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# **PILOT AND FEASIBILITY STUDIES** FOR THE IMPLEMENTATION OF **LITTER IMPACT INDICATORS** IN THE MSFD AND RSCs OSPAR-MACARONESIA, HELCOM AND BARCELONA

*Indicator "Litter ingestion by sea turtles"*
*Indicator "Entanglement of biota with marine debris"*
*Indicator "Micro-plastic ingestion by fish and sea turtles"*

INDICIT deliverable n° D.2.5 of Activity 2

By INDICIT consortium

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**Under the coordination of CNRS (C. Miaud, G. Darmon) February 2018**

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## Foreword

This report is part of the work of the INDICIT consortium funded by the European Union (agreement n° 11.0661/2016/748064/SUB/ENV.C2; https://indicit-europa.eu/). INDICIT (“*Implementation of the indicator of marine litter on sea turtles and biota in Regional Sea Conventions and Marine Strategy Framework Directive Areas*”) is a two-years project (February 2017-January 2019) which overarching aim is to develop a set of standardized tools for monitoring the impacts of litter on marine fauna as bio-indicators in order to support the implementation of EU’s Marine Strategy Framework Directive (MSFD) and other international environmental policies aiming at protecting the marine environment, especially the Barcelona and the OSPAR Regional Sea Conventions. This program is ensured by a consortium composed of biologists from 7 countries (France, Greece, Italy, Portugal, Spain, Tunisia and Turkey) in interaction with experts from other international programs. In order to get the results operational for the long term monitoring of marine litter impacts, INDICIT is advised by its Policy Officer and an External Advisory Board, which includes 14 members, being representatives of the RSCs and Member States’ national administrations and scientific experts in marine litter.

The present report is included in the Activity 2 “*Acquiring and using scientific knowledge to develop the indicators of litter impact at the (sub)regional and the whole MSFD spatial scale*”. CNRS (C. Miaud, G. Darmon), who coordinates this Activity, took charge of the coordination of the 3 studies, each being presented in a separate report. The first report, led by CNRS (G. Darmon, C. Miaud) concerns the “**Litter ingested by sea turtle**”. The second report, led by MNHN (F. Claro) concerns the “**Entanglement with marine litter by biota**”. The third report concerns the “**Micro-plastic ingestion by fish and sea turtles**”, which was led by ISPRA (C. Silvestri, M. Matiddi) for fish and ULPGC (A. Liria Loza) for sea turtles. All INDICIT partners were involved to provide data and comments and improve these reports. The EAB and the PO provided feedback and advice to better fall within an operational implementation of the monitoring of marine litter impacts.

The reports aim to provide a situational analysis for each of the 3 indicators, at the beginning of the program. To this end, a literature review was performed on a global scale (Entanglement and Micro-plastic ingestion indicators) or more specifically in the areas targeted by INDICIT (indicator Litter ingested by sea turtles). The possible available data were also considered in order to assess the indicators’ criteria and better identify the lack of knowledge that must be filled for the development of the indicators. An inventory of the existing methods for the collect of data and of the available networks has also been made.

# General introduction

Plastic items compose more than 80% of the litter observed offshore and on beaches. They are omnipresent and accumulate for years to centuries in the seas. More than six hundred species (invertebrates, cetaceans, fish, sea birds, sea turtles) are known to be impacted, especially by ingestion and entanglement. Anthropogenic debris induce direct mortality or indirect effects related to a decrease in individuals' reproduction and chance of survival because of difficulties to e.g. feed, move or escape predators. The issue is certainly larger, with more impacted species than being currently observed, and with related risks of cascading effects that may jeopardize the sustainability of the marine ecosystems.

In light of the abundance and impacts of anthropogenic litter in the marine environment, several directives have been implemented at the regional, European or international levels. The Marine Strategy Framework Directive aims at achieving the Good Environmental Status (GES) of the EU's marine waters by 2020. For its Descriptor 10 ("Marine Litter"), GES will be achieved when the "*properties and quantities of marine litter do not cause harm to the coastal and marine environment*". Marine litter is here defined as the "*items that have been made or used by humans, deliberately discarded into the sea or on beaches, brought indirectly to the sea with rivers, sewage, storm water or winds or accidentally lost at sea notably in bad weather*". As for the other 10 Descriptors of environmental health, the Descriptor 10 is declined in a set of detailed indicators and criteria to support the Member States in monitoring the pollution by marine litter. As a measure of the progress in the achievement of GES, these indicators allow to evaluate the efficiency of restoration measures.

The Descriptor 10 is composed of two types of indicators aiming to measure the abundance and distribution of marine litter and their impacts on the environment and the fauna. The indicator 10DC3 (formerly D10.2.1) concerns the litter ingested by sea turtles. To appreciate the environmental state, indicators must be quantifiable and accurate, and clearly defined according to criteria (or conditions of use). For an operational monitoring on the long term, samples and data must be easily collected. The protocols must be standardized so that a homogenised approach can be implemented by large monitoring networks, thus allowing to assess temporal trends, regional differences and compliance with a set target for acceptable ecological quality. Common standard protocols must thus be used at the entire area including the whole MSFD and RSCs (Figure 1).

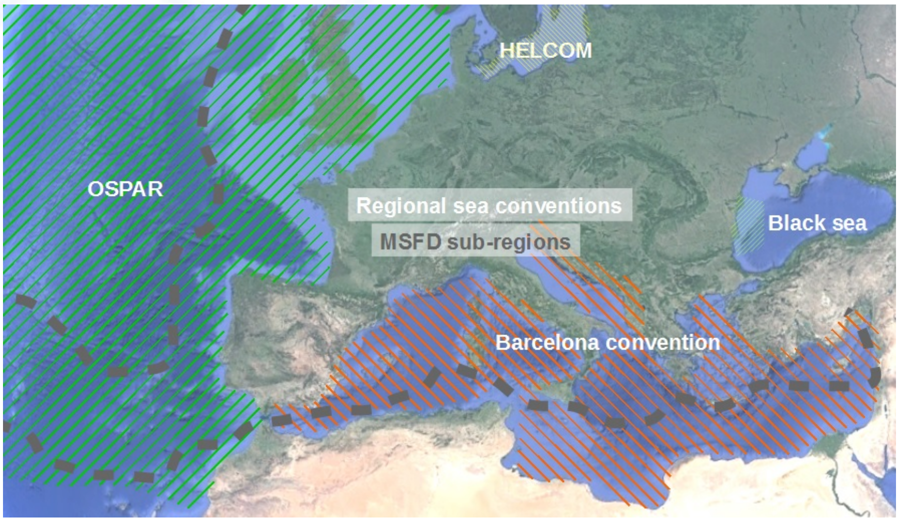


**Figure 1**. Area considered by the INDICIT program

Marine taxa such as sea turtles have a wide geographic range (Atlantic and Mediterranean), use different habitat compartments (neritic to pelagic) and are known to ingest marine debris. While the litter ingested by fulmar *Fulmarus glacialis* is currently used within the OSPAR RSC, this indicator cannot be used beyond the North Sea area since the species is absent in other areas. Given their propensity to ingest debris, their wide distribution and the large range of habitats used during their life, sea turtles, in particular the loggerhead species *Caretta caretta*, were proposed as possible indicator to cover a wider area. As a consequence, the MSFD Task Group on Marine Litter proposed "Litter ingested by sea turtles" as an impact indicator for D10. However, the initial assessment of GES (threshold below which the GES is supposed to be reached or temporal trend to achieve the GES) and the methodological standard procedures for the use of this indicator yet need to be implemented. The Commission Decision (2010/477/EU) expresses the need for further development based on emerging knowledge and regional experience. Therefore, for the Indicator 10DC3, the primary task for the implementation of appropriate monitoring programmes for marine litter impacts, is to develop tools to be used for investigating trends in the amount and composition of litter ingested by marine animals that should cover all the MSFD marine regions: define GES, criteria (i.e. biological constraints (e.g., which individuals to consider), spatial and temporal units, sample size) and develop networks trained for the collection of standard and exploitable data. Moreover, other impacts beside the ingestion of litter, must still be considered, as the entanglement of marine organisms in litter (indicator 10DC4 of the revised Commission Decision). Considering the specific impact of micro-litter (defined as the particles inferior to 5 mm" in the new Commission Decision (2017/848)), that are known to be ubiquitous but for which the considerable engendered damages are just starting to be assessed, appears also crucial. The relevance and feasibility of the implementation of two new indicators targeting the specific impacts of litter related to entanglement and ingestion of micro-plastics have to be evaluated.

