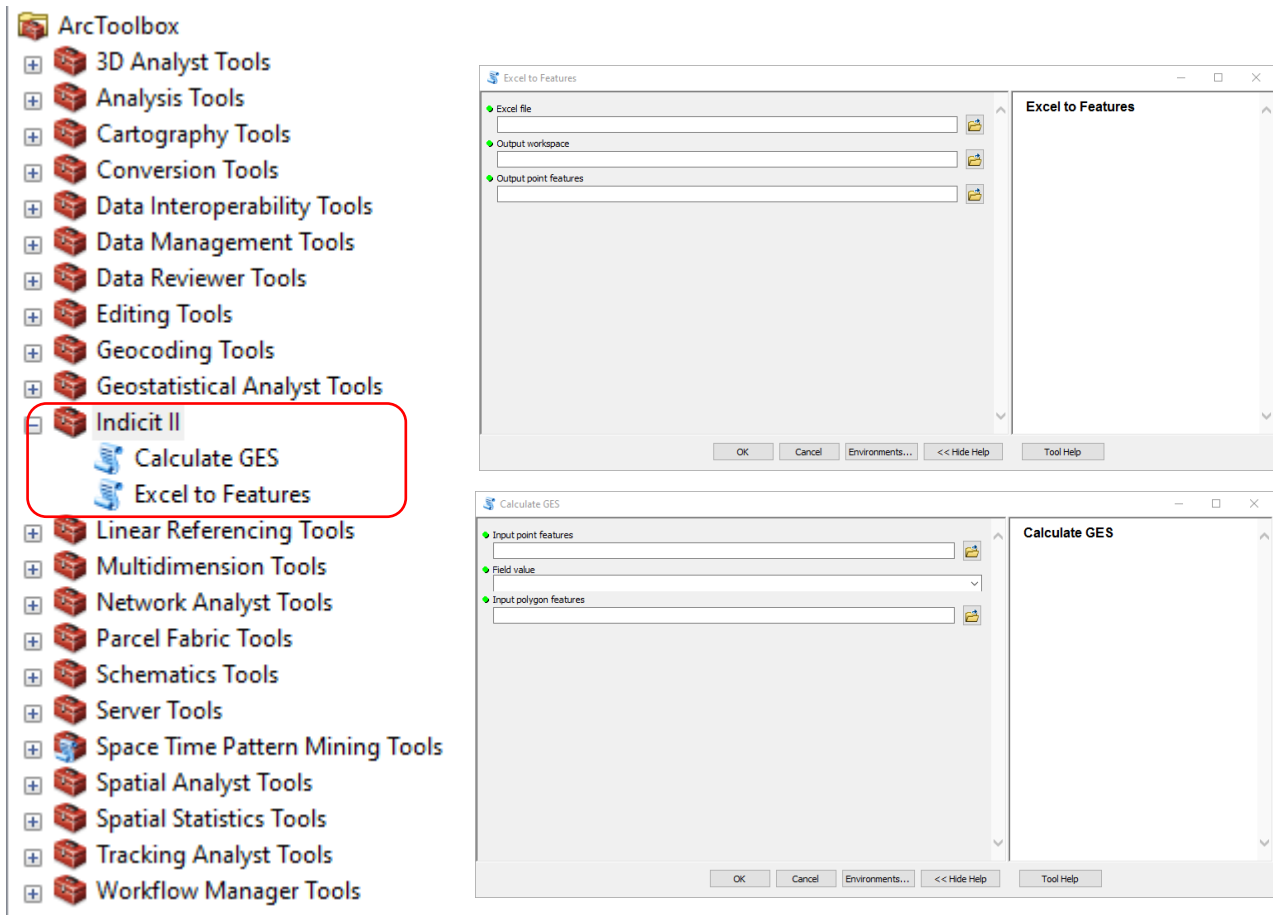
The GIS tool has the potential to be continuously fed with new data on ingested litter by people involved in the MSFD implementation in each member state. At any time, the tool can show whether each MRU has reached GES or not. At HCMR, the online version of the tool will be uploaded on the website of the Institute of Oceanography.

In order to facilitate the same visualisations and calculations locally (per desktop) the **ArcGIS desktop Toolbox** currently comprises of three distinct script Tools:

1. the “Excel to Features” script which converts strictly formatted excel rows to a point Feature Class contained in a defined from the user output File Geodatabase
2. the “Append to existing Features” script which appends newly collected excel data to existing point Feature Class (previously created)
3. the “Calculate GES” script which estimates GES / no GES Status and calculates the distance from GES for every MRU contained in a corresponding polygon Feature Class available in the same File Geodatabase.

The implementation is currently based on Python programming with the predominant use of the arcpy module, available at every ArcGIS for Desktop application.

Each partner can insert new data on litter ingestion as well as information on national stakeholders involved in the implementation of the MSFD. Moreover, the desktop tool could be used by the competent authorities in order to have a quick overview of the state of national waters as regards D10.



**Figure VII. 2.** A screenshot of the ArcGIS desktop Toolbox.

More details of the technical aspects of both tools are presented in Deliverable 5.8. Due to the Covid-19 situation, the development of the GIS tool was delayed and consequently not discussed in detail between INDICIT II partners. For this reason, the HCMR team is happy to keep in touch with all partners over the next six months and answer any questions or comments regarding the operation or improvement of the tool. The tool can support the monitoring practices, if it is adopted by the agencies responsible of the MSFD implementation, as the uploaded data could be incorporated into a European database such as the EMODNET. In Greece for example, where HCMR has undertaken the MSFD monitoring, the GIS tool will be connected with the National Oceanographic Data Center (EMODNET collaboration). The tool is based on a GIS functioning allowing selecting specific areas (e.g. with specific stakeholders, areas and sub-areas at the national or subnational scales). The observed trends in e.g. ingestion of litter can thus be evaluated at the scale of the defined pilot areas.

## VIII. Means needed and provided for the monitoring of the indicators

### VIII.1. Evaluation of the means needed for monitoring the indicators

The monitoring of the indicator “ingestion” (turtles and fish) and “entanglement” was made possible thanks to the EU funding of INDICIT and INDICIT-II projects. We have tried to evaluate the means needed for the monitoring for each partner in their respective country. There is a strong heterogeneity of situations (e.g. historical presence of active standing networks, number of volunteers involved, sources of funding). We summarise the situations in order to give some proposals for the sustainability of the monitoring. The detailed cost evaluation for France, Cyprus, Spain, Italy and Tunisia is provided in appendix 6.

The description of the main successive tasks and means needed to record the quantity/quality of ingested litter by sea turtle is:

#### *Phase 1: Collection of turtles in the field:*

Management of the stranding networks (Volunteer or funded time)

Volunteer times (in surveillance e.g. beach visit) and time to go collecting the dead turtle (stranded) or live turtle (e.g. in port in case of by catch) and considering the first measures, photos and time to record the observation sheet

Travel cost

Schemes of local network organisation and contacts

#### *Phase 2: Reception at the rescue centre (living individuals) or place for necropsy (dead individuals)*

Times at the rescue centre (Volunteer or funded time)

Storing cost at the necropsy lab (freezing of specimens pending necropsy)

#### *Phase 3: Ingested marine litter collection (necropsy or faeces collection)*

Times at the rescue centre (Volunteer and funded time) for faeces collection and storing

Times at the necropsy lab (Volunteer or funded time) for the measures of biometry and health parameters, the dissection, the digestive tract extraction, the litter and food sampling in the digestive tract and faeces, measuring (e.g. mass, volume, etc.) and storing.

#### *Phase 4: collection and analysis of data (digestive tracts or faeces)*

Times at the rescue centre or research lab (Volunteer or funded time) for analysis of litter in faeces (identification and counting) and storing of the sampled items.

Times at the necropsy lab or research centre (Volunteer or funded time) for analysis litter in the digestive track (identification and counting) and storing of the sampled items.

#### *Phase 5: Banking of data*

Times (Volunteer or funded time) for cleaning and updating the data in the dedicated data base.

The partners provided evaluation of costs in their respective countries. If some costs are relatively similar (e.g. small materials for the necropsy, for the identification and counting of litter items in the digestive tracks and

faeces, large differences are observed concerning the personal costs, the volunteer involvement and the type of facility (e.g. rescue centre, veterinary laboratory, research laboratory) where the analysis (necropsy, digestive tract extraction, content of the digestive tract analysis) are performed. The regulation and the empowerment of staff vary among countries.

We can consider the following costs for the successive phases of the protocol (Table III.4):

*Phase 1:* The management of the standing network is considered as a mid-time position for a project manager (e.g. ± 25 000 euros salary + taxes). Volunteers can participate (surveillance for stranded turtles' detection) which can be evaluated in each country on the basis of an annual charge of 800 hr/year. The volunteer times (including travel costs) directly depend on the size of the area covered by the stranded network.

*Phase 2:* The management of the live turtles at rescue centre is allowed thanks to volunteers and/or permanent staff (when available, with funding from different sources e.g. city, departments, region, according to the structure in charge of the rescue centre). The dead turtles are stored close to the place of discovery (availability of freezer in a local facility such as research lab, aquarium, etc.) or carried to the place where the necropsy will be performed (travel cost above).

For phases 1 and 2, volunteers can be mobilised. However, teaching is needed and the activity of the stranding networks cannot be based only on volunteers' involvement. A perennial recording of the indicator needs permanent position funding.

*Phase 3:* For the dead turtles, the main costs are the personal cost for necropsy. It is evaluated that it takes about 5 hours with two persons to perform the complete necropsy leading to the extraction of the digestive tract according to the INDICIT standard protocol. This would lead, on the basis of 30 turtles analysed per year, to a budget of about 6000 euros/year. For the live turtles, the costs correspond to the times at the rescue centre (Volunteer or funded time, see above on the funding of the rescue centre) for faeces collection.

*Phase 4:* For the analysis of litter in the stored digestive tracts and in the faeces, this task can be achieved by various persons (volunteers, funded technicians) who have been trained in the method. On the basis of 30 digestive tracts and ½ hours per DT, the budget can be evaluated in each country according to this needed time. about 450 euros/year. For faeces, the samples are collected every day during 2 months after the arrival of the individuals at the rescue centre. It needs about 15 minutes per individual per day. All faeces sampled are stored per individual and analysed together. The time required for analysing the litter depends on litter abundance in the samples and can take until 1 h. The budget can be evaluated in each country according to this needed time.

*Phase 5:* the time to enter the data into the database should not be underestimated. It included the validation of the data, and the experience gained during the INDICIT and INDICIT-II projects shows that the corrections to be made are numerous and time consuming. A time was invested to clean the entire INDICIT and INDICIT-II dataset, included data from 1988 and the time required should now be reduced. It is recommended to train dedicated persons for verifying and recording the data. The cost has to be included in the work charge of the funded project manager in charge of the indicator implementation in each country (when this position exists).

Types of cost	Phase 1: Collection of turtles in the field		Phase 2: Reception at the rescue centre or place for necropsy		Phase 3: Marine litter collection (necropsy or faeces collection)		Phase 4: Collection and analysis of data (digestive tracks or faeces)		Phase 5: Banking of data
	turtle alive	dead turtle	turtle alive	dead turtle	turtle alive	dead turtle	from carcasses	from faeces	
PERSONAL COST									
Technician cost <sup>1</sup>						x		x	x
Project manager cost <sup>2</sup>	x	x	x	x	x	x	x	x	x
Volunteer's Times <sup>3</sup>	x	x	x	x	x			x	
Travel cost <sup>4</sup>	x	x		x					
Turtle accomodation in the rescue center <sup>5</sup>			x		x		x		x
Turtle storage <sup>6</sup>		x		x		x			
LABORATORY COST									
Facility renting <sup>7</sup>						x	x		
Cold chamber or freezer				x		x			
SMALL MATERIAL	x	x			x	x	x	x	

**Table VIII.1.** Summary of location of cost for evaluating the indicators (ingestion/entanglement) in sea turtles. <sup>1</sup>time as technician in the facility (research lab, vet lab), <sup>2</sup>time as project manager, i.e. in charge of the stranding network and/or rescue centre, <sup>3</sup>time as cost of volunteer investment, <sup>4</sup>the mean cost of going to the stranding place (dead turtle) or e.g. pier (live turtle) in e.g. vehicle consumption, <sup>5</sup>mean cost of maintaining turtle in the rescue centre (functioning cost of the rescue centre, food, etc...), <sup>6</sup>mean cost for storing dead turtle, <sup>7</sup>mean cost for renting the facility (e.g. vet lab for necropsy). The list of “small material” is provided in Appendix 6.

## VIII.2. Means provided by the member states for the monitoring of the indicators

The partners provided, when available, the means that are allocated to monitor the indicators in their respective country. The summary is provided below:

**In Tunisia**, the Marine Biodiversity Laboratory of the INSTM is leading a national program on the conservation of sea turtles in Tunisia. The implementation of the INDICIT protocol (ingestion and entanglement for sea turtles) was possible thanks to the sea turtle rescue centre and the National Stranding Network for sea turtles and cetaceans. After INDICIT II, these indicators will benefit of these facilities and will continue to be monitored as a research activity in this national program. Funding of research activities of the laboratory are covered by the Ministry of Agriculture, Hydraulic Resources and Maritime Fisheries. Moreover, INSTM is a scientific partner in the EU funded program COMMON (COastal Management and MOnitoring Network for tackling marine litter in the Mediterranean). From 2019 to 2022, Monitoring ingestion of marine debris represents one of the research activities included in this program. On the other hand, the Marine Litter MED II project funded by the European Union will be executed from 2021 to 2023 by the UNEP/MAP-Barcelona Convention Secretariat. It further supports the implementation of the Regional Plan on Marine Litter Management in the Mediterranean particularly on southern countries such as Tunisia.

**In France**, two stranding networks with trained volunteers, are in charge of alerting when a turtle is observed at sea or recovered dead or living. They are RTMMF (coordinated by SHF) in the Mediterranean façade and RTMAE (coordinated by La Rochelle aquarium) for the Atlantic façade. The alive individuals are being taken to rescue centres and dead bodies are stored in freezers before necropsies been realised in partnership with veterinarian laboratories. A big work for collaborating with fishermen is conducted by the rescue centres in order to recover the turtles after bycatch and information. The rescue centres, one on the Atlantic façade (CESTM) hosted by La Rochelle Aquarium, two on the Mediterranean façade (CestMed and CRFS) are NGO auto-funded, from private funds, awareness actions and private studies. There is an ongoing project for the opening of a rescue centre in Corsica (no rescue centre at this time) in partnership with CARI NGO. The funding of these rescue centres varies each year depending on the subsidies obtained. The implementation of the indicator (ingestion and entanglement for sea turtles, litter in faeces) is funded by French Agency for Biodiversity (OFB) which supports the monitoring of litter impacts on sea turtles for both Atlantic and Mediterranean facades. For the Mediterranean façade, OFB signed an agreement (3 years, 2020-2022) with the NGO French Herpetological Society (SHF) in charge of knowledge, protection and education about the French herpetofauna for coordinating and distributing funds to its partners rescue centres, veterinarian and research laboratories (as sub-contractors). This funding includes a project manager full-time position (40 K€/year) for the management of the stranding network (Mediterranean façade), the participation to the necropsies and to the laboratory analyses of litter collected in the digestive tracts and in faeces, collected by volunteers, rescue centres and veterinarian laboratories.

**In Cyprus**, the collection of stranded (dead & alive) turtles, necropsy, collection of the digestive tract and measures (mass, volume, etc.) are achieved through a veterinary (Society for the Protection of Turtles, SPOT) and University of Exeter (UK). With support of MAVA foundation, SPOT has expanded its network and increased its capacity. SPOT is looking to include more participatory fishers and public engagement. However, funding mechanisms need to be considered for this. The analysis of litter collected in the digestive track (type, mass, etc.) is done by the University of Exeter (UK). Stranding work will be funded by SPOT for a baseline but additional funds are needed for a long-term maintenance and expansion. There is no further specific funding after INDICIT II.

**In Portugal (Azores)**, the collection of stranded (dead) turtles is achieved through a stranding network funded by regional government. The rearing and care to injured turtles at the rescue centre is mainly funded by private and regional agencies. The necropsies (no veterinarian lab) have no continuous funding, which come from research projects. The analysis of litter collected in the digestive track (type, mass, etc.) and the analysis of litter collected in faeces have no continuous funding, which come from research projects. National and international research projects provide funding for human resources and consumables.



**In Greece**, in case the assessment of D10C3 indicator is included in the National MSFD Monitoring Program, the HCMR will be funded by the Ministry of the Environment (General Secretariat for the Natural Environment – GSNE) under contract. Currently, the collection of stranded (dead) turtles is funded by HCMR under sub-contract. Breeding and care of injured turtles are mainly funded by the Ministry of the Environment (General Secretariat for the Natural Environment, GSNE). The necropsy, collection of the digestive tract and measurements (mass, volume, etc.) are performed in veterinarian labs funded from HCMR under sub-contract. The analysis of ingested litter and faeces are funded by the contract between the HCMR and the General Secretariat for the Natural Environment (Greek Agency in charge of the implementation of the MSFD). This contract includes a 100 % of full-time position (management of the stranded networks, analysis of the collected digestive tracts of the dead sea turtles). The funds for the sub-contract between HCMR and the rescue centre depend on the number of collected sea turtles each year. Similarly, the funds for the sub-contract between HCMR and the Veterinarian labs depend on the number of the necropsied sea turtles each year.

**In Spain**, the monitoring Programme for Marine Litter in Biota (coded as BM-8), both through ingestion and entanglement indicators, is included in the MSFD Monitoring Strategy for Marine Litter. After finalisation of INDICIT protocols and recommendations, a national protocol will be developed and agreed to start the regular monitoring on those indicators at the national level. The organisation of the stranding network is regionally based and is characterised by a significant heterogeneity among regions in terms of human resources, equipment and practices at the Rescue Centres. On June 2021, the Ministry for the Ecological Transition, in collaboration with Universidad de Valencia and Universidad de las Palmas de Gran Canaria (all Spanish INDICIT partners) organised an online meeting with regional governments and INDICIT II stakeholders to prepare a national workshop to discuss the pathway towards a national protocol, opportunities and needs. This workshop was held in Valsain, Segovia, on October 13<sup>th</sup>-14<sup>th</sup> (fig. VIII.1). A total of 20 representatives from almost every region in Spain attended and engaged in the discussions. As a result, the Ministry will develop a diagnosis on strengths and needs, as well as a tailor-made protocol that considers national specificities, in order to fully implement the monitoring programme. This will include an agreement on data use and other necessary steps. In parallel, the Ministry for the Ecological Transition will finance the strengthening of resources, both human and technical, at the Rescue Centres, through a call for projects.