The present study aims at compiling the existing knowledge and available human and logistical means in order to (1) develop and implement the indicator "Litter ingested by sea turtle", and (2) assess the relevance of two new indicators "Entanglement with debris by marine biota" and "Micro-debris ingested by marine biota". For each indicator, a literature review, compiled with data/expert knowledge, are used. For "Entanglement with debris by marine biota", the literature review considers all the taxa relevant at the MSFD and RSC scales. For "Micro-debris ingested by marine biota", the study targets especially sea turtles, since the monitoring could be performed by the similar networks than for "Litter ingested by sea turtles". Fish is also considered in order to evaluate if the collection of samples and data could be easier and cover a wider area. As defined in the MSFD's Guidance on Monitoring of Marine Litter in European Seas[1], we considered all items superior to 1 mm, without differentiation between micro-litter (i.e. items of size ≤5 mm) and macro-litter in order to be in line with the Fulmar indicator. The differentiation of micro (≤5 mm), meso (5-25 mm) and macro- litter (≥25mm) is proposed as optional parameters. To study micro-litter, we specifically target the items of size between 1 and 5 mm for sea turtles, since they can be easily recognizable and collected by the networks. The 3 pilot studies will consider the existing or necessary networks and the logistical means for the implementation of each of the three indicators, analyse the available literature and raw data and for "Entanglement with debris by marine biota", expert knowledge was elicited from surveys.

The first report is used as a basis for the definition of GES and criteria of the indicator Litter ingested by sea turtles, the main objective of the INDICIT program. In parallel of the standard data collected for this indicator, optional data are proposed to be collected on sea turtles by our networks, on: 1) the ingested debris, classified by size: micro-debris (1-5mm), meso-debris (5-25mm) and macro-debris (>25mm), and 2) the litter causing entanglement. An updated version of the reports 2 and 3 will be proposed by the end of the INDICIT program to integrate the results obtained from these optional data.

[1] Hanke, G., Galgani, F., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R., Van Franeker, J.A., Vlachogianni, T., Palatinus, A., Scoullos, M., Veiga, J.M., Matiddi, M., Alcaro, L., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J., Liebezeit, G., 2013. Guidance on Monitoring of Marine Litter in European Seas. European Commission. Available online: http://hdl.handle.net/10508/1649.

**Table 1:** Target species and area, litter size and type of impact considered in the state-of-art report for the indicator Litter ingested by sea turtles, and in the pilot studies the indicator for Entanglement with debris by marine biota and the indicator Micro-plastics ingested by fish and sea turtles

| Indicator (report) | Species | Litter size | Impacts | Region |
|---|---|---|---|---|
| Litter ingestion by sea turtles (1) | Sea turtles (loggerhead, leatherback) | > 1 mm | Ingestion | MSFD, OSPAR RSC, Barcelona RSC |
| Entanglement with marine debris (2) | All taxa | Size limits (as well as litter types) has to be defined | Entanglement | Global, with specific evaluation in MSFD, OSPAR and Barcelona RSCs |
| Micro-plastic ingestion (3) | Sea turtles | 1-5 mm | Ingestion | |
| | Fish | ≤ 5 mm | | |

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# GENERAL INDEX

# PILOT STUDY FOR THE IMPLEMENTATION OF INDICATOR **"LITTER INGESTED BY SEA TURTLES"** IN THE AREAS OF THE MARINE STRATEGY FRAMEWORK DIRECTIVE AND THE REGIONAL SEA CONVENTIONS **OSPAR AND BARCELONA**


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INDICIT
INDICATOR • IMPACT • TURTLE

# INDEX

# I - CONTEXT

This report is a deliverable of INDICIT Activity 2 ("Acquiring and using scientific knowledge to develop the indicators of litter impact at the (sub)regional and the whole MSFD spatial scales"). Its objective is to assess the available knowledge and networks for the implementation of the indicator "Litter ingested by sea turtles" in the areas covered by the Marine Strategy Framework Directives and the Regional Sea Conventions OSPAR and Barcelona. While the relevance of this indicator has been validated, further development is needed, in particular to provide an initial assessment of the indicator Good Environmental Status and criteria, that are the possible biological constraints (e.g., individual size), the spatial and temporal units and the sample size. This examination of the situation is made from a literature review and a descriptive analysis of data, when available, targeting especially the INDICIT target areas in order to consider the possible specific environmental, species and logistic constraints of this area compared to the global situation. The literature at the global scale will be considered to better understand the biological factors which may influence litter ingestion in sea turtles and thus the identified indicator criteria.

This report is coordinated by CNRS, supported by the INDICIT partners who provide published and grey literature, gathered on the INDICIT private area, as well as comments to improve the report. The External Advisory Board also provided feedback by email and during the INDICIT 3rd meeting held in Montpellier in February 2018. I. "Litter ingested by sea turtles", a relevant indicator of marine litter impact

The Commission Decision 2010/477/EU and the New Commission Decision 2017/848/EU proposed for the Descriptor 10 ("Marine litter") of the Marine Strategy Framework Directive (MSFD), the Indicator "Trends in the amount and composition of litter ingested by marine animals". Galgani et al. (2010) suggested that pilot studies for bio-monitoring should consider marine turtles due to their propensity to ingest litter, particularly in the Mediterranean Sea. In 2011, Matiddi et al. proposed to verify the use of the loggerhead turtle *Caretta caretta* (Linnaeus 1758) as a potential indicator species for monitoring marine litter ingestion in the Mediterranean Sea, alike the *Fulmarus glacialis* (Linnaeus, 1761) in the Northern European waters for the OSPAR Regional Sea Convention (RSC) (van Franeker & Meijdoom, 2002). After a pilot study conducted in 2012 by an Italian team of ISPRA, evaluating the methods and the potential sources of bias of such an indicator, the expert researchers of the Technical Subgroup on Marine Litter (TSG-ML) established by the DG ENV of the European Commission, proposed the loggerhead turtle among the target indicators for litter ingestion by biota (Galgani et al., 2013).

Several criteria actually gave this species the potential to be a relevant indicator of marine litter impacts:

- Its opportunistic feeding behaviour, making the species prone to ingest debris items (Dell'Amico & Gambaiani, 2013; Camedda et al., 2014; Schuyler et al., 2014);

- Its use of several marine horizontal and vertical habitats during its life history, from oceanic to neritic areas (Casale et al., 2008), at the surface and the first tens of meters of the upper water column;

- Its large spatial distribution including the OSPAR/Macaronesia RSC, the Barcelona Convention, as well as the whole MSFD areas (Witt et al., 2007);

- The ability for rescue, stranding and authorized research networks to collect individuals/samples.