In the Valencia region (Mediterranean side), the collection of stranded (dead) turtles and turtles dead in rescue centres is coordinated by the University of Valencia (with their own means) and with the support of the regional government and the collaboration of the Valencia rescue centre at Oceanogràfic (Valencia region). In other regions, the stranding networks are coordinated by rescue centres (Catalonia: CRAM; Balearic Islands: Palma Aquarium; Murcia: Centro recuperación El Valle (public-regional government), and Almería province, Andalusia: Equinac). Regional networks provide data and samples (e.g. faeces) to the UVEG team for analysis. The rearing and care to injured turtles and turtles by-caught by fishermen and brought to ports are funded by 2 regional governments and 3 private companies, but in agreement and with economical support of regional governments. The necropsy, collection of the digestive tract and measures (mass, volume, etc.) are made at the University of Valencia (UVEG). The analysis of litter collected in the digestive tract (type, mass, etc.) and of litter collected in faeces are made at the University of Valencia (UVEG). The funding comes from a contract renewed annually between the regional government of the Valencia region (area of biodiversity) and UVEG for the coordination of the stranding network and necropsies (30 k€).

**In Italy**, ISPRA is supporting the Ministry of Environment (today Ministry of Ecological Transition) for the Marine Strategy Framework Directive implementation. Within MSFD ISPRA is leading the National stranding network on sea turtle applying the INDICIT protocol for monitoring litter ingested by Loggerhead turtle *Caretta caretta* on death and alive turtles, considering also entanglement on marine mammals as experimentally. Data collection is performed in all the three Italian sub-regions (Western Mediterranean Sea-Adriatic Sea- Central Ionian Sea) involving 8 different stakeholders (Research Institutes and Rescue Centres). In Sardinia, CNR-IAS collects all the sea turtles of the region, both alive and dead individuals, and provide the data to ISPRA. Data are uploaded by the stakeholders on the National Centralised Information System and validated by ISPRA. Finally, data are provided to the European Commission (EMODNET) and to the Unep/Map Barcelona Convention (INFO/RAC). The cost of the National monitoring programme for litter ingestion is around 192 K€ year.



**Figure VIII.1.** Twenty representatives from almost every Spain region attended a meeting with the Ministry for the Ecological Transition to discuss the content of a call for project to strengthen the resources (human and technical) for the implementation of the MSFD indicators “ingestion and entanglement”.

## IX. Summary of INDICIT-II main output

### Marine litter, a major concern

**Anthropogenic marine litter** is having an **alarming impact on marine fauna** with several hundred species described to be affected by litter, primarily by **ingestion** and **entanglement**. The European Commission's Marine Strategy Framework Directive (MSFD, 2008/56/EC) aims to evaluate the pressure of marine litter and actions to reduce this pollution in order to **recover 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. **Sea turtles** are used as an **indicator** of the impact of **macro-litter** (items > 5 mm) by ingestion in the framework of the MSFD (for Criteria D10C3) and the OSPAR (indicator 10.2.1) and Barcelona (ECAP pilot indicator 18) Regional Sea Conventions (RSCs). **Micro-litter** (< 5 mm) is also widespread in the marine environment, making it inevitably more readily accessible to marine fauna by ingestion than macro-litter. Recent studies have demonstrated that micro-litter ingestion occurs in various fish species, including species of commercial importance. OSPAR, HELCOM, and Barcelona Conventions are currently scoping **the use of fish for this indicator**. In 2017, the UNEP/MAP updated the Report "Defining the most representative species for IMAF candidate indicator 24 (UNEP, 2017)" underlining the potential of fish as bio-indicators for monitoring ingestion of litter. However, none of the proposed species have been selected to plan a common monitoring strategy. A literature review and analysis of data collected during INDICIT project are already available, the report "State of the art: Indicator micro-plastic debris ingested by marine turtle and fish" (Silvestri et al., 2018) gives several guidelines for the testing of this indicator with several fish species as well as sea turtles. The impacts caused by **entanglement**, for example reduced moving capacity, inability to feed, injuries and lacerations, which can lead to fast or slow mortality, are also concern to many marine organisms. The INDICIT report "State of the art: Indicator entanglement with marine debris by biota (Claro et al., 2018) analysed the data available in epibenthic invertebrates (corals), fish (Elasmobranches), sea birds, marine mammals and sea turtles and provided guidelines for the test of this indicator with sea birds, marine mammals and sea turtles.

### The INDICIT project

The INDICIT-II project is a follow-up of the INDICIT project (1st February 2017 – 31st January 2019). INDICIT-II project started the 2<sup>nd</sup> February 2019 and finished 31<sup>st</sup> July 2021. The total budget represented 1 243 670,09 euros, considering an allocation of the UE DG Environment of 921 399.31 Euros added to the 20% of partners' co-funding."

The INDICIT II consortium was comprised of **12 partners from 6 EU countries** (France, Greece, Italy, Portugal, Spain, United Kingdom) and **2 non-EU countries** (Turkey, Tunisia) in addition to **2 authority representatives** from France and Spain. 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. The general objectives of INDICIT II were (1) to capitalise the practical outcomes related to **networking**, development of **standardised tools**, gathering of standard data and constraints and **GES assessments** for the Indicator "**Litter ingested by sea turtles**", (2) to support the implementation of the indicators "**Entanglement of sea turtles, sea birds, and marine mammals in floating debris**" and "**Micro-plastic ingestion by fish and sea turtles**", (3) to support the next 6-year cycle of MSFD implementation by testing the indicators (especially the more advanced "Litter ingested by sea turtles") in response to **National Programs of Measures** (PoMs) in several pilot areas.

## The INDICIT project main results

### Strengthening the implementation of the indicators.

Following the high mobilisation of the stakeholders during the INDICIT project, the new INDICIT-II project has allowed for the pursuit of the strengthening of the networks in charge of collecting specimens and data on litter ingestion and entanglement. Most of training sessions had to be cancelled due to the global covid-19 pandemic, but short training sessions were performed by email (e.g. dissemination of protocols) or online conferences (e.g. to provide support for dissection of dead animals or the identification of ingested items). **For the indicator « litter ingestion by sea turtle »**, the open-access publication of the protocol (Matiddi et al., 2019), automatically translated into 17 languages, has facilitated considerable dissemination use. **For the indicator « entanglement »**, the pilot study conducted on INDICIT project (2017-2019) provided the first step for a standard protocol. Following discussions on adequate definitions of entanglement, criteria to distinguish entanglement from bycatch and adaptation of the list of litter typologies based on the MSFD litter classification, a standard protocol with instructions on how to measure and identify the litter was produced and disseminated to stakeholders. An adaptation of this protocol was also proposed for the analysis of pictures/video available in social media platforms. For the **indicator “microlitter ingested in fish”**, the first step was to identify the analytical steps needed for a monitoring. A questionnaire was sent to a large group of researchers in Europe, to have an overview of what is considered important for the development of a micro-litter ingestion monitoring strategy. A pilot study was then performed by 5 partners with 2 fish species and different laboratory practices. A standard protocol was consolidated, and is now available for dissemination and use.

### Quantification of the indicators

Indicator **“Litter ingested by sea turtles”**. Among the 1103 individuals collected since 1988 with the 113 collaborating institutions, **69.24% of loggerhead turtles were found to ingest macrolitter**, and 56.62 % when considering plastics only. Among the 802 individuals collected since 2013 (date of the publication of MSFD guidance with procedures to extract litter in turtles' digestive tracts), these percentages increased respectively to **73.46% and 58.99%**. Loggerhead turtles ingested a mean of **31.5g** (dry mass) of both synthetic and natural materials. Plastics (mean 1.94 g) accounted for **38.7% of the mass of all ingested material and 93.8% of the ingested litter**. The abundance of ingested litter represented a mean of 6.7 pieces, with plastics corresponding to 95.5% of them (6.2 pieces; max=200). Most of ingested items were soft and hard plastics, such as fragments of food packaging, bags, cups, caps, cotton buds, lollipop sticks, balloons, finger rinse wipes, sanitary napkins or filters from waste treatment plants for example. The threadlike litter were generally items from fishing activities, such as fragments of lines and nets. The occurrence and dry mass of plastic ingestion did not differ between the Atlantic and the Mediterranean areas. The abundance of ingested litter is significantly higher in the Atlantic compared to the Mediterranean areas. Due to methodological limitations (e.g. no control of contamination), the micro-plastics recorded were in the range 1-5 mm. **Loggerhead turtles ingested a mean of 0.77 pieces** (26% of the total number of ingested plastics). The majority of these plastic pieces was white or transparent, dark and more rarely light coloured.

**Indicator “Entanglement”**. The “Social media Protocol for entanglement” allowed for the collection of more than **800 links with images from 743 individuals of marine fauna** (538 sea turtles, 144 cetaceans, 46 seabirds, 10 seals and 5 from other taxa), where 55,9% (n=415) were entangled animals. The highest number of entangled sea turtle were found in the Canary Islands (Atlantic Spain), the Spanish Mediterranean and Italy. Despite the low number of images found on Cyprus, all the animals were entangled. For the standard protocol shared with stakeholders, the main issue was the big differences in data collected. In many cases, databases were not updated and not homogenised, so the raw data were not available for the INDICIT project, but many stakeholders have started collecting data with the standard protocol in 2020. On the other hand, several stakeholders worked hard to extract the parameters needed in the protocol from their databases, e.g. 2055 entangled sea turtles stranded in the Canary Islands (Spain) from 1989 to 2019 or 56 entangled sea turtles stranded in the Valencia region (Spain) from 1995 to 2020. **Among the 2391 entangled animals analysed, the great majority was sea turtles (98,9%), and only 27 were entangled cetaceans, from Italy (23) and Azores (4)**. Among sea turtles, the loggerhead turtle was the most impacted by entanglement in the European waters, with

35,5% (n=2193) for this species registered from 1990 to 2020, instead of the 8,5% (n=19) green turtle and 12% (n=6) leatherback turtle. The type of litter causing entanglement was not identified in many cases. Litter that was identified were fishing nets, fishing lines and ropes, strings and cords, originating from fisheries and maritime sources. Heavy-duty sacks, plastic bags, packing rings and plastic sheeting greenhouse were also observed, this kind of litter originated mainly from land-based sources. Concerning the other taxa, some data were available only for Cetacean in Azores and Italy 74% (n=23) of these observations in Italy were entanglements in fishing nets and lines.

**Indicator “microliter ingestion in fish”.** Following recommendation on sampling and laboratory procedure, three species were selected for this indicator, *Scomber colias* (pelagic), *Merluccius merluccius* (demersal), and *Mullus barbatus* (benthic). For *Scomber colias* (n=163), *Mullus barbatus* (n=197) and *Merluccius merluccius* (n=153), **the occurrence of microliter was 40%, 63% and 57,3% respectively, i.e. one fish per every two fish have ingested micro-litter.** The highest occurrence (57% in *Mullus barbatus* and 83% in *Scomber colias*) **could be related to the environmental contamination**, due to the presence of a large river closed to the sampling area. Contrary to this, fish collected far from the mainland and without strong local anthropogenic pressures (Sardinia Island) exhibited the lowest occurrence (16.7% in *Mullus barbatus* and 46.7 in *Scomber colias*). The mean number of ingested micro-litter is 1.8, 2.9 and 2.0 items in *Mullus barbatus*, *Scomber colias* and *Merluccius merluccius* respectively.

#### Biological constraints on the indicators and evaluation of the health of impacted specimens.

The evaluation of the indicators on a scale never prospected before (in term of spatial distribution and sample size) allowed INDICIT II to test the role of biological constraints such as sex, size, locations on the parameters (occurrence, mass, number of items, types of litter). Moreover, the MSFD descriptor « litter » also ask for looking at health effects.

**Indicator “Litter ingested by sea turtles”.** The large dataset allowed for the testing the spatial effect (e.g. countries, areas such as Mediterranean basins) with interactions of Seasons, sex and growth stages, and individual conditions (with different body conditions index). Overall, these factors and their interactions were not determinant in explaining the variability of the recorded parameters on ingestion of macrolitter. **The widespread and high occurrence** recorded highlights that sea turtles are strongly exposed to macrolitter in all the studied marine areas and at all their aquatic life stages. **As plastics represents a mean of 38.8% of all ingested material**, this high ratio could negatively impact growth, reproduction and reserve accumulation, and ultimately **population dynamics**. Levels of pollutants and biomarkers activity were measured in live loggerhead turtles reared in Aquarium and rescue centres, controlling for plastic ingestion thanks to faeces analysis. Tissues (liver and fatty tissue) of dead turtles (of which the digestive track was analysed for the presence of plastics) were also used for analysis of the phthalate levels and the quantification of porphyrins. The levels of porphyrins were also evaluated in the faeces of specimen in three rescue centres and in an aquarium. Altogether, the area (French/Spain) and condition (hospitalised/captive) show statistical differences in plastic tracers (e.g. phthalates, PBDEs, PBT compounds) in tissues. **Most of the physiological alterations to the organisms analysed were related to the immune system.** The samples available could not be used to evaluate the health status of the loggerhead turtles in the different areas of their geographic distribution but give important output for future analysis. The **multi-tier approach** (detection on marine litter ingestion, accumulation of PAEs, biological endpoints) would be able to **separate populations** living in area characterised by different marine litter pressure. Defining the health status of sea turtle with the ecological approach should consider their cumulative stress (exposure to marine litter, legacy and emerging contaminants, etc.).

#### Indicator “Entanglement”.

In the loggerhead turtles, the highest percentages (42,9%) of animals affected by entanglement presented a “medium” degree of impact, with typical injuries of deep cuts affecting epidermis and dermis, with different degree of infection. Among highly impacted, 10,5% suffered flipper amputation or severe body deformations and 8,1% dead due to entanglement. Among the litter derived from fisheries and maritime sources, **the fishing lines presented the greatest impact**, with 15,5% of the turtles suffering severe impacts (amputations, bone

fracture, eye loss) and 19% died. Among the litter derived from land-based sources, **plastic bags presented the highest mortality** (50% extreme severity), followed by other land-based (28,6%) and packing strapping bands (14,3%). **Fishing lines** and **heavy-duty sacks** produce severe impacts because these materials are thin and very resistant, presenting a greater capacity to cut the skin, producing deeper wounds in a very short time.

#### **Indicator “microliter ingestion in fish”.**

The objective was to standardise and consolidate the protocol, and recommendations to use “standard” fish (sex, size) prevent analysing the potential role of biological constraints. Similarly, the impact of microlitter ingestion on the health of fish remains to be evaluated. We observed that the highest value of occurrence (76.7%) and abundance (2.26 items) were in the samples of *Merluccius merluccius* where the size (mean 68.3 cm) was strongly higher than in other countries (max 30 cm).

### Evaluating the Good Environmental Status

For the indicator “**Litter ingested by sea turtles**”, three 3 scenarios were analysed as Good Environmental Status (GES):

GES 1 (based on the occurrence/number of pieces of ingested litter): There should be less than X% of turtles with more than Y g (or pieces) of ingested plastics” (this GES is similar to the “Fulmar approach” (OSPAR Ecological Quality Objective scenario - OSPAR EcoQO).

GES 2 (based on ingested plastics and food items): There should be not more than a ratio of x of ingested plastic litter / food remains in the digestive tract. The use of this ratio ingested plastics/food remains” is a tentative to connect this GES with health, as it is expected that ingesting plastics could alter the feeding behaviour.

GES 3 (based on dose-response relationship): There should be less than X g (or pieces) of ingested plastics”, X being the threshold at which an impact on health is detected.

According to the data availability and needed to evaluate a change within a six years interval, **GES 1 is selected** as “There should be not more than 26% of individuals with > 2 pieces of ingested plastic litter or **there should be not more than 26% of individuals with > 0.32 g of ingested plastic litter** (dry mass). GES evaluated with dry mass is recommended as weighting is more standardised than counting ingested items. As the lowest occurrence and quantities of ingested litter were found in Eastern Mediterranean Sea, this area serves as pristine area (i.e. reference), e.g. as target for the GES of other areas. The other evaluated GES are tentatively connected with the health of Individuals. For GES 2, the quantity of food remains would be more systematically recorded by stakeholders to increase the sample size needed to calculate ratios which could be compared between regions and over the years. GES 3 is also connected with health, but the threshold of ingested plastic that could alter the health of individual is not yet evaluated.

For the indicator “**entanglement**” and for loggerhead turtles, 3 scenarios are proposed as GES, which can be tested in the future with the disseminated standard protocol:

GES 1 (based on the number of individuals): **The number of individuals** impacted by entanglement in each region during a 6-year cycle **will be reduced**. The baselines from each region (entangled loggerheads from 2013 to 2020) can be obtained by the currently observed respective numbers.

GES 2 (based on the frequency of occurrence): **The FO% of entangled turtles** in each region during a 6-year cycle **will be reduced**. Baselines from each region (entangled loggerhead turtle from 2013 to 2020) can be obtained by the currently observed frequency of entanglement. Each region needs to be analysed separately, and the variations on FO% from main threats (bycatch, entanglement, boat collisions, human interaction, etc.) needs to be considered.

GES 3 (based on the frequencies of litter typologies): **The FO% of specific litter typologies** (fishing nets, fishing lines, ropes, stings, cords and heavy-duty sacks) entangling turtles in each region during a 6-year cycle

**will be reduced.** Baselines for the main types of litter on each region (from 2013 to 2020) can be obtained by the currently observed frequency of the different types of litter.

For the indicator “**microliter ingestion in fish**”, the possible GES scenario could be “There should be less than X % of fish having ingested micro-litter per assessment area (GSA, Country, MSFD Subregion). X % is the mean value of the occurrence of ingested litter for pelagic, demersal and benthic species in the pristine area (area with the lowest FO% currently observed). The number of sampling stations must be representative of the entire area assessed, with species representatives of the pelagic, demersal and benthic habitats.

### Pilot areas and assessment of effectiveness of measures

The strategy to evaluate the **capacity** of the indicators (ingestion and entanglement) to **evaluate the effect of the Programs of measures (PoMs)** implemented in each country was to select pilot areas, i.e. areas where the indicators can be evaluated, where litter abundance and sources can be described (synergies with MEDESEALITTER project in the Mediterranean areas and CleanAtlantic project in the Atlantic areas), and where PoMs are implemented. The INDICIT protocols differentiate plastic categories of litter ingested by sea turtles or causing entanglement according to the MSFD guidelines, e.g. **USE SHE** (remains of sheet, e.g. from bag, agricultural sheets, rubbish bags), **USE FRA** (fragments, broken pieces of thicker type plastics), **USE THR** (threadlike materials, e.g. pieces of nylon wire, net-fragments) as examples. **The precise description of these litter categories allows a connection with specific measures** such as the ban of plastic bags (category USE SHE), the ban of the single use plastic (category USE FRA) or the ban of fishing gear deposition at sea (category USE THR).

**The selection of pilot areas** was performed during the first 6 months of the INDICIT-II project, with previously collected data (for ingestion), expertise and empirical knowledge of partners and experts, and at the end of INDICIT-II project, utilising **a standardised methodology**. This decision tree has three entrances: (1) “**Pollution levels**” which concerns the main source of pollution affecting the area (main rivers and cities, existence of litter gyre/sub-gyre) and all relevant information on litter source in the area. (2) “**Turtles**” which concerns the sea turtles’ feeding activity and the capacity to collect information (e.g. ingestion/entanglement) in the area (e.g., presence of stakeholders and capacity to collect specimens (dead or live), with active stranding networks and rescue centres). In the case of absence of stakeholders or incapacity to collect turtle or to analyse ingested litter, this information is used in the decision tree to make proposal (e.g., network to be deployed, etc.). (3) “**PoM**” which concerns the implementation of the PoMs that can potentially influence the quantity/quality of litter that is ingested/causing entanglement.

The decision trees allowed the proposal of areas where **capacity building is needed** (e.g. North Corsica in France, MPA Zakynthos in Greece), **areas for high pollution** (e.g. Golf of Lion in France, the Tyrrhenian Sea in Italy, the Balearic Islands and continental façade in Spain for the Mediterranean Sea). In the Atlantic, the Canary Islands and the Azores Archipelago were evaluated as areas with high pressure but the large influence of oceanic currents imply that a significant part of the litter is coming from extra-European countries, preventing the assessment of PoM. Turkish waters were proposed as **pilot area with low pressure** as the frequency of occurrence of litter ingestion by sea turtles was the lowest observed in the studied areas of the Mediterranean Sea. In Greece waters, the frequency of occurrence of litter ingestion by sea turtles is in the average. Acquiring more data in these areas will enable confirming these areas **as low or moderate pressure** (which may vary locally according to spatial configuration and river inputs) and contributed to assess implemented PoMs.

### Implementation of the indicators in RSC monitoring and MS/MSFD administrations

In the framework of the descriptor 10 (the properties and quantities of marine litter which do not cause harm to the coastal and marine environment) of the MSFD, the INDICIT project has supported the implementation of the criteria “the impacts cause by ingestion” (D10C3) and “entanglement or other injuries to marine fauna” (D10C4).

**The indicator macro-litter ingested by sea turtle is now implemented**, with an effective network of stakeholders, standard protocol, GES calculation and proposed target for each country, areas (e.g. basins) in the Southern Atlantic (OSPAR IV area, OSPAR common indicator) and in the Mediterranean (ECAP pilot indicator 18 of the Barcelona convention). **The use of standard typology** for litter description allows **connecting the ingested litter with programs of measures** such as the reduction or banning of plastic bags, single use plastics, etc. This indicator is particularly relevant to evaluate the efficiency of such EU and national policies. This indicator is included in the National MSFD Monitoring program in France, Greece, Italy, Portugal, Spain, with funds from national/regional governments.

**Micro-litter ingested by marine animals** is also part of the D10C3, and expert researchers of the MSFD TG-ML have identified fish has potential focus species. **The INDICIT project has validated a standard protocol** with three species (*Scomber colias*, *Merluccius merluccius* and *Mullus barbatus*). With the provided practical recommendation, this new indicator can now be implemented at the MSFD scale, allowing GES scenario proposals.

The criteria D10C4 « **Entanglement in marine litter** » is not yet implemented in the MSFD area. Due to the feasibility study, the identification of stakeholders and the development of standard protocols, **this indicator can now be implemented**, especially with sea turtle as focus species, at the MSFD scale. GES scenarios are proposed and need validation. This indicator is complementary to the indicator “ingestion of macro-litter”. **The typology of the observed macro-litter differs** between these two indicators, which **increase the potential evaluation of the MS programs of measures**.

## INDICIT-II communication

The INDICIT website (<https://indicit-europa.eu/>, available until April 2022 and then managed by France and accessible as resource centre) provides the description of the project (objectives, partners), documents (deliverables, technical tools such as standard protocols for stakeholders, tutorials), and more general information on awareness (documentary, pedagogical tools, challenge, images on litter ingestion, entanglement, and people in action). The private area allowed partners to feed the database with the recorded parameters (ingestion and entanglement) allowing the statistical analysis. A dedicated page has been created in April 2020 to encourage the general public to send photos, videos and links that they had found posted on social media relating to the entanglement of marine wildlife in plastic pollution (the INDICIT Challenge).

Standard protocols to monitor entanglement of sea turtles and biota in marine debris (with data collected in the field and data collected in social media), and a harmonised protocol to monitor micro-litter ingestion in marine fish have been produced, complementing the protocol to monitor litter ingestion in sea turtle produced during the first INDICIT project.

The INDICIT-II project is present on Facebook and tweeter, with one to two posts once a week, containing news related to the INDICIT II project, work by different partners and other related current international studies or news about the issue of marine plastic pollution and biota.

The comic “Carrie, a brave turtle in a changing world” was produced, designed to raise awareness of the impact of plastic pollution on marine biota like sea turtles. It was disseminated by partners and stakeholders during their respective activities with large audience, scholars, etc.

An exhibition with high quality images gathered during INDICIT I and INDICIT II, and an exhibition catalogue have been produced. The images of plastic pollution, pressures on marine biota, and scientists working in the field have been exposed as a virtual exhibition during the lockdown period ([kunstmatrix.com](http://kunstmatrix.com)), and has been opened in Rome (Italy) in September 2021. The exhibition and catalogue are now available for presentation in the other partners’ countries.



## INDICIT-II synergies

After selecting connected or complementary projects during the first 6 months of the project, closed collaborations have been implemented (with the signature of memorandum of understanding) with Plastic Busters MPA (2018-22, to share protocol to collected samples for ecotoxicological analysis in order to be feasible for stakeholders outside the field of research and to collect the samples), LIFE EUROTURTLES (2016-2021) and LIFE MEDTURTLES (2020- ), to help in identifying and mobilising the stakeholders (rescue centers, stranding networks) for implementing the monitoring of the indicators, MISTIC SEAS III (2019-2021) and Blue Circular Economy and Circular Ocean (2015-2021) for sharing the INDICIT data base (Body Condition Index for healthy turtles) and data collected on entanglement of sea turtles, marine birds and mammals in order to be uploaded on the web site developed by the former project.

The results of INDICIT programs have been shared with the Marine Litter MED II (2020-23) program which aims to reduce and prevent the production of marine litter in the Mediterranean. The evaluation of especially GES, baselines and threshold for litter ingestion by sea turtles and entanglement in litter for marine biota strongly support the development of the IMA Candidate Indicator 24.

A strategic collaboration can be highlighted with the CleanAtlantic project (2017-2020), about data storing, security and availability. A platform Data Litter (DALI, developed by IFREMER, France) for collecting data on litter (on beach, sea floor, surface, ingested and causing entanglement) at the OSPAR scale is developed. The INDICIT partners have been involved in order to test the web interface for data uploading and INDICIT II agreed to be included in the marine litter knowledge database.

As experts in marine biology, many partners were also directly involved in complete and/or ongoing programs (such as MEDSEALITTER (MED-INTERREG), MEDCIS (DG ENV), DeFishGear (IPA ADRIATIC), MISTIC SEAS III (DG ENV), Plastic Busters MPAs (MED-INTERREG)), and projects funded under the same call as the INDICIT-II project (e.g. MEDREGION, QUIETMED).

## Recommendations:

- **Indicator macrolitter ingested by sea turtles:**

### ***Data collection:***

Collect standard measurements such as dry mass and number of pieces of litter.

Collect data on fat, plastron shape, body mass, lengths (especially Standard curved carapace length, injuries) in order to evaluate the health of individuals.

Collaboration with veterinarians is also encouraged to better identify the parameters to collect for evaluating body condition and health before death.

### ***Ecotoxicological approach:***

Investigate the parameters (marine litter ingestion, accumulation of PAEs, biological endpoints) on both dead and hospitalised organisms.

Considered cumulative stress (marine litter, climate change, exposure to legacy and emerging contaminants, etc.) for a correct definition of the health status of the species.

### ***Good environmental status proposal:***

Several scenarios have been tested and the updated proposed GES is: There should be not more than 26% of individuals with > 0.32 g of ingested plastic litter (dry mass). Although the abundance of ingested plastics provides better powerful tests compared to the dry mass of ingested plastics, the INDICIT II consortium recommends to keep the dry mass for setting the threshold values at this stage because this parameter is the most commonly collected by stakeholders with least confusion or biases. We also encourage collecting data on abundance (number of pieces) with a careful training. The power analyses can be updated after collecting more data on all parameters.

- **Indicator microlitter ingested by sea turtles:**

**Data collection:**

Select the range 1-5 mm to analyse the micro-litter, as it was impossible to manage cross-contamination for litter size < 1 mm.

Includes all litter debris from 1 mm, as with the Fulmar indicator, in order to be able to compare these two indicators in their common area (OSPAR).

At this time, this indicator cannot be implemented as a routine protocol on most of the countries considered.

- **Indicator « entanglement »**

**Data collection:**

A better development of networks with more engaged stakeholders is necessary within certain areas/countries (creating/reinforcing stranding networks and rescue centres).

The inclusion of pictures of individuals on stranding protocols is the better way to avoid confusion on litter classification.

Fishermen, who are key to the recovery of sea turtles in certain areas, should be more involved.

1-2 experts on marine litter and marine fauna could be involved as “focal points” in each country/region to coordinate data collection by stakeholders, harmonise litter classification involved on entanglement (review pictures, identify new litter typologies, etc.)

Additional data are needed, as the majority of the data collected about entanglement was on loggerhead turtle.

Data on other species, such as green and leatherback turtle, or other taxa (cetaceans, seals and seabirds), are promising and would be collected during the next years following the standard protocol developed by the INDICIT II consortium.

**Good environmental status proposal:**

GES evaluation and scenarios can only be tested with loggerhead turtles. Further analyses are needed to evaluate the indicator’s constraints.

Three GES scenarios can be tested with the type of data collected for entanglement data: (1) The number of individuals impacted by entanglement in each region during a 6-year cycle will be reduced, (2) The occurrence of entangled loggerhead turtles in each region during a 6-year cycle will be reduced and (3) The occurrence of specific litter typologies (fishing nets, fishing lines, ropes, stings, cords and heavy-duty sacks) entangling loggerhead turtles in each region during a 6-year cycle will be reduced.

- **Indicator « microlitter ingestion in fish »**

**Data collection:**

A minimum of two species in different marine compartment (*i.e.*, pelagic and benthic) are needed to assess the investigated areas. Even demersal species should be chosen to improve information on micro-litter pollution.

Establishing a lower limit for the collected micro-items (e.g. 100 µm) is fundamental to produce comparable data.

Secondary contamination must be reduced as much as possible and blank control should be performed at step of the protocol.

People in charge of the monitoring must follow all the steps of the monitoring process, including fishes catch, in order to verify the goodness of the samples.

**Good environmental status proposal:**

More data is needed to obtain a representative baseline and propose a threshold value.

A possible GES scenario could be “There should be less than X % of fish having ingested micro-litter per assessment area (e.g. GSA, Country, MSFD Subregion), where X % is the average value of the three different FO% (pelagic, demersal, benthic) in the pristine area.

## X. References

- Alomar, C., Deudero, S., 2017. Evidence of microplastic ingestion in the shark *Galeus melastomus* Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. *Environ Pollut*, 223, 223–229. <https://doi.org/10.1016/j.envpol.2017.01.0150>
- 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>
- Angiolillo, M., G erigny, O., Valente, T., Fabri, M.-C., Tambute, E., Rouanet, E., Claro, F., Tunesi, L., Vissio, A., Daniel, B., Galgani, F., 2021. Distribution of seafloor litter and its interaction with benthic organisms in deep waters of the Ligurian Sea (Northwestern Mediterranean). *Sci Total Environ*, 788, 147745. <https://doi.org/10.1016/j.scitotenv.2021.147745>
- Ara jo, 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.
- Attademo, A.M., Cabagna-Zenklusen, M., Lajmanovich, R.C., Peltzer, P.M., Junges, C., Bass o, A., 2011. B-esterase activities and blood cell morphology in the frog *Leptodactylus chaquensis* (Amphibia: Leptodactylidae) on rice agroecosystems from Santa Fe Province (Argentina). *Ecotoxicology* 20, 274–282.
- Attia El Hili H., Kaberi H., Kaska Y., Matiddi M., Monzon-Arguelo C., Moussier J., Ostiategui P., Paramio L., Avio, C.G., Pittura, L., d’Errico, G., Abel, S., Amorello, S., Marino, G., Gorbi, S., Regoli, F., 2020. Distribution and characterization of microplastic particles and textile microfibers in Adriatic food webs: General insights for biomonitoring strategies. *Environ. Pollut.* 258, 113766. <https://doi.org/10.1016/j.envpol.2019.113766>
- Baini, M., Martellini, T., Cincinelli, A., Campani, T., Minutoli, R., Panti, C., Finoia, M.G., Fossi, M.C., 2017. First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber. *Anal. Methods* 9, 1512–1520.
- 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.
- Bianchi, J., Valente, T., Scacco, U., Cimmaruta, R., Sbrana, A., Silvestri, C., Matiddi, M., 2020. Food preference determines the best suitable digestion protocol for analysing microplastic ingestion by fish. *Mar Pollut Bull*, 154, 111050. <https://doi.org/10.1016/j.marpolbul.2020.111050>
- 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>
- Bray, L., Digka, N., Tsangaris, C., Camedda, A., Gambaiani, D., de Lucia, G. A., Matiddi, M., Miaud, C., Palazzo, L., Pérez-del-Olmo, A., Raga, J. A., Silvestri, C., Kaberi, H., 2019. Determining suitable fish to monitor plastic ingestion trends in the Mediterranean Sea. *Environ Pollut*, 247, 1071-1077. <https://doi.org/10.1016/j.envpol.2019.01.100>
- Caliani, I., Campani, T., Giannetti, M., Marsili, L., Casini, S., Fossi, M.C., 2014. First application of comet assay in blood cells of Mediterranean loggerhead sea turtle (*Caretta caretta*). *Marine Environmental Research* 96, 68–72.
- 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>
- Carpentieri, P., Colloca, F., Cardinale, M., Belluscio, A., Ardizzone, G., 2005. Feeding habits of European hake (*Merluccius merluccius*) in the central Mediterranean Sea. *Fishery Bull*, 103(2), 411-416.
- Casale P. 2011. Sea turtle by-catch in the Mediterranean. *Fish and Fisheries* 12: 299-316.
- 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>
- Casini, S., Caliani, I., Giannetti, M., Marsili, L., Maltese, S., Coppola, D., Bianchi, N., Campani, T., Ancora, S., Caruso, C., Furi, G., Parga, M., D'Agostino, A., Fossi, M.C., 2018. First ecotoxicological assessment of *Caretta caretta* (Linnaeus, 1758) in the Mediterranean Sea using an integrated nondestructive protocol. *Science of The Total Environment* 631–632, 1221–1233.
- 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., Pham C.K., Loza A. L., Bradai M.N., Camedda A., Chaieb O., Darmon G., de Lucia G.A.5,
- 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.
- Compa, M., Ventero, A., Iglesias, M., Deudero, S., 2018. Ingestion of microplastics and natural fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) along the Spanish Mediterranean coast. *Mar Pollut Bull*, 128, 89-96. <https://doi.org/10.1016/j.marpolbul.2018.01.009>

- Cowger, W., Booth, A.M., Hamilton, B.M., Thaysen, C., Primpke, S., Munno, K., Lusher, A.L., Dehaut, A., Vaz, V.P., Liboiron, M., Devriese, L.I., Hermabessiere, L., Rochman, C.M., Athey, S.N., Lynch, J.M., De Frond, H., Gray, A., Jones, O.A.H., Brander, S., Steele, C., Moore, S., Sanchez, A., Nel, H., 2020a. Reporting guidelines to increase the reproducibility and comparability of research on microplastics. *Appl. Spectrosc.* 74(9), 1066-1077. <https://doi.org/10.1177/0003702820930292>
- 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 déchets ingérés par les tortues marines (D10-2-1) et d'un bon état 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 le 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*.
- Dehaut, A., Cassone, A.-L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., Paul-Pont, I., 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environ. Pollut.* 215, 223-233. <https://doi.org/10.1016/j.envpol.2016.05.018>
- 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](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>
- Di Giacomo, R. F., Koepsell, T. D., 1986. Sampling for detection of infection or disease in animal populations. *J Am Vet Med Ass*, 189 (1), 22-23.
- Di Natale, A., Molinari, A., Öztürk, B., Srour, A., Pollard D., 2011. *Merluccius merluccius*. The IUCN Red List of Threatened Species 2011. e.T198562A9031395. Downloaded on 08 July 2020. <https://www.iucnredlist.org/es/species/198562/9031395>
- 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>