The MSFD GES Technical Group on Marine Litter recommends to formulate a Good Environmental Status (GES) for the MSFD (Claro et al., 2014; Matiddi et al., 2015; 2017), based on the number and the amount of debris items (≥ 1 mm) found in the dead sea turtles' digestive tract, thanks to a standardized protocol (Galgani et al., 2013). The proposed operational objective for the indicator "Litter ingested by sea turtles" of ECAP (EI 18) in the Regional Action Plan for the Mediterranean Sea on marine litter, is a significant decrease of the number of individuals having ingested litter as well as the weight of their ingested litter. The maximum threshold proposed was of 40-60% occurrence of litter ingestion among the necropsied loggerhead turtles with a maximum of 1 to 3 g of ingested debris items, at the scale of the whole Mediterranean basin (UNEP, 2015). Nonetheless, later, the Commission Decision for MSFD expressed the need for further development and knowledge, in the light of a concrete implementation of the indicator. Indeed, there are still several gaps for the definition of a GES (threshold or trend) and the application criteria of the indicator (Claro et al., 2014). The GES should correspond to the target parameters to be controlled for a significant decrease, e.g., the percentage of individuals having ingested litter and the quantity of debris items (e.g., in term of dry mass) found in the dead turtles' digestive tract or in the alive turtles' faeces (Galgani et al., 2013; Camedda et al., 2014). The indicator criteria correspond to:

- the biological constraints, which are the factors that can affect the probability to ingest litter (e.g., the development stage of the individual; Schuyler et al. 2015);

- the spatial scale of use, in which no significant difference in GES must be detectable;

- the temporal scale of use, which is the temporal window at which a significant trend in GES can be detectable (e.g, 5 years for the OSPAR indicator based on the litter found in the fulmar *Fulmar glacialis* (Linnaeus, 1761) digestive tract; Van Franeker & Meijdoom, 2002);

- the sample size, which is the minimum number of individuals which should be considered to detect significant spatio-temporal trends in GES.

Due to its abundance in the Atlantic area (Duguy et al., 2002) and also its propensity to ingest debris (Schuyler et al., 2014; Dell'Amico & Gambaiani, 2013), the relevance of the leatherback turtle *Dermochelys coriacea* as an indicator, in addition the loggerhead, was also proposed to be assessed (Claro et al, 2014).

In order to support the European Commission for the implementation of the indicator "Litter ingested by sea turtles" in the OSPAR, the Barcelona RSCs and MSFD areas, the INDICIT consortium (INDICator Impact Turtle, project n° 11.0661/2016/748064/SUB/ENV.C2, 2017-2019, https://indicit-europa.eu/) aims at developing of a large network trained to collect standardized data based on a harmonized approach, and to assess the indicator GES and criteria.

As a starting point, this report aims at compiling the existing knowledge at the beginning of the project and identifying the available logistical means for the implementation of the indicator on litter ingested by sea turtles, specifically *Caretta caretta* and *Dermochelys coriacea*, on the OSPAR, the Barcelona convention and the MSFD areas. In order to determine the indicator GES and criteria, the first objective is to compile the peer-reviewed and grey literature on: i) the frequency of individuals having ingested litter; ii) the quantities of litter ingested by turtles; iii) the biological and environmental factors which may affect the probability of litter ingestion, as well as iv) the spatial and temporal variations in the frequency of occurrence and the ingested quantities. In order to assess the possibility of the implementation of this indicator, the second goal of this report is to evaluate the existing network involved in the collection of dead and/or alive individuals in each INDICIT partners' country.

# II – LITERATURE REVIEW

## 1. Method

All partners participated in the collection of literature (grey and published), under the coordination of CNRS, who prepared the report with the collaboration of ULPGC. The report was reviewed by the partners and will be submitted to the External Advisory Board at the next INDICIT meeting (February 2018).

The review consisted in investigating the literature published in peer-reviewed journals and in popular science magazines, books, as well as the literature not found in the official scientific channels, such as technical and expertise reports, theses, posters, slides of oral presentations, minutes of meetings, conference proceedings and practical feedback and expertise collected from interviewed people (e.g., biologists from rescue centres). Internet tools (e.g., Google, Google Scholar, ISI Web of Knowledge) were used to explore search strings with keywords including “plastic” OR “litter” OR “marine debris” AND “sea turtle” OR “loggerhead” OR “leatherback” AND “ingestion” OR “ingest” OR “digestive”. The objective of this study being to identify the factors to consider for a concrete implementation of the indicator, we selected the references among the large amount of literature on the topic at the worldwide scale, according to the areas on which the INDICIT program is committed to and where sea turtles are present (MSFD, Barcelona and OSPAR RSCs). If relevant, the INDICIT partners’ local networks were also contacted. Documents were searched firstly by using the English language, secondly by completing with the INDICIT partners’ respective country official language (Arab, French, Greek, Italian, Portuguese, Spanish and Turkish).

Following the JRC (2013) definition, we considered both macro (≥ 5mm) and micro (1-5 mm) debris. The literature found was then compiled in order to assess the indicator criteria, as the parameters necessary to establish the GES:

- the percentage of individuals having ingested litter corresponding to the frequency of occurrence,

- the weight, volume, number of items and other parameters relative to the amount of debris among the individuals having ingested debris.

A synthesis on the possible biological constraints was performed, based, if possible, on descriptive statistical analyses. We searched the influence of the factor which may lead to stratify the samples:

- the developmental stage, determined by the turtle size (Campani et al. 2013; Casale et al. 2008; Schuyler et al. 2012, 2014), which may influence the individual’s behaviour (e.g., spatial distribution, diet and ability of prey discrimination) and thus the probability of ingesting litter;

- the individual sex, to verify if females and males are as likely to ingest litter (a hypothesis being that females could compensate for the energetic costs of vitellogenesis and egg-laying by eating more and thus be more prone than males to ingest litter);

- the individuals’ area of birth, which may influence their spatial distribution and feeding areas, those of the South-Western Mediterranean basin being more probably juveniles originating from the Atlantic (Clusa et al, 2014);

- the health status and mortality condition, which could have impacted the satiety and the feeding behaviour.

We also examined the reported sample sizes, the time term trends of litter ingestion by sea turtles and the type of record (i.e., stranding versus capture, and type of fishing gear). The results were compared according to the protocol, considering either dead or alive individuals (Galgani et al., 2013), and considering for the latter, the digestive transit duration, e.g., as evaluated from the procedure of the MSFD guidelines (Galgani et al., 2013).

## 2. Results found in the literature review for marine litter ingested by the loggerhead and the leatherback turtles in the Barcelona and OSPAR RSCs and the MSFD areas

The review includes literature until July 2017.

### a) The loggerhead turtle

#### *i. Description and sorting of the literature identified*

Sixty-three reports on litter ingestion by the loggerhead turtles in the Barcelona Convention and the OSPAR RSCs as well as the entire MSFD area were found in 59 references reviewed (Table 1, Appendix 1). The studies were performed in 3 countries in the Atlantic area (France, Portugal and Spain) and 10 in the Mediterranean area (Croatia-Slovenia, France, Greece, Italy, Spain, Malta, Morocco, Tunisia and Turkey).

**Table 1:** Number of records related to litter ingested by the loggerhead turtles (occurrence and/or ingested quantities) in the Barcelona and OSPAR RSCs and in the MSFD areas (details in Appendix 1).

| Type of reference | Atlantic area | Mediterranean area |
|---|---|---|
| Reports or abstracts of report | 2 | 4 |
| Conferences (slideshow or proceedings of conferences or workshops) | 8 | 15 |
| Unpublished data | 2 | 3 |
| Peer-reviewed articles | 11 | 12 |
| Non-peer reviewed articles | 4 | 0 |
| Books or book chapters | 1 | 0 |
| Posters | 0 | 1 |
| *Total* | 28 | 35 |

All available documents dealt with 1 to 906 loggerheads (12 studies considering both species) (Calabuig et al., 2004). Individuals' curved carapace length (CCL) varied from 9.3 to 155 cm (method of measure basely specified). The oldest mention to litter ingestion in a loggerhead turtle was in 1967 in the Madeira Islands, Portugal (Brongserma, 1968, 1969 in Balazs, 1985). The ingested litter was mostly described from necropsies (43 records, Appendix 1), less often by systematic observations of the rescued individuals' excretions by oral cavity or faeces (14 records), and rarely from other types of observation (e.g., 1 observation at sea). The two protocols, using both necropsies of dead individuals and observation of alive individuals, were rarely compared (but see Camedda et al., 2014; Casale, 2016; Darmon & Miaud, 2016). The use of a systematic protocol, such as the one proposed in the MSFD guidelines (Galgani et al., 2013), was quite rare and fairly recent, thus providing rough estimations on the prevalence of litter ingestion in sea turtles at the global level but accurate at the local level (Camedda et al., 2014; Nicolau et al., 2016; Matiddi et al., 2017).