- Enders, K., Lenz, R., Beer, S., Stedmon, C. A., 2017. Extraction of microplastic from biota: Recommended acidic digestion destroys common plastic polymers. *ICES J Mar Sci*, 74(1), 326-331. <https://doi.org/10.1093/icesjms/fsw173>
- 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>
- Flower, J.E., Norton, T.M., Andrews, K.M., Nelson, S.E., Parker, C.E., Romero, L.M., Mitchell, M.A., 2015. Baseline plasma corticosterone, haematological and biochemical results in nesting and rehabilitating loggerhead sea turtles (*Caretta caretta*). *Conservation Physiology* 3, cov003.
- Foekema, E. M., De Gruijter, C., Mergia, M. T., van Franeker, J. A., Murk, A. J., Koelmans, A. A., 2013. Plastic in North Sea fish. *Environ Sci Technol*, 47(15), 8818-8824. <https://doi.org/10.1021/es400931b>
- 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., Baine, 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>
- Froese, R. and D. Pauly, Editors. 2019. FishBase. World Wide Web Electronic Publication. [www.fishbase.org](http://www.fishbase.org), version (12/2019).
- 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](https://doi.org/10.1007/978-3-319-16510-3_2)
- Galgani, F., Hanke, G., Werner, S., De Vrees, L., 2013. Marine litter within the European Marine Strategy Framework Directive. *ICES J Mar Sci*, 70(6), 1055-1064. <https://doi.org/10.1093/icesjms/fst122>
- 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>
- Garcia-Garin, O., Vighi, M., Aguilar, A., Tsangaris, C., Digka, N., Kaberi, H., Borrell, A., 2019. Boops boops as a bioindicator of microplastic pollution along the Spanish Catalan coast. *Mar Pollut Bull*, 149, 110648. <https://doi.org/10.1016/j.marpolbul.2019.110648>
- Ghaffar, A., Hussain, R., Khan, A., Abbas, R.Z., Asad, M., 2015. Butachlor induced clinico-hematological and cellular changes in fresh water fish *Labeo rohita* (Rohu). *Pak. Vet. J.* 35.
- Grandchamp, B., Deybach, J.C., Grelier, M., De Verneuil, H., Nordmann, Y., 1980. Studies of porphyrin synthesis in fibroblasts of patients with congenital erythropoietic porphyria and one patient with homozygous coproporphyrin. *Biochimica et Biophysica Acta (BBA) - General Subjects* 629, 577–586.
- 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 environmental management or an important concept with no practical application? *Ecosystems* 9, 113. <https://doi.org/10.1007/s10021-003-0142-z>
- Guerranti C., Baine M., Casini S., Focardi S., Giannetti M., Mancusi C., Marsili L., Perra G., Fossi M.C., 2014. Pilot study on levels of chemical contaminants and porphyrins in *Caretta caretta* from the Mediterranean Sea. *Marine Environmental Research* 100, 33 – 37.
- Güven, O., Gökdağ, K., Jovanović, B., Kıdeys, A. E., 2017. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. *Environ Pollut*, 223, 286-294. <https://doi.org/10.1016/j.envpol.2017.01.025>
- 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>

- Hermsen, E., Mintenig, S.M., Besseling, E., Koelmans, A.A., 2018. Quality Criteria for the Analysis of Microplastic in Biota Samples: A Critical Review. *Environ. Sci. Technol.* 52, 10230–10240. <https://doi.org/10.1021/acs.est.8b01611>
- Hermsen, E., Pompe, R., Besseling, E., Koelmans, A. A., 2017. Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. *Mar Pollut Bull*, 122(1-2), 253-258. <https://doi.org/10.1016/j.marpolbul.2017.06.051>
- Herrera, A., Stindlová, A., Martínez I., Rapp, J., Romero-Kutzner, V., Samper, M. D., Montoto, T., Aguiar-González, B., Packard, T., Gómez, M., 2019. Microplastic ingestion by Atlantic chub mackerel (*Scomber colias*) in the Canary Islands coast. *Mar Pollut Bull*, 139, 127-135. <https://doi.org/10.1016/j.marpolbul.2018.12.022>
- 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>
- Kühn, S., van Franeker, J. A., O'Donoghue, A. M., Swiers, A., Starckenburg, M., van Werven, B., Foekema, E., Hermsen, E., Egelkraut-Holtus, M., Lindeboom, H., 2020. Details of plastic ingestion and fibre contamination in North Sea fishes. *Environ Pollut* 257, 113569. <https://doi.org/10.1016/j.envpol.2019.113569>
- 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](https://doi.org/10.1007/978-1-4613-8486-1_10)
- Lajmanovich, R.C., Junges, C.M., Attademo, A.M., Peltzer, P.M., Cabagna-Zenklusen, M.C., Basso, A., 2013. Individual and mixture toxicity of commercial formulations containinglyphosate, metsulfuron-methyl, bispyribac-sodium, and picloramon *Rhinella arenarum* tadpoles. *Water Air Soil Pollut.* 224,1404.
- Law, K.L., Thompson, R.C., 2014. Microplastics in the seas. *Science* 345, 144–145. <https://doi.org/10.1126/science.1254065>
- Lin, Z.-P., Ikonomou, M.G., Jing, H., Mackintosh, C., Gobas, F.A.P.C., 2003. Determination of Phthalate Ester Congeners and Mixtures by LC/ESI-MS in Sediments and Biota of an Urbanized Marine Inlet. *Environmental Science & Technology* 37, 2100–2108.
- Lopes, C., Raimundo, J., Caetano, M., Garrido, S., 2020. Microplastic ingestion and diet composition of planktivorous fish. *Limonl Oceanogr Letters*, 5, 103-112. <https://doi.org/10.1002/lo2.10144>
- Lusher, A., Hollman, P., Mendoza-Hill, J., 2017. Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. *FAO Fisheries and Aquaculture Technical Paper*. No. 615. Rome, Italy (2017).
- Matiddi, M., de Lucia, 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., Indicit C., 2019. Data Collection on Marine Litter Ingestion in Sea Turtles and Thresholds for Good Environmental Status. *J Vis Exp*, 147, e59466.
- Matiddi, M., de Lucia, 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).
- Matiddi, M., Hochscheid, S., Camedda, A., Bains, 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., van Franeker, J. A., Sammarini, V., Travaglini, A., Alcaro, L., 2011. Monitoring litter by sea turtles: An experimental protocol in the Mediterranean. In: *Proceedings of the 4th Mediterranean Conference on Sea Turtles*. 7-10 November, Naples Italy.
- 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*.
- MEDSEALITTER DELIVERABLE 4.6.1, 2019. Common monitoring protocol for marine litter. [www.interregmed.eu/medsealitter](http://www.interregmed.eu/medsealitter)
- Miller, E., Sedlak, M., Lin, D., Box, C., Holleman, C., Rochman, C.M., Sutton, R., 2021. Recommended best practices for collecting, analyzing, and reporting microplastics in environmental media: Lessons learned from comprehensive monitoring of San Francisco Bay. *J. Hazard Mater.* 409, 124770. <https://doi.org/10.1016/j.jhazmat.2020.124770>
- 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.
- 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., Scoullou, 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 TG Marine Litter 2013, François Galgani, Georg Hanke, Werner, Stefanie, Oosterbaan, Lex, Nilsson, Per, Fleet, David, Kinsey, Susan, Thompson, Richard C., van Franeker, Jan, Vlachogianni, Thomais, Scoullou, Michael, Mira Veiga, Joana, Palatinus, Andreja, Matiddi, Marco, Maes, Thomas, Korpinen, Samuli, Budziak, Ania, Leslie, Heather, Gago, Jesus, Liebezeit, Gerd, 2013. Guidance on Monitoring of Marine Litter in European Seas. EUR 26113. Luxembourg (Luxembourg). Publications Office of the European Union. JRC83985. <http://publications.jrc.ec.europa.eu/repository/handle/111111111/30681>
- 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>
- Novillo, O., Raga, J. A., & Tomás, J. (2020). Evaluating the presence of microplastics in striped dolphins (*Stenella coeruleoalba*) stranded in the Western Mediterranean Sea. *Marine pollution bulletin*, 160, 111557.
- 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).
- 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.
- Palazzo, L., Coppa, S., Camedda, A., Cocca, M., De Falco, F., Vianello, A., Massaro, G., de Lucia, G. A., 2021. A novel approach based on multiple fish species and water column compartments in assessing vertical microlitter distribution and composition. *Environ Pollut*, 272, 116419. <https://doi.org/10.1016/j.envpol.2020.116419>



- Pereira, J. M., Rodríguez, Y., Blasco-Monleon, S., Porter, A., Lewis, C., Pham, C. K., 2020. Microplastic in the stomachs of open-ocean and deep-sea fishes of the North-East Atlantic. *Environ Pollut*, 265 (A), 115060. <https://doi.org/10.1016/j.envpol.2020.115060>
- 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>
- Prata, J.C., Reis, V., da Costa, J.P., Mouneyrac, C., Duarte, A.C., Rocha-Santos, T., 2021. Contamination issues as a challenge in quality control and quality assurance in microplastics analytics. *J. Hazard Mater.* 403, 123660 <https://doi.org/10.1016/j.jhazmat.2020.123660>
- R Core Team, 2017. R: A Language and Environment for Statistical Computing.
- Renner, G., Nellessen, A., Schwiers, A., Wenzel, M., Schmidt, T.C., Schram, J., 2019. Data preprocessing & evaluation used in the microplastics identification process: A critical review & practical guide. *Trends Anal. Chem.* 111, 229-238. <https://doi.org/10.1016/j.trac.2018.12.004>
- Renner, G., Schmidt, T. C., Schram, J., 2017. Analytical methodologies for monitoring micro(nano)plastics: Which are fit for purpose? *Curr Opin Environ Sci Health*, 1, 55-61. <https://doi.org/10.1016/j.coesh.2017.11.001>
- Revuelta O., Silvestri C., Sozbilen D., Tòmas J., Tsangaris C., Vale M., Vandeperre F. and Miaud C., 2018 - State of the art and feasibility study for the implementation of the indicator "Entanglement of biota with marine debris" in the areas of the Marine Strategy Framework Directive and the regional sea conventions OSPAR, HELCOM AND BARCELONA. INDICIT, Deliverable n° D.2.6 of the European Project "Implementation of the indicator of Marine Litter Impact on Sea Turtles and Biota in Regional Sea Conventions and Marine Strategy Framework Directive Areas" ([indicit-europa.eu](http://indicit-europa.eu)). (Grant agreement 11.0661/2016/748064/SUB/ENV.C2. <https://indicit-europa.eu/indicit-documents>
- Rochman, C. M., Tahir, A., Williams, S. L., Baxa, D. V., Lam, R., Miller, J. T., Teh, S. J., 2015. Anthropogenic debris in seafood: plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Sci Rep*, 5, 14340. <https://doi.org/10.1038/srep14340>
- 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](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: *Foundations of Environmental Law and Policy*. Revesz, Richard L., New York, N.Y., pp. 52–58.
- Schaumburg, L.G., Poletta, G.L., Siroski, P.A., Mudry, M.D., 2012. Baseline values of Micronuclei and Comet Assay in the lizard *Tupinambis merianae* (*Teiidae, Squamata*). *Ecotoxicological Environmental Safety* 84, 99–103.
- 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>
- Silvestri, C., Dalù, M., Piermarini, R., Matiddi, M., Darmon, G., Liria Loza, A., Claro, F., Attia, E., Hili, H., Bradai, M. N., Camedda, A., de Lucia, G. A., Kaberi, H., Kaska, Y., Paramio, L., Pham, C. K., Sozbilen, D., Tòmas, J.,

- Tsangaris, C., Vale, M., Vandeperre, F., Miaud, C., 2018. Pilot Study for the Implementation of Indicator “Micro-plastic Ingestion by Fish and Sea Turtles”. INDICIT, Deliverable n° D.2.5 of the European Project “Implementation of the indicator of Marine Litter Impact on Sea Turtles and Biota in Regional Sea Conventions and Marine Strategy Framework Directive Areas” (indicit-europa.eu). (Grant agreement 11.0661/2016/748064/SUB/ENV.C2. <https://indicit-europa.eu/indicit-documents>)
- 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>
- Torre, M., Digka, N., Anastasopoulou, A., Tsangaris, C., Mytilineou, C., 2016. Anthropogenic microfibres pollution in marine biota. A new and simple methodology to minimize airborne contamination. *Mar Pollut Bull*, 113 (1-2), 55-61. <https://doi.org/10.1016/j.marpolbul.2016.07.050>
- Tsangaris, C., Digka, N., Valente, T., Aguilar, A., Borrell, A., de Lucia, G. A., Gambaiani, D., Garcia-Garin, O., Kaberi, H., Martin, J., Mauriño, E., Miaud, C., Palazzo, L., del Olmo, A. P., Raga, J. A., Sbrana, A., Silvestri, C., Skylaki, E., Vighi, M., Wongdontree, P., Matiddi, M., 2020. Using Boops boops (osteichthyes) to assess microplastic ingestion in the Mediterranean Sea. *Mar Pollut Bull*, 158, 111397. <https://doi.org/10.1016/j.marpolbul.2020.111397>
- 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>
- Valente, T., Sbrana, A., Scacco, U., Jacomini, C., Bianchi, J., Palazzo, L., de Lucia, G. A., Silvestri, C., Matiddi, M., 2019. Exploring microplastic ingestion by three deep-water elasmobranch species: A case study <sup>[1]</sup>from the Tyrrhenian Sea. *Environ Pollut*, 253, 342-350. <https://doi.org/10.1016/j.envpol.2019.07.001>
- van Franeker, J. A., 2004. Save the North Sea Fulmar-Litter-EcoQO Manual Part 1: Collection and Dissection Procedures. Wageningen, Alterra, Alterra-rapport 672 pp.
- 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. *Environ Pollut*, 159, 2609-2615. <https://doi.org/10.1016/j.envpol.2011.06.008>
- van Franeker, J. A., Meijboom, A., 2002. Alterra-rapport. Litter NSV, Marine Litter Monitoring by Northern Fulmar; a Pilot Study, vol. 401, 72 pp.
- 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.
- Worm, B., Lotze, H.K., Jubinville, I., Wilcox, C., Jambeck, J., 2017. Plastic as a persistent marine pollutant. *Annu. Rev. Env. Resour.* 42, 1-26. <https://doi.org/10.1146/annurev-environ-102016-060700>
- 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>.

## Appendixes

## Appendix 1: list of deliverables

### Activity 1

Number	Deliverable name	Coordinator	Month	Original deadline	% progress	New deadline
D1.1	Inception report	EPHE	M2	31/03/2019	100	-
D1.2	Progress report RP1	EPHE	M6	15/08/2019	100	-
D1.3	Progress report RP2	EPHE	M12	15/02/2020	100	-
D1.4	Progress report RP3	EPHE	M20	15/08/2020	100	15/02/2021
D1.5	Progress report RP4	EPHE	M24	15/01/2021	100	15/07/2021
D1.6	Final report	EPHE	M26	31/03/2021	<i>This report</i>	30/09/2021
D1.7	Minutes of KOF and Workshop#1	EPHE	M2	31/03/2019	100	-
D1.8	Minutes of 1st intermediate meeting	INSTM	M6	15/08/2019	100	-
D1.9	Minutes of 2nd intermediate meeting	EPHE	M12	15/02/2020	100	-
D1.10	Minutes of 3 <sup>rd</sup> intermediate meeting	PAU-DEKAMER	M18	15/08/2020	100	Included in the new RP
D1.11	Minutes of dissemination meeting	PAU-DEKAMER	M18	15/08/2020	100	Included in the new RP
D1.12	Minutes of Final meeting	PAU-DEKAMER	M18	15/02/2021	100	15/08/2021
D1.13	Consortium agreement	EPHE	M3	30/04/2019	100	-
D1.14	Communication and dissemination plan	UNEXE	M2	31/03/19	100	-
D1.15	Communication Progress report RP1	UNEXE	M6	15/08/19	100	-
D1.16	Communication Progress report RP2	UNEXE	M12	15/02/20	100	-

### Activity 2

Number	Deliverable name	Coordinator	Month	Original deadline	% progress	New deadline
D2.1	Inception report	EPHE	M2	31/03/2019	100	-
D2.2	Progress report RP1	EPHE	M6	15/08/2019	100	-
D2.3	Progress report RP2	EPHE	M12	15/02/2020	100	-
D2.4	Progress report RP3	EPHE	M20	15/08/2020	100	15/02/2021
D2.5	Progress report RP4	EPHE	M24	15/01/2021	100	15/07/2021
D2.6	Training session (workshop with stakeholders involved in implementation)	UVEG/CNR	M12	15/02/2020	75	31/01/2021
D2.7	List Pilot areas/tested PoMs	UVEG/CNR	M9	15/11/2019	100	-
D2.8	List of stakeholders + google map	UVEG/CNR	M12	15/02/2020	100	-
D2.9	Final list of stakeholders + google map	UVEG/CNR	M24	15/02/2021	100	15/07/2021
D2.10	GES and indicators' units and constraints	UVEG/CNR	M24	15/07/2021	100	15/07/2021
D2.11	Report on assessment in pilot areas	UVEG/CNR	M24	15/07/2021	100	15/07/2021
D2.12	Final common database	UVEG/CNR	M24	15/07/2021	100	15/08/2021

### Activity 3

Number	Deliverable name	Coordinator	Month	Original deadline	% progress	New deadline
D3.1	Inception report	ULPGC/INSTM	M2	31/03/2019	100	
D3.2	Progress report RP1	ULPGC/INSTM	M6	15/08/2019	100	
D3.3	Progress report RP2	ULPGC/INSTM	M12	15/02/2020	100	
D3.4	Progress report RP3	ULPGC/INSTM	M18	15/08/2020	100	15/02/21
D3.5	Progress report RP4	ULPGC/INSTM	M24	15/02/2021	100	15/07/21
D3.6	Training session (workshop with stakeholders involved)	ULPGC/INSTM	M12	15/02/2020	75	15/09/20
D3.7	List of stakeholders + google map	ULPGC/INSTM	M12	15/02/2020	100	15/09/20
D3.8	Final list of stakeholders + google map	ULPGC/INSTM	M24	15/02/2021	100	15/07/21
D3.9	Standard procedures (protocol entanglement)	ULPGC/INSTM	M12	15/02/2020	100	15/09/21
D3.10	Final standard procedures (protocol)	ULPGC/INSTM	M24	15/02/2021	100	15/07/21
D3.11	Final common standard database	ULPGC/INSTM	M24	15/02/2021	100	15/07/21
D3.12	Report on baselines and target species/taxa	ULPGC/INSTM	M24	15/02/2021	100	15/07/21
D3.13	Report on conclusions from assessment in pilot areas	ULPGC/INSTM	M24	15/02/2021	100	15/07/21