Since almost no raw data was available to perform statistical analyses, only averages (i.e. grand means, calculated from the means of the means provided by the prospected literature), with standard deviations (thereafter noted "sd", which provide an overview of data spread), were calculated. The studies concerning only 1 individual, those for which the procedure employed to assess litter ingestion was not specified, and those whose data were probably included in other references, were removed from our analyses. In order to describe the ingested litter, we classified the debris items according to the MSFD guidelines (Galgani et al., 2013; Appendix 1). The class "TAR" (oil, tar as well as other pollutants) was not considered as litter. The ingestion of only "HOO" (hook), with or without "THR" (threadlike materials, e.g. fishing line), with no other plastic or rubbish items, was considered as active fishing and excluded from the analyses. After the removal of such records, 32 records remained, including 5 in the Atlantic and 25 in the Mediterranean, which respectively 4 and 3 used the MSFD protocol.

### *ii. Occurrence and spatiotemporal variation in litter ingestion*

- **Averages**

The frequency of occurrence of litter ingestion was considered as the number of individuals having ingested litter among all individuals having been tested. The frequency presented above may be badly estimated: On one hand, most of the identified publications did not use a standard protocol such as the MSFD protocol (Galgani et al., 2013) and did not specify if the necropsies were performed systematically on all the individuals found dead. On the other hand, necropsies were sometimes impossible because of the decomposition level of the individuals, which can under-estimate the frequency: Pham et al. (2017) showed that the frequency of litter ingestion, which they actually estimated at 83 % in 24 individuals necropsied between 1996 and 2016 in the Azores (Portugal), could have reached 91 % if non-complete individuals for which no necropsy was possible, would have presented ingested debris items.

The frequency of debris occurrence varied from 5 to 100 % in the literature. From the averages reported in the literature considered (see Appendix 1), the occurrence averaged 46.82 ± 29.27% (N = 30 sources of information), all protocols considered. The average, weighted by studies' sampling size, was 39.4 ± 22.18% (means without considering sample size, are presented in Table 3). However, providing accurate estimations must consider raw data, which were not available for this study.

- **No difference between necropsies and observation of alive individuals**

The frequency of litter ingestion was sometimes reported for the whole tested individuals, both alive and dead with no differentiation between protocols, e.g., Casale et al. (2008) found 48.1% of individuals having ingested debris by combining analysis of both alive individuals and necropsies and Camedda et al. (2015) found 21.92% of them. The 20 studies using necropsies found between 5 and 100% of individuals with ingested litter, with mean of 51.55 ± 29.66% and a weighted mean of 37.96 ± 5.36%. The 6 studies found using observations of live individuals' faeces reported on average between 9 and 93.75% of individuals with ingested litter, with a mean of 36.27 ± 31.94% and a weighted mean of 32.25 ±3.66%. A Student t test for the comparison of the mean occurrences between necropsies and observations of faeces did not reject the null hypothesis of an equivalence, comparatively to a greater mean occurrence using the former protocol compared to the second (t= 1.04; df= 7.78; p= 0.33; idem for variance analysis with a Fisher test). Nonetheless, the test was performed from the means provided in the found studies and not from the raw data, which are necessary to determine if the data can be combined or must be stratified according to the protocol. For now, as no difference in the frequency of ingested litter between the two protocols was found, the data were combined for further analyses.

- **Spatial variation**

Using the mean frequency of occurrence, we found between 18.75 and 88% of individuals having ingested litter in the Atlantic area and between 5 to 100% in the Mediterranean, with a respective average of 62.26 ± 31.68% (weighted mean of 63.13 ± 9.58%) and 44.45 ± 28.8% (weighted mean of 38.13 ± 4.24%; respectively N = 4 and 26 found records). We found no significant difference neither in variances (Fisher test: F = 1.21; df = 3; p = 0.65) nor in means (Student t test: t= 1.06; df = 3.80; p = 0.35). For the mainland France, we also found no significant difference of frequency of occurrence between the Atlantic and the Mediterranean façades both when considering the whole data collected from 1995 (respectively 88% and 78.6%) and the standard data obtained from 2013 (83.3 % versus 87%).

An analysis of the variance at the country level, asserting a homogeneous production of marine litter at this scale, showed also no difference in the frequency of occurrence among countries (df = 9; F = 1.61; p =0.17; Table 2 and Figure 1; test realized from means calculated per country with uncertainty that data were not redundant between the records presented in Appendix 1). It was not possible to compare the sites within the countries because the raw data were not available. Within the mainland France, Darmon et al. (2016) found no difference in the frequency of occurrence between the sites in the French Mediterranean façade.

**Table 2:** Minimum, maximum, number of records, grand means and means weighted by number of records, ±standard deviations, of the occurrence of litter ingestion in loggerhead sea turtles reported in the literature targeting the Barcelona and OSPAR RSCs and the MSFD areas (from Appendix 1).

| Country | Minimum (%) | Maximum (%) | Number of records | Mean ±sd (%) | Weighted mean ±sd (%) |
|---|---|---|---|---|---|
| Croatia-Slovenia | - | - | 1 | 35.2 | |
| France (Med + Atl) | 48.21 | 100 | 4 | 78.05 ±22.18 | 64.81 ±8.98 |
| Italy (continents + islands) | 12.09 | 93.75 | 11 | 42.23 ±30.76 | 39.39 ±10.32 |
| Malta | - | - | 1 | 20.2 | |
| Morocco | 53.8 | 71.4 | 2 | 62.6 ±12.44 | 59.9 ±4.31 |
| Portugal (continent + Azores) | 18.75 | 83.33 | 3 | 44.95 ±12.44 | 58.52 ±8.65 |
| Spain (East + Balearics) | 37.5 | 83.4 | 2 | 60.45 ±32.61 | 62.4 ±11.48 |
| Tunisia | 9 | 50 | 4 | 27.59 ±32.46 | 16.95 ±7.09 |
| Turkey | - | - | 1 | 5 | |

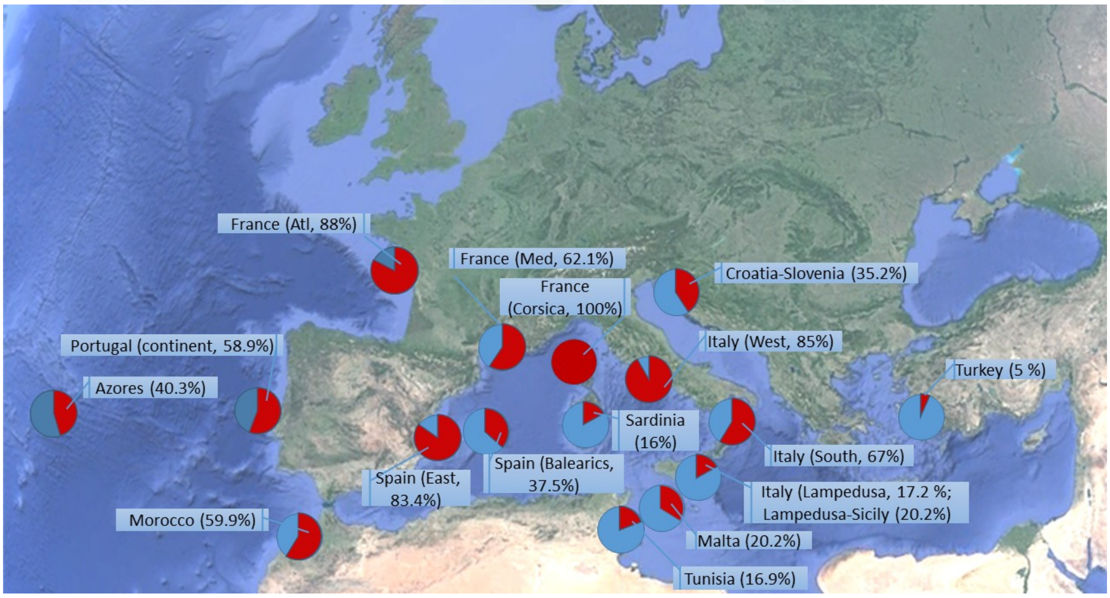


**Figure 1:** Frequency of occurrence of plastic ingestion in the loggerhead turtle *Caretta caretta* in the countries for which records were found (see Appendix 1; in red: proportion of individuals having ingested debris compared to the total of the tested individuals, in blue).