### Activity 4

Number	Deliverable name	Coordinator	Month	Expected date	% progress	New deadline
D4.1	Inception report	ISPRA/FRCT	M2	31/03/2019	100	
D4.2	Progress report RP1	ISPRA/FRCT	M6	15/08/2019	100	
D4.3	Progress report RP2	ISPRA/FRCT	M12	15/02/2020	100	
D4.4	Progress report RP3	ISPRA/FRCT	M18	15/08/2020	100	15/02/2021
D4.5	Progress report RP4	ISPRA/FRCT	M24	15/02/2020	100	15/02/2021
D4.6	Training session (workshop with stakeholders involved in implementation) MODIFIED in VIDEOTUTORIAL	ISPRA/FRCT	M14	30/12/2020	50	15/07/2021
D4.7	List of stakeholders + Google map	ISPRA/FRCT	M12	15/02/2020	100	
D4.8	Final list of stakeholders + google map	ISPRA/FRCT	M24	15/02/2021	100	15/07/2021
D4.9	Standard procedures (protocol micro/fish)	ISPRA/FRCT	M12	15/02/2021	100	
D4.10	Final standard procedures (protocol)	ISPRA/FRCT	M24	15/02/2021	100	15/07/2021
D4.11	Final common standard database	ISPRA/FRCT	M24	15/02/2021	100	15/07/2021
D4.12	Report on baselines and target fish species	ISPRA/FRCT	M24	15/02/2021	100	15/07/2021
D4.13	Report on Indicator "Micro-debris ingested by sea turtles" (relevance, methodology, baselines (if relevant))	ISPRA/EPHE	M24	15/02/2021	100	15/07/2021

## Activity 5

Number	Deliverable name	Coordinator	Month	Original deadline	% progress	New deadline
D5.1	Inception report	HCMR/EPHE	M2	31/03/2019	100	-
D5.2	Progress report RP1 for Activity 5	HCMR/EPHE	M6	15/08/2019	100	-
D5.3	Progress report RP2 for Activity 5	HCMR/EPHE	M12	15/02/2020	100	-
D5.4	Progress report RP3	HCMR/EPHE	M18	15/08/2020	100	15/02/2021
D5.5	Progress report RP4	HCMR/EPHE	M24	15/02/2021	100	15/07/2021
D5.6	List of identified projects for collaboration, meetings, type of data and available means	HCMR/EPHE	M6	15/08/2019	100	-
D5.7	Report on interactions with identified projects, experts and representatives	HCMR/EPHE	M24	15/02/2021	100	15/07/2021
D5.8	Report on methodology for integrative tools	HCMR/EPHE	M24	15/02/2021	95	15/07/2021

## Appendix 2 – Up-date of the programmes of measures in each country used for the decision trees

### France

- Law on energetic transition and green growth **17 August 2015**
- Decree n° **2016-379, 30 March 2016** (Decree published 31 March 2016, JORF n°0076 du 31 mars 2016) on the procedures for implementing the limitation of single-use plastic bags.
- **1st July 2016**, plastic bags with a thickness of less than 50 micrometres will be prohibited in stores. In exchange, consumers will be offered reusable plastic bags (over 50 micrometres) or paper, cardboard or fabric bags;
- **1st July 2017**, ban of non-compostable plastic bags less than 50 micrometres thick, intended for the packaging of goods at the point of sale other than checkout bags, in particular bags distributed on the shelves, as well as packaging in non-biodegradable and non-compostable plastic, for sending the press and advertising (blistered)
- JORF n°0202 31 august 2016 text n° 4
- **Decree n° 2016-1170 30 August 2016** relating to the procedures for implementing the limitation of disposable plastic cups, glasses and plates
- **Decree n° 2019-1451 24 December 2019** on the ban of certain single-use plastic products (JORF n°0300 27 December 2019 text n° 20). Ban of single-use plastic product: product made entirely or partially from plastic and which is not designed, created or placed on the market to accomplish, during its lifetime, several journeys or rotations by being returned to a producer to be refilled, or which is not designed, created or placed on the market to be reused for a use identical to that for which it was designed.
- Packaging and packaging waste, goblets and glasses, disposable kitchen plates for the table, forks, knives, spoons and chopsticks, meal trays, ice cream pots, salad bowls and boxes, straws, glass or beaker lids.

### Italy

- Law on non-biodegradable plastic bags
- **3 August 2017**, decree n°123 – ban of non–biodegradable plastic bags
- Implementing provisions of Directive (EU) 2015/720 of the European Parliament and of the Council of 29 April 2015 amending Directive 94/62 / EC as regards the reduction of the use of light plastic bags. Infringement procedure no. 2017/0127)
- Art. 9 -bis (Ban on the marketing of plastic bags).
- **Since January 1, 2018**, Italian law requires the exclusive use of biodegradable plastic for "ultralight" bags with which bulk products such as bread, vegetables and fruit are weighed and priced.
- Law on ban of cotton buds and microbeads
- **Law 27 December 2017** n. 205 “State budget for the financial year 2018 and multiannual budget for the three-year period 2018-2020”; art. 1 paragraph 545, 546
- **January 1, 2019** ban on the production and sale of cotton swabs with a plastic core (Shops are really selling only cotton buds with a paper/cardboard core)
- **January 1, 2020** ban on the production and sale of microbeads in cosmetic
- Law on ban of single-use plastic (not yet transposed in Italy)
- EU directive SUP 2019/904 (Single Use Plastic)
- Italy by **July 2021** must stop the production of Disposable Plastic (art. 17)
- Prohibited plastics: straws, plastic cutlery and plates, sticks for inflatable balloons, cotton buds, palette for mixing cocktails, bone-degradable plastic bags, and polystyrene foam containers for food.
- Legge Salvamare (not yet in force): The Assembly of the Chamber (House of Representatives), in the session of 24 October 2019, approved the draft law of the A.C. Government. 1939-A and abb., containing "Provisions for the recovery of waste at sea and inland waters and for the promotion of the circular economy (« save sea law »)", which now passes to the Senate examination.



- Professional fishermen (in particular those who use trawling net gears recover the most of sinking marine litter, but also those with gill/trammel nets) will be authorized to bring marine litter found at sea in their gears to the port so to be recycled, when possible, with other kind of waste. In this way, fishermen should avoid discarding marine litter.

## Spain

### **Regional Law 14/2018, of June 18, on waste and its taxation. Autonomous community of Navarre.**

**Article 23.** Use of plastic bags, single-use plastic products and products packaged in single-dose or single-use capsules

- As **of July 1st 2018**, (a) The delivery of plastic bags to consumers free of charge at the point of sale of goods or products, as well as for home delivery or for online sales, shall be prohibited, with the exception of very lightweight plastic bags. (b) Traders shall charge a fee for each non-compostable plastic bag provided to the consumer. In determining the price of plastic bags, traders may take as a reference the guide prices laid down in the regulations in force.
- From **January 1st 2020**, the delivery of plastic bags to the consumer at the point of sale of goods or products, as well as home delivery or delivered for sale online, is prohibited, with the exception of compostable plastic bags that meet the requirements of the standard UNE-EN 13432:2000 or equivalent and these may not be delivered or distributed free of charge.
- **From July 3rd 2021**, the sale of products packaged in single-dose or single-use capsules, which are not covered by Directive 94/62/EC on packaging and packaging waste and Directive (EU) 2019/904 of the European Parliament and of the Council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment, shall be prohibited, provided that they are made of organically or mechanically non-recyclable materials.

**Royal Decree 293/2018 of May 18, on the reduction of consumption of plastic bags and establishing the register of producers.** Ministry of agriculture and fisheries, food and environment

**Article 4.** Measures to reduce the consumption of plastic bags

- As **of 1 July 2018**, (a) The delivery of plastic bags free of charge to consumers at the point of sale of goods or products shall be prohibited, with the exception of very lightweight plastic bags and plastic bags with a thickness of 50 microns or more containing 70% or more recycled plastic. c) Traders shall charge a fee for each plastic bag provided to the consumer. In determining the price of plastic bags, traders may take as a reference the guide prices set out in Annex I.
- From **1 January 2020**, (a) The supply of plastic carrier bags to consumers at the point of sale of goods or products shall be prohibited. (b) Plastic bags with a thickness of 50 microns or more shall contain a minimum of 50 % recycled plastic.
- As from **1 January 2021**, the delivery of light and very light plastic bags to the consumer at the point of sale of goods or products shall be prohibited, unless they are made of compostable plastic. Traders may also choose other packaging formats to replace plastic bags.

## Portugal

- Law 82-D/2014, single use plastic bags charges.
- Law 69/2018, establishment of an incentive system for the return of non-reusable plastic beverage packaging, to be implemented by **December 31<sup>st</sup> 2019**, and a deposit system for non-reusable plastic, glass, ferrous metal and aluminium beverage packaging, mandatory from **January 1<sup>st</sup> 2022**.
- Law 76/2019, single use plastic ban from restaurants and supermarkets.

## Greece

- Law 2939/2001, **6 August 2001** (OJ 179/A/6-8-2001): "Packaging and Alternative Management of Packaging and other products, Establishment of a National Organization for the Alternative Management of Packaging and other

products, and other provisions”. The purpose of this law was to introduce measures for the management of packaging and other products with a view to the re-use or recycling of their wastes.

- Introduction of Eco-Tax for plastic bag
- Law 4496\2017, **8 November 2017** (OJ 170/8-11-2017): “Amendment of Law 2939/2001 on Alternative management of packaging and other products, adaptation to Directive 2015/720/EU, regulation of issues of the Greek Recycling Agency and other provisions”.
- The government has introduced a so-called Eco-Tax of 4 cents for every plastic bag. No charge for plastic bags were imposed for shopping in the open markets and kiosks. As of **2019**, the charge raised to 7 cents. There is also the “National Strategy of the Circular Economy (in Greek)”: <http://www.ypeka.gr/LinkClick.aspx?fileticket=R7N5HFvj2dM%3D&tabid=37&language=el-GR>.

### **Tunisia**

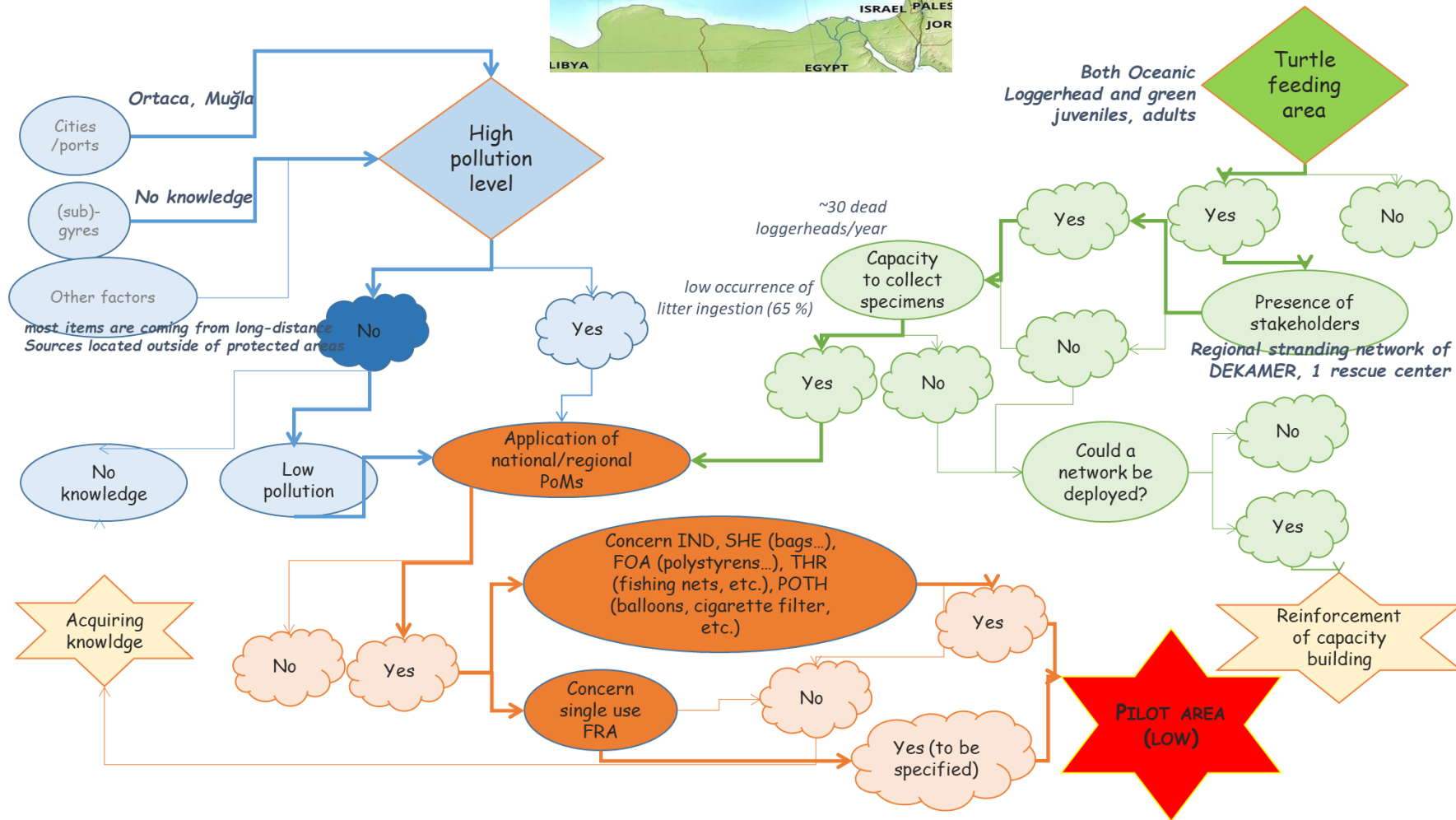
- Framework law 96-41 of **10 June 1996** on waste management
- Decree 97 – 1102 of *2 June 1997* fixes the conditions and the arrangements for the collection and the valorization of the packaging waste. The main products recycled are: Plastic, metal, tires, paper and textile
- 2003: Ecotaxes of 5% from the company revenues importing or producing plastic (particularly granules or semi-final plastic products)
- Decree 2005-2317 of **22 August 2005** on the creation of the National Agency on waste management (ANGed)
- Conventions of **March 1, 2017** and **March 1, 2018**, ban of bags in single-use plastic from supermarkets (with a thickness of less than 40 microns) and the ban of plastic have been prohibited at the exit of the boxes in pharmacies.

### **Turkey**

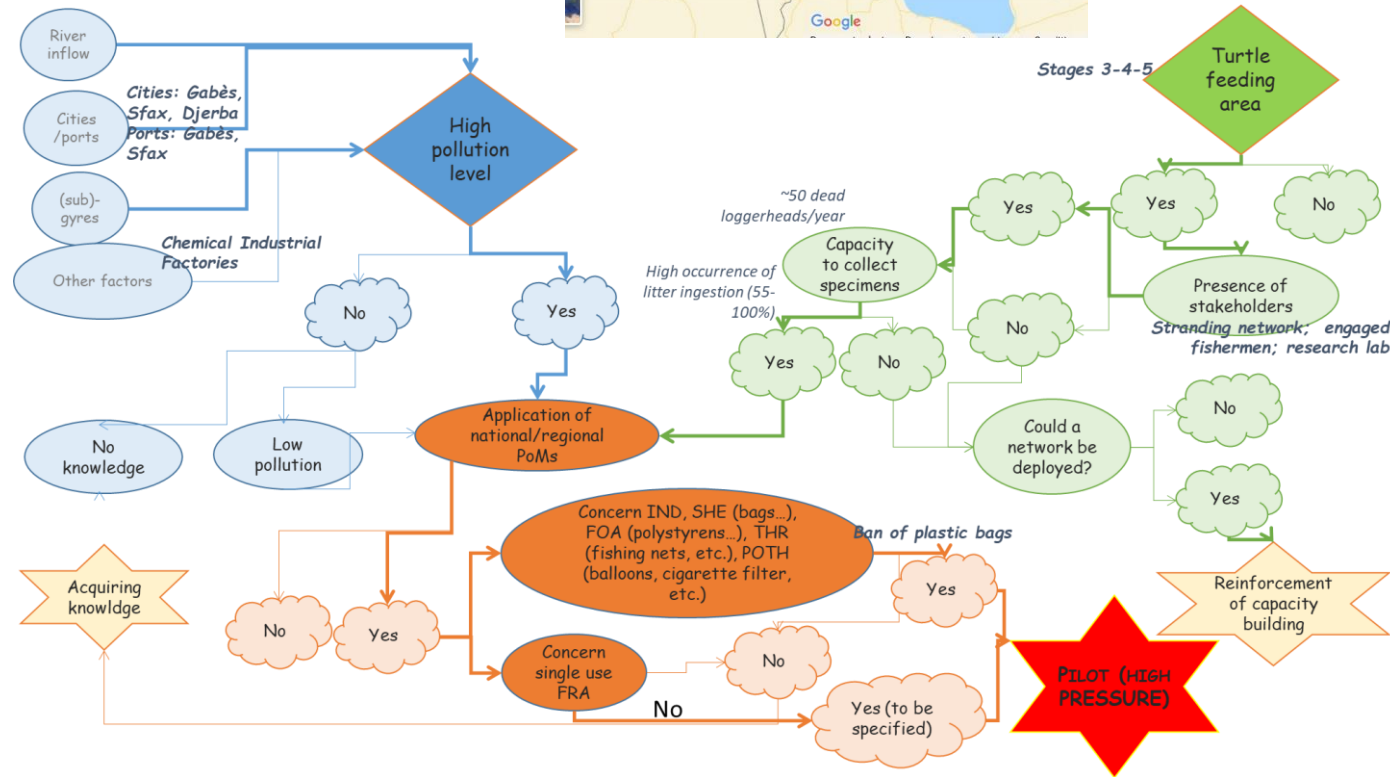
- The Law on Environment was changed (Law n° 2872, Annex 13) and the decree was published on 10.12.2018. Free plastic bags were prohibited in stores since **January 1, 2019** and penalties started for stores that do not comply with the ban. Plastic bag usage reduced by 77% in the first 11 months of the ban, according to the officials of the Ministry of Environment and Urbanisation. This is the only legal regulation for plastic waste for now in Turkey.

Appendix 3 – Decision tree for the identification of pilot areas (indicator  
litter ingestion is sea turtles

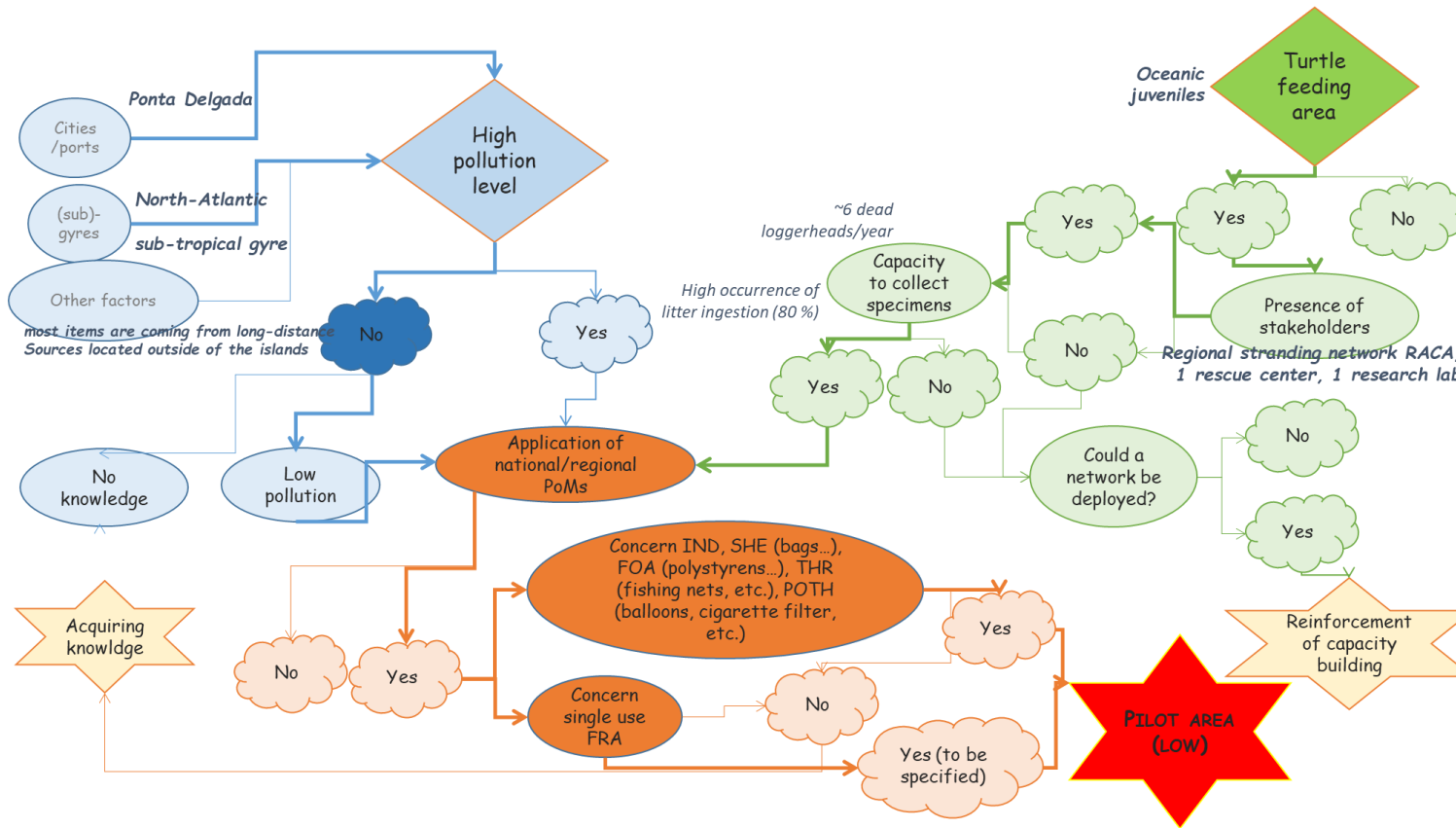
# Turkey, area Dalyan



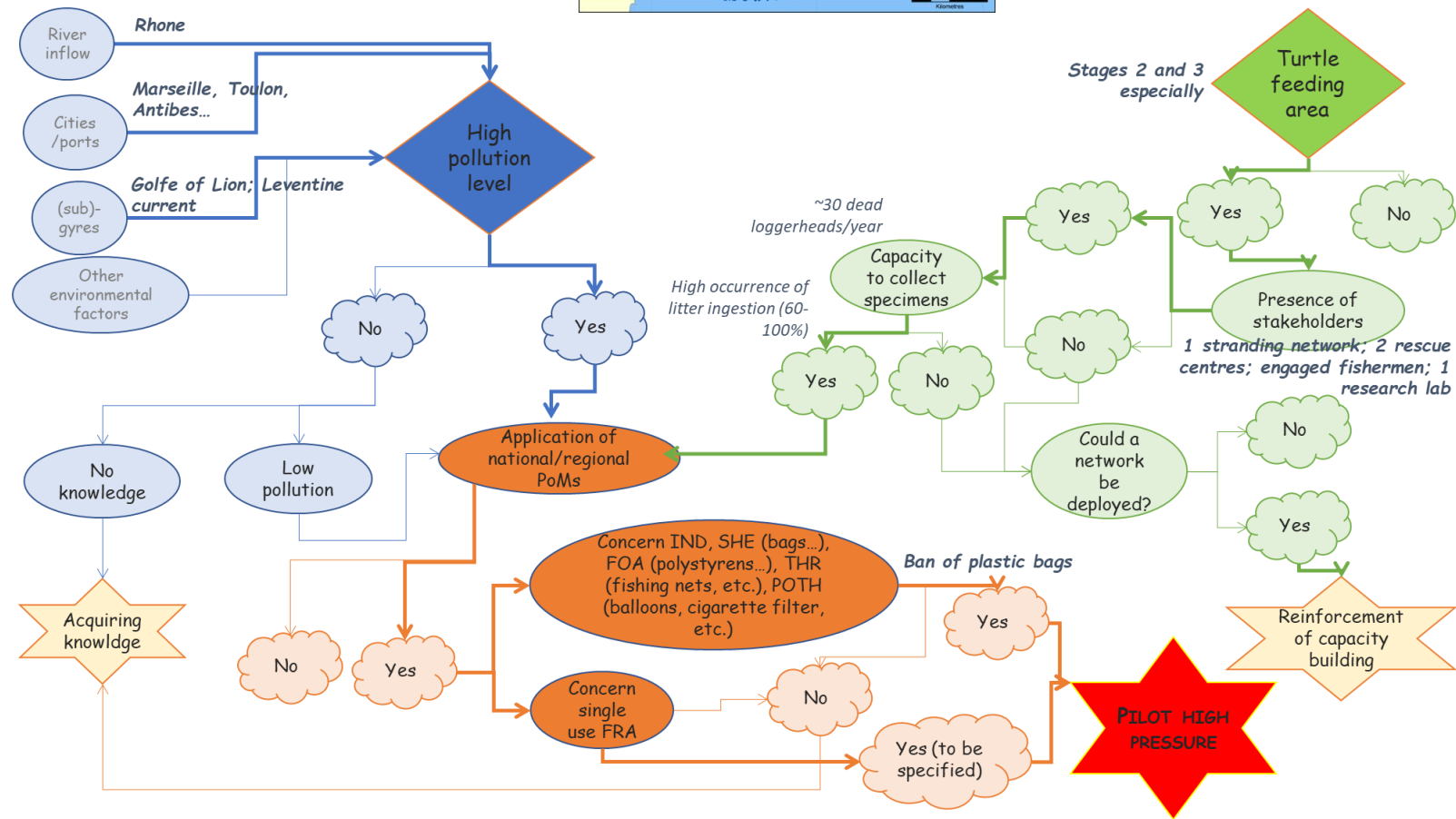
# Tunisia, area Gulf of Gabes



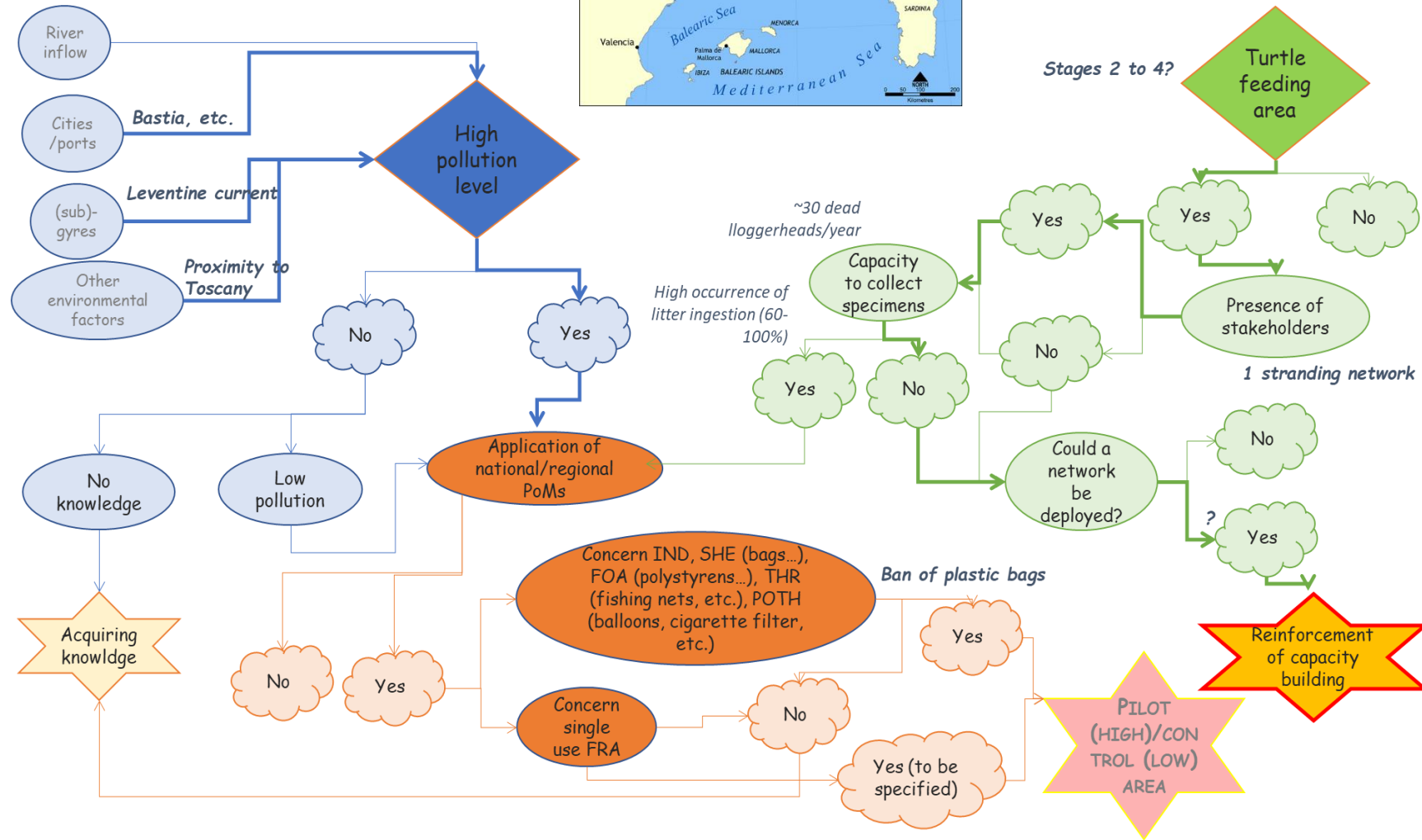
Portugal, area Azores



# France, area Golf of Lion

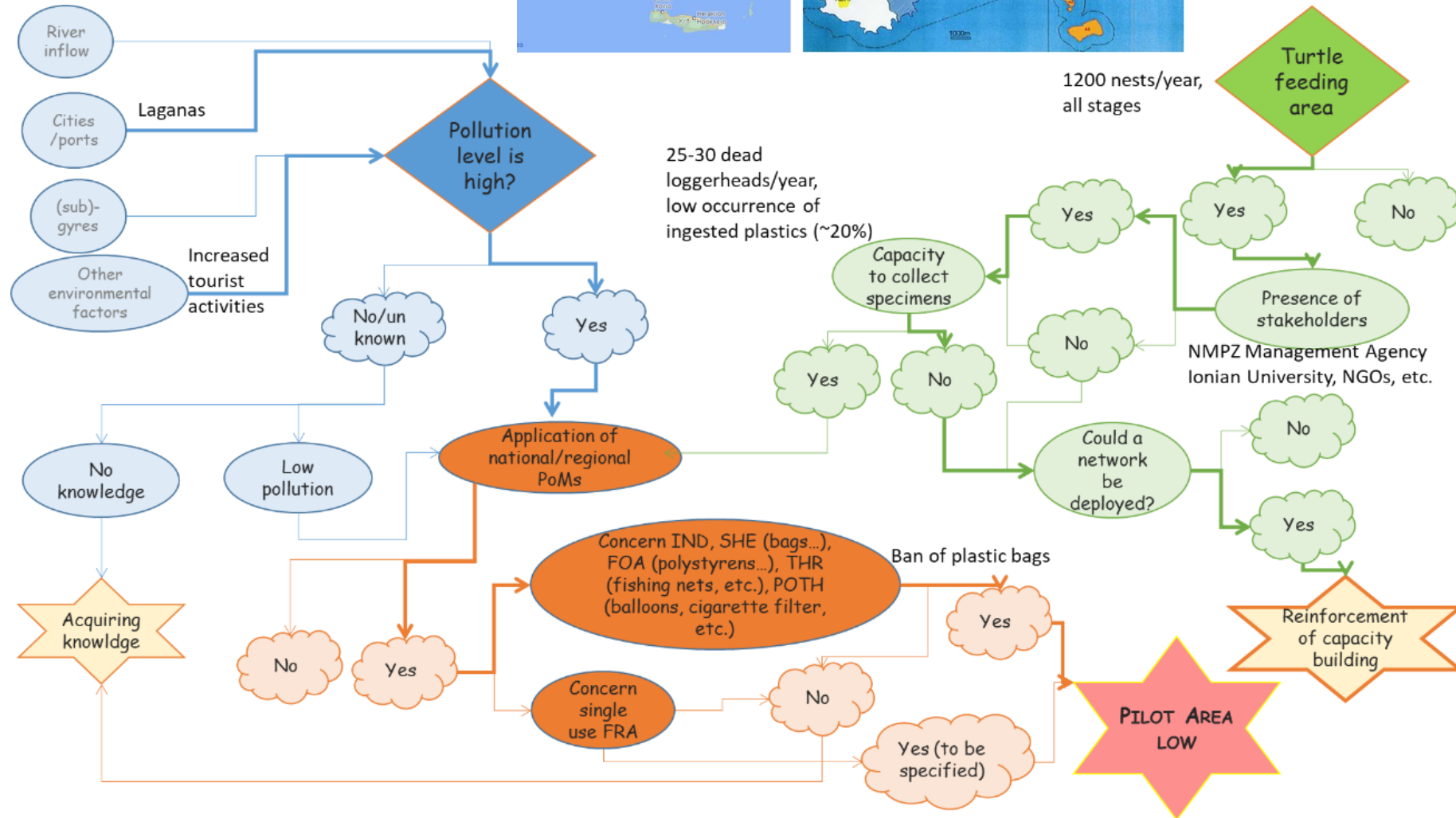


# France, area Corsica

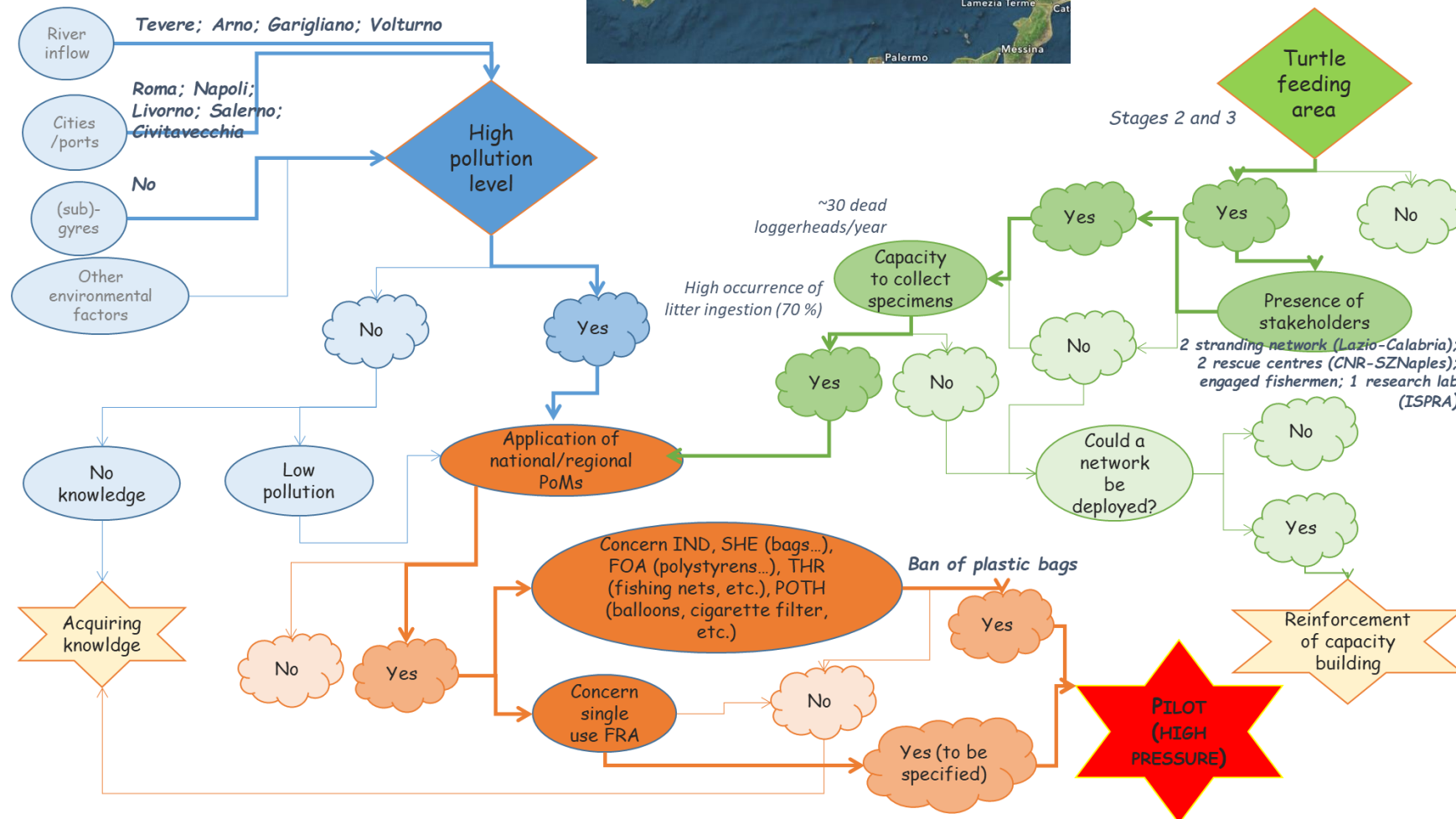




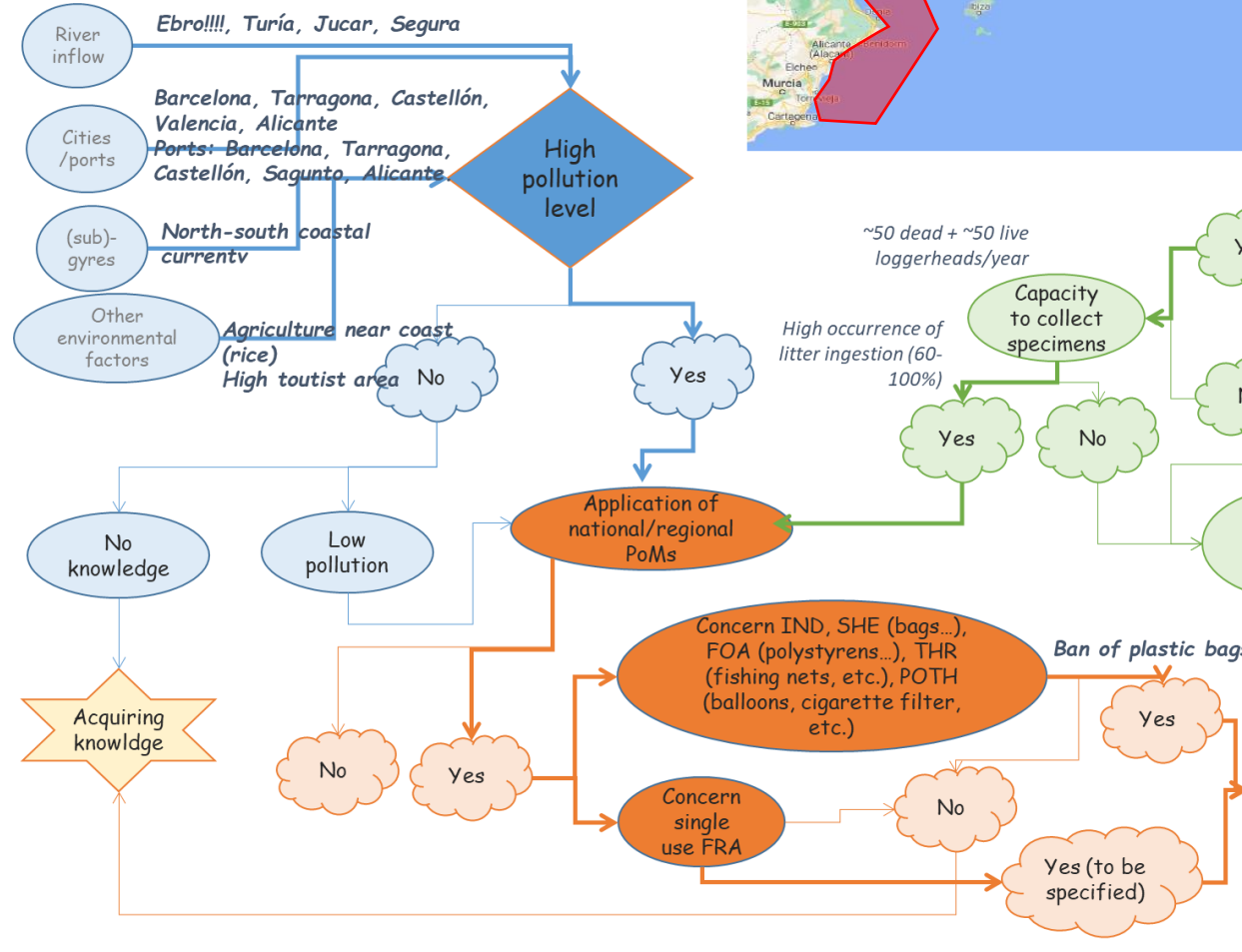
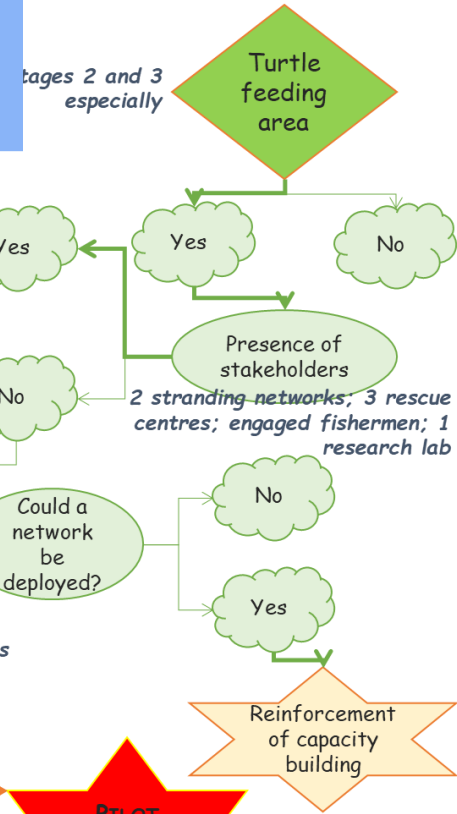
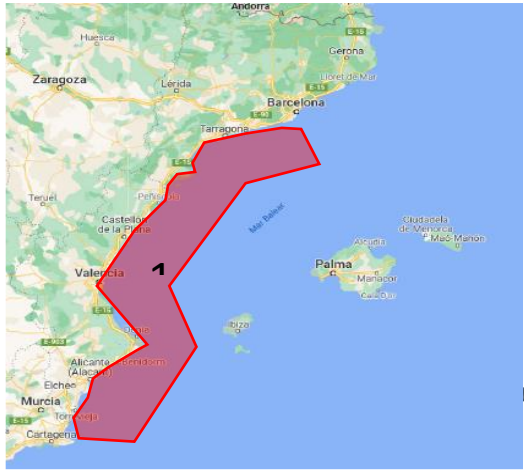
Greece, area National Marine Park of Zakynthos



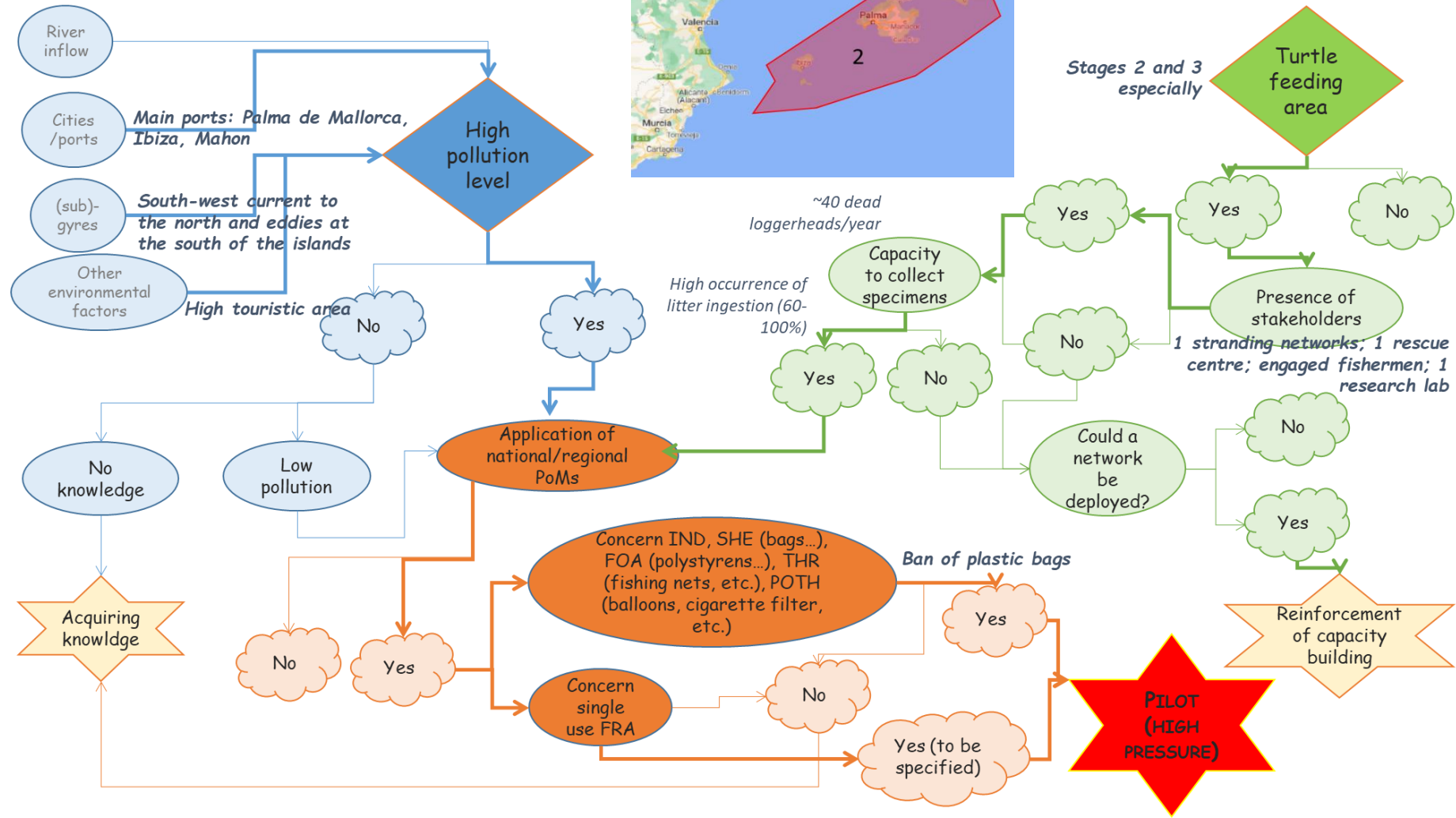
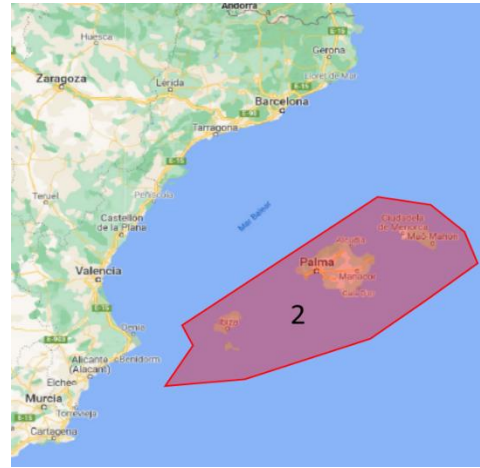
# Italy, Tyrrhenian Sea



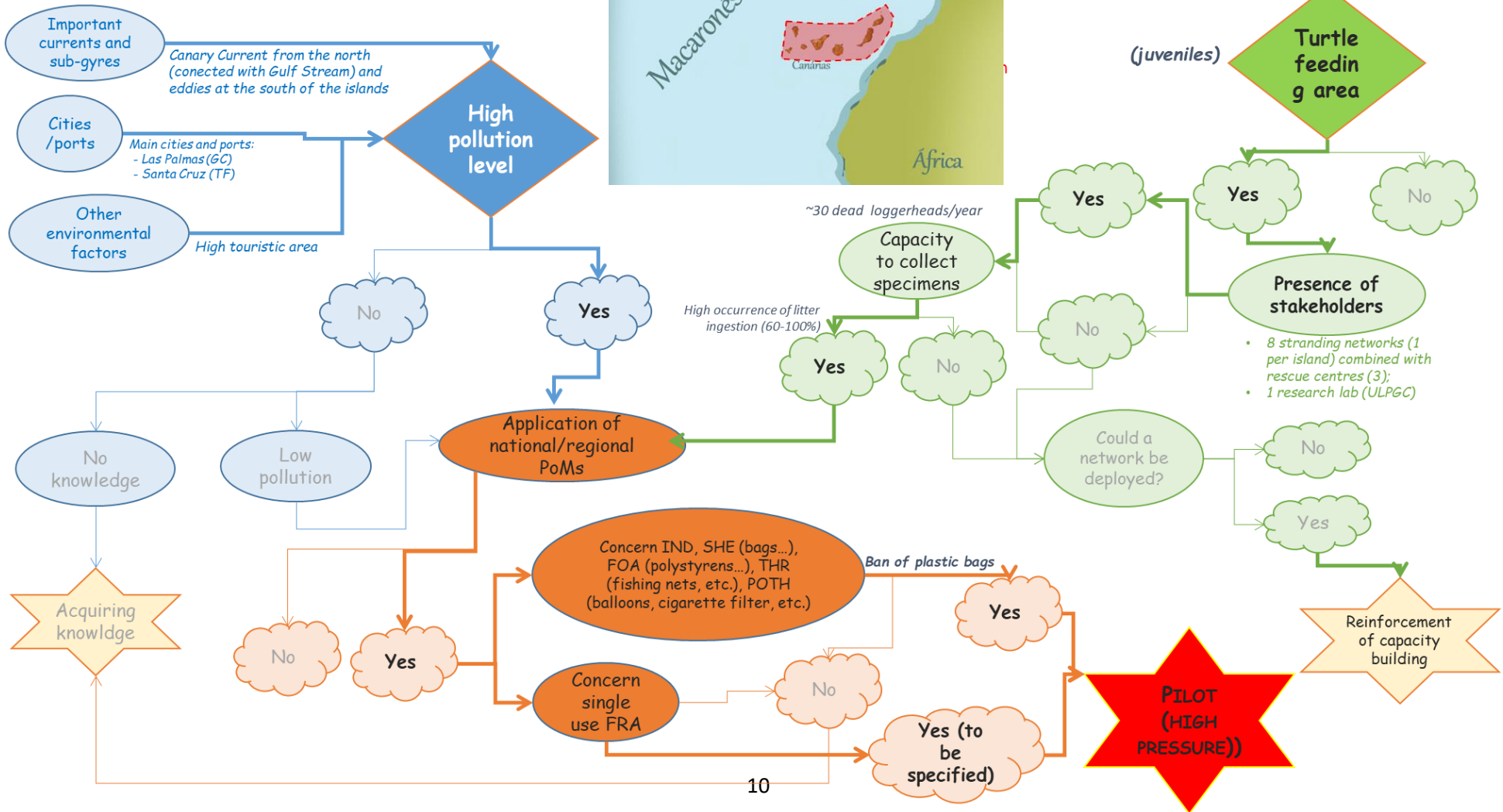
Spain Mediterranean: central area



# Spain Mediterranean: Balearic Islands



# Spain Canary Islands



Appendix 4 – List of the projects with which INDICIT II collaborated in order to share data, information, tools and knowledge

PROJECT	OBJECTIVES	AREA	WEBSITE	CONTACT NAME	Interactions with INDICIT II
<b>Plastic Busters MPAs (2018-2022)</b>	To contribute towards maintaining biodiversity and preserving natural ecosystems in pelagic and coastal MPAs, by defining and implementing a harmonized approach against marine litter.	MED	<a href="http://plasticbusters.unisi.it/">http://plasticbusters.unisi.it/</a>	Cristina Fossi	The interlink with PB MPAs will enable evaluating the impact on more sea turtles and fish individuals and possibly consider a larger panel of toxins. Data on health were exchanged during 2021 in terms of mammals/sea turtles/fish. INDICIT II data will contribute to the “pool” of samples collected in the MED for sea turtles. The protocols of analysis and assessment of the risk of exposure have been finalized and shared. A subcontract with Univ of Siena has been signed and a number of samples of tissues and organs from collected sea turtles were shipped to the University of Siena for the ecotoxicological tests.