- **Annual variations**

The authors of the consulted sources worked on a variable number of years, and data were hardly comparable, a standardized approach being barely employed. A first exploratory analysis on the non-standardized data found in the literature showed no trend of the frequency of occurrence over years ($R^2 = 0.02$; $p = 0.54$; Figure 2), even considering data after 2000s ($R^2 = 0.15$; $p = 0.09$; Figure 2). However, raw data are necessary to perform a powerful statistical test.

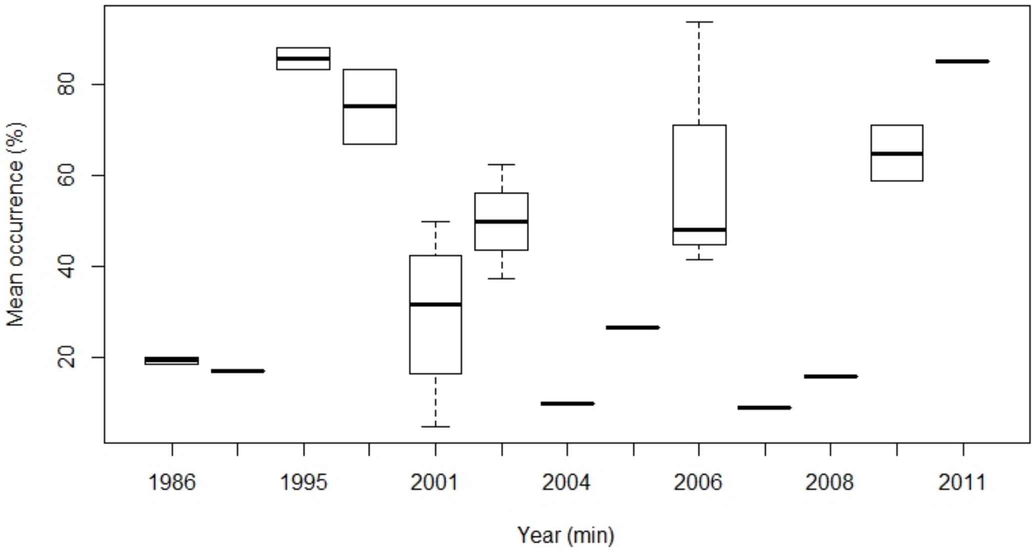


**Figure 2:** Frequency of occurrence of plastic ingestion in the loggerhead turtle *Caretta caretta* over years (boxplots represent the median, 1st and 3rd quartiles and the 5th and 95th centiles for the extreme values; on the x-axis, we considered the first year of the study).

In France, Darmon & Miaud (2016) found no difference before and after the systematic use of the MSFD standardized protocol (from 2013) when considering data collected from necropsies but significant variations when considering data collected from faeces, underlining the need of more data to verify their test. For the accurate analysis of the faeces, all the water must be filtrated and discharged from tanks in order to consider all the individuals' excretion and not only the floating part, which if so, could lead to underestimation of the excreted litter.

### *iii. Quantification of ingested litter*

- **Measures barely comparable among studies**

Four parameters were usually found in literature: the number of items, the mass (generally the dry mass), the volume and the size (Table 3). However, the measures were not systematically assessed, and only few authors provided several or all these parameters (Appendix 1). As we did not have access to the raw data but only to the means (or the median; e.g. Casale et al., 2016), we were not able to perform statistical tests but only exploratory and descriptive analyses. Moreover, the calculation method of the means reported in the literature, either based only on the individuals having ingested litter or on all the sampled individuals, could be different among authors and not systematically presented.

- Dry mass: The mass of litter ingested by an individual can sometimes be high (e.g., 47.5 g in Casale et al. (2008); 78 g in Nicolau et al. (2013, 2016)). Nicolau et al. (2013, 2016) noticed that 96% of individuals ingested a mean mass of less than 5 g of debris. The methodology to assess the dry mass should be standardized (drying duration).

- Volume: Only 3 studies presented the mean wet volume of ingested litter. Tomás et al. (2002) specified that individuals generally ingested less than 20 ml, which is far much higher than the volume noted by Darmon et al. (2015): 5.4 ml of debris items in the faeces of rescued individuals, 4.8 ml on average in the necropsied individuals in the French Mediterranean façade, and 6.9 ml in the necropsies individuals in the French Atlantic façade. The volume may appear difficult to measure especially for floating items such as those of the SHE category.

- Number of items: Nicolau et al. (2013, 2016) obtained 19.6 items and evaluated that 76.8% of individuals had less than 10 items in their digestive tract. The methodology to assess the number of items should be standardized, since the plastic items could fragment by chewing or with the movement of the digestive tract.

- Size: Various units were proposed in literature, most of the authors assessed the length in cm, but others measured the surface in cm² (e.g., Gramentz, 1988 or Revelles et al., 2007). The size of the ingested plastics appeared sometimes really high (16.9 cm (Nicolau et al., 2013, 2016); sometimes more than 20 cm (Bradai, 2017); 17 cm² (Gramentz, 1988)). The size of debris items depended on the category and softness/hardness (threadlike materials (THR, Galgani et al., 2013), being generally the longest). Darmon & Miaud (2016) found, on the basis of the mean of the largest length of the item face, a size up to 17.9 cm in the category THR, 7.2 cm in PAP, 4.4 cm in FRA and 6.1 cm in SHE found in dead individuals, and up to 8.8 cm in THR, 5.1 cm in PAP, 2.9 cm in FRA and 8.4 cm in SHE excreted in live individuals. Nicolau et al. (2013, 2016) noticed that the size of ingested debris items was usually inferior than 80 mm in 95% of individuals. They also reported that the size of ingested debris items decreased throughout the digestive tract, suggesting that items got fragmented during the digestion. A practical methodology to assess debris size should be standardized.

**Table 3:** Global results on litter ingested by sea turtles (minimum – maximum (Range); mean; standard error; number of records) from the consulted literature (see Appendix 1) for the occurrence (proportion of individuals having ingested debris among all tested individuals), the dry mass, the volume, the number of items and the size of ingested litter (mean of the means presented in the literature found). See text for details.

| Parameter | Range | Mean ±sd ; N |
|---|---|---|
| Occurrence | 5 - 100 % | 46.82 ± 29.27%; N = 30 |
| Dry mass | 0.08 - 41.7 g | 7.8 ±12.3 g ; N = 11 |
| Volume | 4.8 - 6.8 ml | 5.7 ±1.05 ml ; N = 3 |
| Number of items | 19 - >26 items | 9.4 ±6.5 items ; N = 9 |
| Size | 1 mm - >20 cm | Variables units provided in literature |

- **Is the dry mass representative of the quantity of ingested litter?**

Darmon & Miaud (2016) calculated a high correlation between the dry mass and the volume of ingested litter in loggerhead, and a relatively important correlation between the mass and the number or the size of the debris items, among the turtles having ingested debris. Considering only 3 references which recorded 3 parameters in the same study, we found a correlation of 0.98 between the dry mass and the volume and of 0.67 between the volume and the number of items, but almost no correlation between the mass and the number of items (0.47). Therefore, we used thereafter, the dry mass to represent the quantity of ingested litter, assuming the authors used the same practical approach to assess it. When acquiring more knowledge and with the raw data, this must be confirmed while considering the litter category.

### - More data needed to compare the dry mass of ingested litter between protocols

It was not possible to compare the protocols with the available literature. Darmon & Miaud (2016) noticed a mean of 1.4 ±2 g in necropsied loggerhead turtles and 0.25 g in live individuals, but they underlined their data was not sufficient to compare their results according to the protocol. The use of a high number of individuals in Camedda et al (2014)'s study, highlighted a homogeneity between both protocols (N=121).

### - No spatial variation in dry mass between and within areas

The mean dry mass was 4.8 ±6 g in the literature targeting the Mediterranean area and 13 ±19.4 g in the Atlantic area (respectively N = 7 and 4 records). A Student t test with a correction factor (Welch-Satterthwaite approximation to the degrees of freedom) for heteroscedasticity (i.e., probably due to highly dispersed data, the variances of the mass measured in these two areas tended to be different (Fisher F = 10.514; df = 3;6, p = 0.02)) detected no difference between the two areas (t = 0.83, df = 3.3301; p = 0.46). The mean dry mass reported in the literature concerning Morocco was particularly high (Table 4), but no difference among the countries in which the dry mass was reported, was found (ANOVA F = 0.204; p = 0.2).

**Table 4:** Minimum, maximum, number of records and mean (± standard deviation, when available) of the mean dry mass (in grams) of the litter ingested by the loggerhead turtles reported in all the literature found, targeting the Barcelona and OSPAR RSCs and the MSFD areas (results from Appendix 1).

| Country | Minimum | Maximum | Number of records | Mean ±sd |
|---|---|---|---|---|
| Croatia-Slovenia | - | - | 1 | 0.08 |
| France | 1 | 8.1 | 3 | 3.97 ±3.55 |
| Italy | 1.3 | 1.6 | 2 | 1.46 |
| Morocco | 11.8 | 41.7 | 2 | 26.75 |
| Portugal | 1.07 | 1.3 | 2 | 1.21 |
| Tunisia | 1 | 15 | 1 | - |

- **More data needed to test for an annual tendency in the dry mass of ingested litter**

The mean dry mass found in the literature targeting the Barcelona and OSPAR RSCs and the MSFD areas did not present a significant positive nor negative tendency over time ($R^2 = 0.2$; $p = 0.3$; Figure 3).

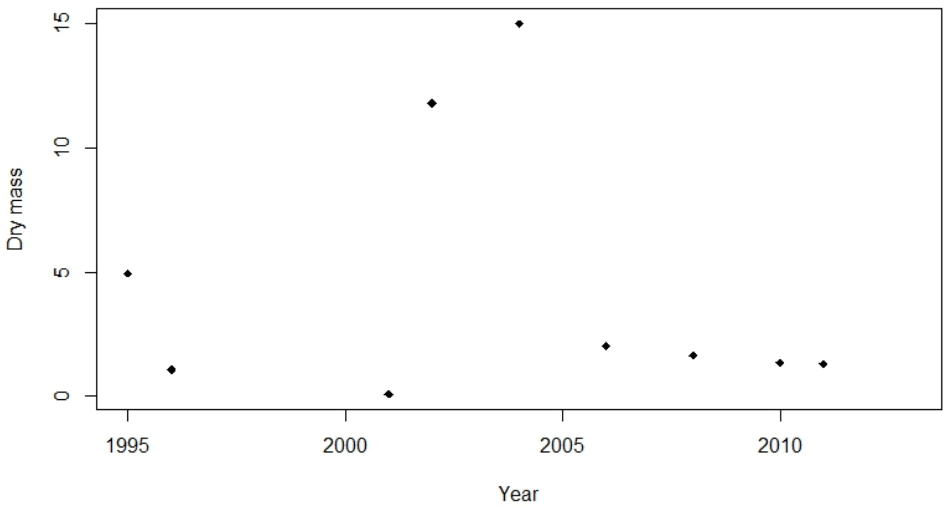


**Figure 3:** Mean dry mass of the litter ingested by the loggerhead turtle *Caretta caretta* over years calculated between the countries (based on Appendix 1, literature targeting the Barcelona and OSPAR RSCs and the MSFD areas); on the x-axis, we considered the first year of the study).

### *iv. Description of ingested debris items*

Plastic was the main type of debris item noticed by the authors with various percentages (13.2% ("user plastics", Camedda et al., 2014); 56.8% (Nicolau et al., 2013, 2016); 63% (Tomás et al., 2002); 69.,4% (Lazar & Gracan, 2011); 76% (Travaglini et al., 2013, in Dell'Amico & Gambaiani, 2013); 91% (Armanasco et al., 2010); 91.7% (Campani et al., 2012 and 2013); Picture 1).


**Picture 1.** Type of debris items found in necropsied sea turtles (from Pham et al., 2017)

For those authors who were able to characterize them, most of them found SHE, THR and FRA according to the categories proposed in the MSFD guidelines (Galgani et al., 2013). For example, in the mainland of France, Darmon & Miaud (2016) calculated that SHE represented 40.9% of the volume of the ingested litter and 29.9% of their dry mass (9.1% of the volume and 5.81% of the dry mass of all the ingested material, debris items and natural food). Matiddi et al. (2017) also found USE SHE as being the most represented category, with 12±2 items on average among the impacted turtles (N = 102), followed by USE FRA and USE THR (respectively 3 ±0.5 and 2 ±1 items). The category beyond plastic that was the most represented, was PAP. Casale et al. (2016) noticed variations in the items' categories between the protocols (from alive or dead individuals), contrary to Camedda et al. (2014), who found a homogeneity in relation to the total abundance, weight and composition of litter sub-categories.

The ingested items were generally soft and of clear colour, mainly white or transparent. For example, Darmon & Miaud (2016) found 44.3% of white-transparent items and 30.4% of dark items, the remaining being multi-coloured or unclassifiable. Matiddi et al. (2017) showed more frequently an ingestion of transparent items (46%), followed by white (21%) then black items (16%), with red and grey being the less ingested colour (1%).

### *v. Biological constraints*

#### - Individual size

Although some authors reported the weight or the growth stage, the most common measure of individual size was the curved carapace length, notch to tip (CCL). Since the authors barely specified how they assessed it, we supposed that there were minimal differences in the measurement approach. The smallest individual had a CCL of 9.3 cm (Portugal, Azores, Frick et al., 2009) and the biggest 91 cm (Tunisia, unpublished data from Bradai et al., INSTM). On average, CCL was 50.1 ±14 cm.

Pham et al. (2017) brought to light a positive correlation between the length of the ingested debris items and the turtle size but Lazar & Gracan (2011) found no effect of individual size on the amount of ingested litter, but they noticed an equal proportion of prey and debris. Darmon et al. (2016) showed no influence of individual mass on the frequency of litter ingestion in 18 necropsied individuals. Camedda et al. (2014) also noticed no correlation between the turtle size class (inferior versus superior to 40 cm) and the mass as well as the abundance of ingested litter.

Unfortunately, because the authors generally did not specify which of the individuals had ingested debris, we were unable to test the influence of CCL on the occurrence of litter ingestion bit this is necessary to determine if data should be separated according to individuals' size. In this way, Matiddi et al. (2017) stratified their sample (N=150; total frequency of occurrence of 68%) in order to discriminate individuals with CCL longer than 40 cm (N=110; frequency of occurrence of 83%) from juveniles, and excluding animals unable to feed, considered sick for a long time before being stranded (N=120; FO=85%).

### - Individual sex

There is no sufficient data to test for the effect of sex on litter ingestion. A few authors tested this effect from their raw data. Mostly based on green turtle *Chelonia mydas*, Bjorndal et al. (1994) found that adult females ingested litter more frequently than adult males, but as much in terms of mass and volume. Darmon & Miaud (2016) detected no difference between sexes both in terms of frequency of occurrence and in mass of ingested litter, but they noted that the differentiation of sexes in juveniles (most of the sampled individuals) is confusing. Lazar and Gracan (2011) also noticed no effect of the sex on litter ingestion.

### - Health status

Travaglini et al. (2013, in Dell'Amico & Gambaiani, 2013) found more debris items in individuals in bad health condition. However, it was not possible in literature, to have information on the individual's health condition before the ingestion of litter, no to assess how the individual's condition or its feeding behaviour would evolve after having ingested debris. Raw data shall be analysed to compare the body condition (CCL, mass, Body Condition Indices, etc.) between the individuals having ingested debris and those who do not.