<b>LIFE EUROTURTLES (2016-2021)</b>	Mediterranean Sea Turtle Biology & Conservation web site for Science and Education	MED	<a href="https://www.euroturtles.eu/">https://www.euroturtles.eu/</a>	Drasko HOLCER & Paolo Casale paolo.casale@unipi.it, paolo.casale1@gmail.com  lifeeuroturtles@gmail.com	LIFE EUROTURTLES is dedicated to the conservation status of sea turtles. INDICIT II and LIFE EUROTURTLES shared experience and knowledge on the methodologies for assessing turtles’ health and how litter affects body condition. The two projects shared also experience to collaborate with fishermen. INDICIT II has shared guidelines/protocols (pdf and videos) for collecting dead and live specimens, especially with rescue centers, stranding networks and laboratories. LIFE EUROTURTLES can define the needs, especially in terms of materials and training, for the monitoring of litter impacts for MSFD and Barcelona RSCs. LIFE EUROTURTLES can provide contact names for stakeholders to be involved in data sharing. A MoU is signed between the two projects
<b>LIFE MEDTURTLES (2020- )</b>	Collective Actions for Improving the Conservation Status of the EU Sea Turtle Populations	MED	<a href="https://medturtles.eu">https://medturtles.eu</a>	Dr Paolo Casale paolo.casale@unipi.it, paolo.casale1@gmail.com	The MEDTURTLES project, a geographical extension of LIFE EUROTURTLES, aims to improve the conservation status of the EU populations of the Habitats Directive priority sea turtle species <i>Caretta caretta</i> and <i>Chelonia mydas</i> . MEDTURTLES and INDICIT II tried to develop collaborative activities in order to strengthen the knowledge on sea turtles (behavior, population dynamics, etc.) and anthropogenic impacts, focusing on the complementarity of the working networks and the dissemination of standard protocols of collecting information and data. A MoU has been signed by the projects’ coordinators.
<b>MISTIC SEAS (2015-)</b>	Reaching Common Grounds on Monitoring Marine Biodiversity in Macaronesia	Macaronesia	<a href="http://mistic-seas.madeira.gov.pt/">http://mistic-seas.madeira.gov.pt/</a>	Maria LP. Martin	MISTIC SEAS aims to improve the implementation of MSFD in Macaronesia. INDICIT II provided a list of identified knowledge gaps regarding litter impacts (missing areas) and shared tools for dissemination. MISTIC SEAS provided spatial data on litter and fauna to improve INDICIT II knowledge on risky areas and indicator’s constraints. FRC and ULPGC as partners shared