Several studies reported that the ingestion of litter rarely caused directly in death in the loggerhead species although this appeared (Table 5). Most of the authors agreed that litter ingestion rather led to indirect negative effects on the individuals' feeding behaviour (buoyancy troubles; Russo et al., 2013) and body condition (e.g., anorexia; Elouar et al., 2008; Travaglini et al., 2013, in Dell'Amico & Gambaiani, 2013), due to the accumulation of debris items in the digestive tract, leading to internal lesions such as ulcers or fibrinous necrotizing enteritis sometimes purulent (Oros et al., 2003, 2004, 2005, 2009), or to the levels of PCB in tissues (Oros et al., 2009; Duguy's reports (references in Appendix 1)). Tomás et al. (2002) observed no lesions or pathologies caused by litter ingested.

**Table 5:** Examples found in literature reporting deaths of loggerhead sea turtles caused by marine litter ingestion

| Number death among all tested individuals | Cause | Reference |
|---|---|---|
| 1 among 95 individuals | Perforation of the digestive tract following the ingestion of 4.4 g of litter (26.6% of the digestive content) | Nicolau et al. (2013, 2016) |
| 1 among 24 individuals | Obstruction caused by a hook | Pham et al. (2017) |
| 1 juvenile among 54 individuals | Litter accumulation in 35% of the digestive content | Lazar & Gracan (2011) |
| 1 among 31 individuals | | Campani et al. (2013) |
| Several among 763 individuals | | Insacco et al. (2011) |

The question of the greater propensity to litter ingestion of individuals in bad health condition (due or not to a previous ingestion of litter), compared to healthy individuals, remains. Acquiring knowledge on the factors determining feeding selectivity and the capacity to differentiate debris from natural preys may provide answer elements.

### - Methods for the collection of individuals

Individuals were collected according to two main collection methods: (1) stranding, with some authors observing marks of entanglement, such as Barreiros & Raykov (2014) and (2) bycatch, with one study including turtles seized by police from a Hotel who bought them to fishermen (Tomás et al., 2002). The observed mean frequency of occurrence was higher in stranded individuals (54.77 ±31.47%; N = 9 records) compared to bycaught individuals (17.43 ±4.62%; N = 3 records) (Student t test for heteroscedasticity: $p = 0.03$; However, there was no significant difference of grand mean occurrences when considering the captures (17.05 ±1.62%; N = 2 records) and the studies with several sampling methods (55.31 ±28.27%; N = 10 records)). Some experts suggested that stranded individuals can be in poorer health condition compared to bycaught individuals, and due to their possible greater propensity to ingest marine litter, considering them for the indicator may over-estimate litter impacts (Claro et al., 2014). At this moment, the number of studies is nonetheless highly variable between the two types of turtle's origin, and the capacity to collect individuals varies among countries, availability and means of the local sea turtle stranding and rescue networks. It thus appears important to pursue the litter collection in all possible individuals, whatever their origin, in order to test the influence of the collection method litter ingestion based on a high quantity of raw data.

### - Information on the digestive transit duration

Most of the authors noticed that they found most of the debris items in the intestines, more than in the stomach and then the oesophagus in necropsied individuals (Brongserma, 1972; Duguy et al., 2002; Tomas et al., 2002; Misfud et al., 2009; Camedda et al., 2014; Casale et al., 2016; Darmon & Miaud, 2016; Bradai, 2017; Matiddi et al., 2017; Pham et al., 2017). For example, Nicolau et al. (2013, 2016) observed 90.8% of debris items in the intestines (which represented 52.8% of the ingested mass of litter found in this digestive section), 8.9% in the stomach (44.8% of the litter mass) and 0.3% of the items in the oesophagus (2.9% of the ingested litter mass). Similar results were reported for the turtles collected in Italy: The occurrence of litter was 75% in the intestines, with a total mass of 127.9 g, followed by 32% in the stomach, with a total mass of 27.8 g, while the oesophagus was the least affected part of the gastro-intestinal tract, with an occurrence of 5% and a total mass of 1.5 g (Matiddi et al., 2017). This suggests either that debris items accumulate in the digestive tract or that the digestion is quite rapid.

As hard and large plastic items can be found in loggerheads' faeces, individuals should be able to excrete plastics and accumulation of debris items is thus not systematic. The few studies found on this topic in our selected area showed that transit duration can be long. Casale et al. (2016) obtained a duration between 1 to 114 days on 47 individuals from their arrival to the first debris defecation and up to 129 days for the last defecation of debris. Camedda et al. (2014) found an expulsion of litter at the rescue centre for longer than a month of hospitalization, with most of the litter expelled within the first two weeks.

Experimentation studies to evaluate the digestive transit duration are pretty rare. Valente et al. (2008) observed a mean digestive transit duration (time between ingestion and first elimination) of 13.25 ± 4.86 days for polyethylene spheres and 8.5 ± 2.73 days for soft flat foam materials in 8 studied simultaneously. They also brought to light an influence of water temperature, the transit time being faster between 20°C and 23.6°C, but no correlation with the minimum straight carapace length or the body mass. On 12 rescued loggerheads of 11.5 kg and 32.5 cm CCL on average, Darmon et al. (2016) noted that the duration of the excretion of a coloured micro-ball from the day when individuals arrived in the rescue centre (according to the MSFD guidelines, Galgani et al., 2013), varied between 13 and 185 days, with a mean of 32.7 ±22.1 days.

The digestive transit duration informs on the duration during which the faeces of live individuals must be collected and analysed for litter ingestion. Moreover, related to individuals' displacements, it may inform on the location where individuals could have ingested litter, and thus help to delimitate the management units in which a GES should be defined. Acquiring knowledge on the capacity and the duration of litter excretion would be necessary, as well as on the degradability of plastics during the digestion, in order to better understand sea turtles' selectivity for plastics (e.g., comparing the colour of debris items in the environment and those found in sea turtles' digestive tracts or faeces). The MSFD guidelines presents an experiment consisting in calculating the time for the excretion of a coloured micro-ball (Galgani et al., 2013). However, the evaluation of the digestive transit duration may be more accurate by determining the plateau or the average of durations obtained from several balls administrated to the individuals at close intervals, e.g. daily, but such an experiment on rescued turtles could lead to ethical issues.

## b) The leatherback turtle

### *i. Description and sorting of the literature identified*

Forty-eight records in 45 references were found on litter ingestion in the leatherback turtles in the Barcelona and OSPAR RSCs and the MSFD area (Table 6, Appendix 1). Eight studies concerned the Mediterranean Sea (France, Greece, Italy and Tunisia), 36 the Atlantic Ocean (England, France, Netherlands, Portugal, Spain and Wales) and 1 did not specify the area but was probably performed in the Atlantic (Morocco, Benhardouze et al., 2014).

**Table 6:** Number of records on litter ingested by the leatherback turtles (occurrence and/or ingested quantities) in the Barcelona and OSPAR RSCs and in the MSFD areas; One study could report several records related to e.g., different protocols or different areas (details in Appendix 1).

| Type of reference | Atlantic area | Mediterranean area |
|---|---|---|
| Reports or abstracts of report | 1 | 1 |
| Conferences (slideshow, proceedings of conferences or workshops) | 2 | 1 |
| Unpublished data | 1 | 1 |
| Peer-reviewed articles | 6 | 2 |
| Non-peer reviewed articles | 29 | 2 |
| Books or book chapters | 0 | 1 |
| Posters | 0 | 1 |
| *Total* | *39* | *9* |

The studies dealt with 1 (N = 14 studies) to 93 individuals (Duguy et al., 2000; French Atlantic raw data analysed by Darmon et al. (2016) for N = 46 individuals) (Table 7). The earliest record was in 1968 (Brongserma (1972), in the region of Friesland (Wadden sea, North Sea) of the Netherlands). Only necropsies were reported, but they were probably not systematically practiced, especially in the oldest studies. Different parameters were used to describe individuals' body condition, more generally the straight carapace length, varying from 96 cm (Duguy, 1989) to 256.5 cm (Eckert & Luginbuhl, 1988; Morgan, 1989).