					<p>data on birds, turtles and mammals. The MoU includes the sharing of data (BCI for healthy turtles)</p> <p>A MoU has been signed by the projects' coordinators.</p>
<b>MEDREGION (2019-2021)</b>	To provide the necessary support to the competent authorities of the MSs for the coordinated implementation of the New GES Decision and to provide a useful platform for the necessary Regional and Sub-regional cooperation, in order to support the further development of PoMs.	MED	<a href="http://www.medregion.eu">http://www.medregion.eu</a>	Popi Pagou, HCMR	<p>MEDREGION aims to support the Competent Authorities of the Mediterranean MS to implement the MSFD. Task 6.3 - Link between D1 and D10 through the pressure criteria of entanglement and floating litter (existing data). Cecilia Silvestri from ISPRA is the leader of the subtask for entanglement and so there was an active connection between the two projects. Shared knowledge, protocols and data on entanglement of sea turtles (INDICIT II) and mammals /other taxa (MEDREGION).</p> <p>Common work on D10C4 - Entanglement</p>
<b>QuietMed &amp; QuietMed 2 (2017-2021)</b>	Underwater noise (D11) for the implementation of the Second Cycle of the MSFD in the Mediterranean Sea	MED	<a href="http://www.quietmed-project.eu">www.quietmed-project.eu</a>	Marta Sánchez <a href="mailto:martasanchez@ctnaval.com">martasanchez@ctnaval.com</a> coordinator, developer of geoportal	<p>INDICIT II has been inspired by the work of QuietMed, and discussed the possibility of close collaboration to develop a GIS tool adapted to D10. Since QuietMed 2 could not incorporate data on D10 in the tool developed for D11, INDICIT II developed a map with data on and information on stranded sea turtles and ingested litter and created a tool which can calculate the average abundance of the ingested litter in each Mediterranean sub region and subsequently assess the distance from GES set for the whole Mediterranean basin. This tool is described in detail in D5.8.</p>
<b>COMMON (2019-2022)</b>	COastal Management and Monitoring Network for tackling marine litter in Mediterranean	MED	<a href="http://www.enicbcmed.eu/projects/common">http://www.enicbcmed.eu/projects/common</a>	Sana Ben Ismail <a href="mailto:sana.benismail@instm.rnrt.tn">sana.benismail@instm.rnrt.tn</a>	<p>The COMMON project aims at applying the Integrated Coastal Zone Management (ICZM) principles to the marine litter management, improving the environmental performance of 5 pilot coastal areas in Italy (2), Tunisia (2) and Lebanon (1). The two projects shared information on management measures and marine litter mainly through common workshops (Tunisia, 20th February 2020: INDICIT II and COMMON projects joint forces to monitor Marine Litter impacts on sea turtles and biota, during a meeting at the sea turtle rescue Centre of INSTM MONASTIR).</p>



<b>CleanAtlantic (2017-2020)</b>	Tackling Marine Litter in the Atlantic Area	ATL	<a href="http://www.cleanatlantic.eu/">http://www.cleanatlantic.eu/</a>	Fundación CETMAR	Ongoing work for data banking for OSPAR based on DALI platform with IFREMER France. Clean Atlantic aimed to improve capabilities to monitor, prevent and remove macro marine litter in Atlantic areas. INDICIT II agreed to be included in the marine litter knowledge database. INDICIT II shared also tools for dissemination among Clean Atlantic stakeholders, as well as results for raising awareness of stakeholders targeted by Clean Atlantic project. Clean Atlantic shared the litter distribution maps in the Atlantic (modeling) with INDICIT.
<b>MEDCIS (2017-2019)</b>	Support Mediterranean MS towards coherent and coordinated implementation of the second phase of the MSFD	MED	<a href="http://medcis.eu/">http://medcis.eu/</a>	Popi Pagou, HCMR	Shared methodology for assessing GES
<b>MEDSEALITTER (2017-2019)</b>	Developing Mediterranean-specific protocols to protect biodiversity from litter impact at basin and local MPAs' scales.	MED	<a href="https://medsealitter.interreg-med.eu/">https://medsealitter.interreg-med.eu/</a>	S. Nuglio	Shared the developed protocols for monitoring ingested ML by sea turtles and fish. Shared data on ingestion litter by sea turtles. EPHE, ISPRA and HCMR as partners in MEDSEALITTER interacted with other experts for the implementation of the protocols and for data sharing.
<b>RIVERSE From rivers to seas</b>	Monitoring and fighting plastics from the River to the Sea	Global	<a href="https://hisaproject.org/riverse/">https://hisaproject.org/riverse/</a>	Gaëlle Darmon	Better knowledge of river litter input to sea (80% of wastes come from the land, transported by rivers). Harmonization of Protocols (WFD, MSFD) Impacts on terrestrial and aquatic fauna. Sharing knowledge with INDICIT II. Improved information for pilot areas.
<b>Blue Circular Economy and Circular Ocean</b>	Creation of a global database on entanglement of all taxa and in several languages based on "birdsanddebris" website	Global	<a href="https://bluecirculareconomy.eu/">https://bluecirculareconomy.eu/</a>  <a href="http://www.circularocean.eu/">http://www.circularocean.eu/</a>	Emma Verling <a href="mailto:emma.verling@ucc.ie">emma.verling@ucc.ie</a>	Waiting for a web designer advice and cost estimation for a new web site wildlifeanddebris (all species / all environments) in 7 languages (INDICIT partners)  MoU is signed between the two projects

## Appendix 5 – List of meetings in which INDICIT-II was involved

Meeting	When	Where	Why	Who	Funding
ISPRA meeting ML	14-15/02/2019	Rome	Monitoring Macro-litter	C. Miaud - M. Matiddi	MEDSEALITTER
WG GES	19-20/03/2019	Brussels	Presentation GES - Ingested Litter	C. Miaud - G. Darmon	DG ENV
ML WG	21-22/05/2019	Berlin	Workshops on protocols for D10C3 and D10C4 and methods for assessing thresholds	G. Darmon- M. Matiddi	INDICIT II
MSFD TGML	26-28/6/2019	Gothenburg	Progress in D10C3 development (GES, constraints)	M. Matiddi	Own funding
MSFD TG ML	11-12 June 2020	On-line	Discussion on threshold values and baselines for entanglement and litter ingestion	Marco Matiddi- Cecilia Silvestri – Helen Kaberi	-
21 <sup>st</sup> Meeting of the COP - UN Environment / MAP – Barcelona Convention Secretariat	December 2019	Naples	Presentation of INDICIT II (Gaetano Leone, the Coordinator of the U Mediterranean Action Plan-Barcelona Convention Secretariat, sent a special thanks for the presentation.	M. Matiddi	-
Joint Plastic Busters MPAs Workshop	14 November 2019	Barcelona	Discussion on ecotoxicology experiments	Catherine Tsangaris	own funding
Intermediate meeting of MEDREGION project	January 2020	On-line	Discussion on D10C4 (Entanglement)	Cecilia Silvestri	-
MSFD TG ML	14 December 2020	On-line	Discussion on the impacts/harm of microplastics and the developed protocols by INDICIT regarding the ingestion by sea turtles and fish	Marco Matiddi- Cecilia Silvestri – Eleni Kaberi	-
CorMon Marine Litter Meeting of the Ecosystem Approach Correspondence Group on Marine Litter Monitoring	30 April 2021	On-line	Presentation of the results on GES scenarios for Indicator “Litter ingested by sea turtles”	Claude Miaud - Gaëlle Darmon	-
EU Green Week MED Pollution, Biodiversity and Health	14 June 2021	On-line	Presentation of INDICIT-II results on Impact of Marine Litter on sea turtles	Olfa Chaieb	-
MSFD TG ML	22-23 June 2021	On-line	Discussion on litter ingestion and entanglement. Potential proposal of baseline and threshold based on INDICIT II outputs.	Marco Matiddi- Cecilia Silvestri - Gaelle Darmon	-

## Appendix 6 – Cost evaluations for the implementation of the indicators

Types of cost	Phase 1: Collection of turtle in the field		Phase 2: Reception at the rescue center or place for necropsy		Phase 3: Marine litter collection (necropsy or faeces collection)		Phase 4: Collection and analysis of data (digestive tracks or faeces)		Phase 5: Banking of data
	turtle alive	dead turtle	turtle alive	dead turtle	turtle alive	dead turtle	from carcasses	from faeces	
<b>PERSONAL COST</b>									
Technician cost <sup>1</sup>						x		x	x
Project manager cost <sup>2</sup>	x	x	x	x	x	x	x	x	x
Volunteer's Times <sup>3</sup>	700/vol/year	700/vol/year	300/vol/year	700/vol/year	300/vol/year	NA	NA	300/vol/year	NA
Displacement cost <sup>4</sup>									
Turtle accommodation in the rescue center <sup>5</sup>			x		x		x		x
Turtle storage <sup>6</sup>		x		x		x			
<b>LABORATORY COST</b>									
Facility renting <sup>7</sup>						x	x		
Stove (35°C)						x		x	
Balance (0,01 g)						x		x	
Cold chamber or freezer				x		x			
<b>SMALL MATERIAL</b>									
Rope (to mark-off the zone)	x	x							
Integral protecting suite		x							
Glasses		x							
Protection mask or shield		x							
Disposable gloves		x							
Boots		x							
Mesuring tape		x							
Bottle/zip bags		x							
Transport bins or containers for the turtle	x	x							
Cooler		x							
Garbage bag		x							
Disposable blouse						x			
Cut-resistant gloves						x			
Sliding caliper						x			
Clamps (at least 6)						x			
sieve 1 mm							x	x	
sieve 5 mm							x	x	
Scissors							x	x	
Clips with claws							x		
Metal containers							x	x	
Dip net mesh 1 mm							x	x	
Graduated test tube (10, 25, 50 ml)							x		

1 times as technician in the facility (research lab, vet lab)

2 times as project manager, i.e. in charge of the stranding network and/or rescue centre

3 times as cost of volunteer investment

4 this is the mean cost of going to the stranding place (dead turtle) or e.g. pier (live turtle) in e.g. vehicle consumption

5 mean cost of maintaining turtle in the rescue centre (functioning cost of the rescue centre, food, etc...)

6 mean cost for storing dead turtle

7 mean cost for renting the facility (e.g. vet lab for necropsy)

## **FRANCE**

### **Rescue Centre**

Rearing and care to injured turtles: Mainly funding from local (city) and regional agencies

Ingested litter: collection of faeces: funding by sub-contracting with SHF (see below)

### **Standing network:**

Collection of stranded (dead) turtles: funding by sub-contracting with SHF (80 % of full-time position, see below)

### **Veterinarian lab:**

Necropsy, collection of the digestive track and measures (mass, volume, etc.): funding by sub-contracting with SHF

### **Research centre (Academic):**

Analysis of litter collected in the digestive track (type, mass, etc.): funding by sub-contracting with SHF

Analysis of litter collected in faeces (type, mass, etc.): funding by sub-contracting with SHF

**SOURCE of FUNDING:** Contract between the Minister of Ecological Transition (French Agency for Biodiversity) in charge of the implementation of the MSFD and the NGO “French Herpetological Society” in charge of knowledge, protection and education about the French herpetofauna.

Contract duration: 2019-2022

### **Funding:**

Contract with SHF: 80 % of full-time position (management of the stranded network in the Med façade and Corsica)

Sub-contract SHF-rescue centre: 3000 €/year (2 rescue centre in continental France and 1 in Corsica)

Sub-contract SHF-Veterinarian lab: 1600€/year

Sub-contract SHF-Research centre: 3000 €/year

## **Cyprus/UK (UNEXE)**

### **Stranding network and lab:**

Collection of stranded (dead & alive) turtles, necropsy, collection of the digestive track and measures (mass, volume, etc.), veterinary collaboration: SPOT Society for the Protection of Turtles (SPOT) & University of Exeter. With support of MAVA SPOT has expanded its network and increased its capacity. SPOT is looking to include more participatory fishers and public engagement; however funding mechanisms need to be considered for this.

### **Research centre (Academic):**

Analysis of litter collected in the digestive track (type, mass, etc.): University of Exeter

**SOURCE of FUNDING:** Stranding work will be funded by SPOT for a baseline but additional funds are needed for a long-term maintenance and expansion. There is no further specific funding after INDICIT II.

## **PORTUGAL (AZORES)**

### **Rescue Centre**

Rearing and care to injured turtles: Mainly funding from private and regional agencies

Ingested litter: collection of faeces: funding by sub-contracting with SHF (see below)

**Standing network:**

Collection of stranded (dead) turtles: funding by regional government

**Veterinarian lab:** none

**Research centre (Academic):**

Necropsies: no continuous funding, funded by research projects

Analysis of litter collected in the digestive track (type, mass, etc.): no continuous funding, funded by research projects

Analysis of litter collected in faeces (type, mass, etc.): no continuous funding, funded by research projects

**SOURCE of FUNDING:** national and international research projects mainly for human resources and consumables

## GREECE

In case the assessment of D10C3 indicator is included in the National MSFD Monitoring Program, the HCMR will be funded by the Ministry of the Environment (General Secretariat for the Natural Environment – GSNE) under contract.

**Rescue Centre**

Breeding and care of injured turtles: Main funding from the Ministry of the Environment (General Secretariat for the Natural Environment – GSNE)

Ingested litter: collection of faeces: funding from HCMR under sub-contract

**Standing networks**

Collection of stranded (dead) turtles: funding from HCMR under sub-contract

**Veterinarian labs**

Necropsy, collection of the digestive tract and measurements (mass, volume, etc.): funding from HCMR under sub-contract

**Research centre (HCMR):**

Analysis of litter collected in the digestive tract (type, mass, etc.): funding from the GSNE under contract

Analysis of litter collected in faeces (type, mass, etc.): funding from the GSNE under contract

**SOURCE of FUNDING:** Contract between the HCMR and the General Secretariat for the Natural Environment – GSNE (Greek Agency in charge of the implementation of the MSFD).

**Funding:**

Contract between the HCMR and the GSNE: 100 % of full-time position (management of the stranded networks, analysis of the collected digestive tracts of the dead sea turtles)

Sub-contract between HCMR and the rescue centre: depending on the number of the collected sea turtles

Sub-contract between HCMR and the Veterinarian labs: depending on the number of the necropsied sea turtles

## **SPAIN (MEDITERRANEAN SEA)**

### **Rescue Centres**

Rearing and care to injured turtles and turtles by-caught by fishermen and brought to ports: 2 funded by regional governments and 3 of private companies but in agreement and with economical support of regional governments.

Ingested litter: collection of faeces:

### **Standing network:**

Collection of stranded (dead) turtles and turtles dead in rescue centres:

Valencia region: Coordinated by the University of Valencia (with their own means) and with the support of the regional government and the collaboration of the Valencia rescue centre at Oceanogràfic.

Other regions: network of collaboration with stranding networks in other regions coordinated by rescue centres: Catalonia: CRAM; Balearic Islands: Palma Aquarium; Murcia: Centro recuperación El Valle (public-regional government), and Almería province, Andalusia: Equinac.

Regional networks provide data and samples from faeces to the UVEG team.

### **Veterinarian lab:**

Necropsy, collection of the digestive tract and measures (mass, volume, etc.): at the University of Valencia (UVEG).

### **Research centre (Academic):**

Analysis of litter collected in the digestive tract (type, mass, etc.): at the University of Valencia (UVEG).

Analysis of litter collected in faeces (type, mass, etc.): at the University of Valencia (UVEG).

**SOURCE of FUNDING:** Contract with the regional government of the Valencia region (area of biodiversity) and UVEG for the coordination of the stranding network.

Contract duration: Renewed annually.

*To be cited as:*

Darmon G., Andrea de Lucia G., Bains M., Bradai M.N., Bray L., Broderick A., Camedda A., Chaieb O., Coppa S., Ducommun L., Duncan E., El Hili H. A., Fossi M.C., Fraija N., Godley B., Gómez M., Jenot B., Kaberi H., Kaska Y., Lagoa J., Loza A.L., Martin J., Martínez-Gil Pardo de Vera M., Massaro G., Matiddi M., Montero Vitores D., Nelms S., Novillo O., Ostiategui P., Panti C., Paramio Martin M.L., Pham C., Piermarini R., Revuelta O., Rodríguez Y., Silvestri C., Sozbilen D., Tomás J., Tsangaris C., Uribarri A.H., Vale M., Vandeperre F., Vencato S. and Miaud C., 2022 - Implementation of the indicator “Impacts of marine litter on sea turtles and biota” in RSC and MSFD areas. Final report of the INDICIT-II project, Directorate-Generale for the Environment, European Union, 170 p. + appendixes.