**Table 7:** Number of individuals evaluated in studies in the literature found. Authors generally reported a case of litter ingestion in 1 individual (14 studies), barely in more than 2 but up to 93 individuals were considered in one reference.

| ***Number of tested individuals*** | ***1*** | ***2*** | ***3*** | ***4*** | ***5*** | ***6*** | ***8*** | ***11*** | ***12*** | ***16*** | ***43*** | ***46*** | ***93*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Number of studies*** | 14 | 6 | 6 | 3 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | 1 |

We employed the same procedure as for the loggerhead turtles to clean the literature results: Only 6 records believed to be reliable and including more than 1 individual were kept. Most of the identified publications did not use a standard protocol such as the one proposed in the MSFD guidelines (Galgani et al., 2013). They also generally did not specify if the protocol they employed was performed systematically, which could badly estimate the calculated averages.

### *ii. Occurrence of litter ingestion*

- **Averages**

Among the literature retained in the framework of this report, only five studies calculated the occurrence of litter ingestion in leatherback turtles. This one varied from 33 to 100 %, with an average of 77.33 ± 28.65% and of 87.13 ± 7.63% by weighting by the studies' sampling size.

- **Spatial variations**

The mean occurrence found in the literature varied from 33 to 93.48% in the Atlantic area of our study (N = 4 references) with an average of 66 ±29.22% (weighted mean of 85.7 ±8.7), and the two studies found in the Mediterranean area showed 100% in 4 individuals in Italy (Travaglini et al., 2006) and 100% in 3 individuals collected in Tunisia (INSTM, unpublished data). Because of the low number of studies found, no statistical test was possible to compare the occurrence of litter ingestion between leatherback turtles on the two areas. An analysis of variance showed no difference in the frequency of occurrence among countries (df = 4; F = 57.13; p = 0.1; Table 8; Figure 4; test realized from the means calculated per country).

**Table 8:** Minimum, maximum and mean (w.m=weighted mean) ±standard deviation (%) of the occurrence of litter ingestion in the leatherback turtle, and number of records, reported in the literature targeting the Atlantic area (results from Appendix 1).

| Country | Minimum | Maximum | Mean ±sd | Number of records |
|---|---|---|---|---|
| England-Netherlands | - | - | 50 | 1 |
| France | 87.5 | 93.48 | 90.49 ±4.23 (w.m=92.59 ±1.52) | 2 |
| Italy | | | 100 | 1 |
| Morocco | - | - | 33 | 1 |
| Tunisia | - | - | 100 | 1 |

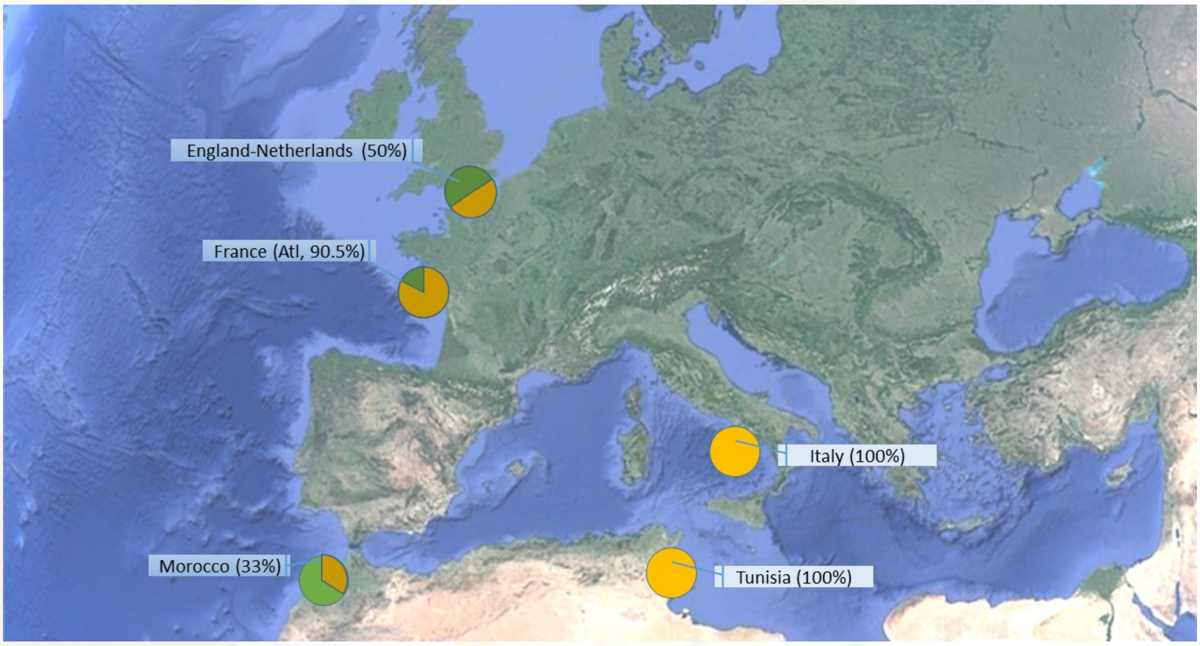


**Figure 4:** Frequency of occurrence of plastic ingestion in the leatherback turtle *Dermochelys coriacea* in the countries for which records were found (see Appendix 1; in yellow: proportion of individuals having ingested debris relative to 100% of the tested individuals, in green).

- **Annual variations**

The first record dated from 1968 and concerned a 244 cm individual (Brongserma, 1972). Data reported in literature were not really comparable, since it would have required the use of a standardized protocol. The low number of data thus prevented to test the temporal tendency of the occurrence over years (Figure 5). Raw data are necessary to perform a powerful statistical test.

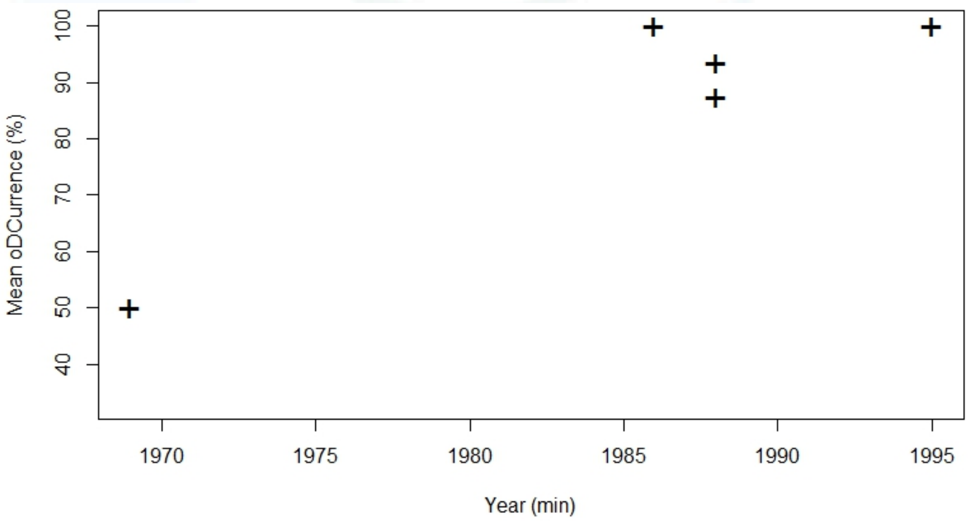


**Figure 5:** Mean occurrence of litter ingestion in the leatherback turtle *Dermochelys coriacea* over years from the literature found in the Barcelona and OSPAR RSCs and the MSFD areas.

Data provided by the French Atlantic stranding network RTMAE and the sea turtle rescue centre of the Aquarium La Rochelle CESTM, who covered the entire French Atlantic coast, especially the Charente Maritime region, did a standardized monitoring since 1980's to nowadays (Figure 6). However, no clear tendency appeared (non-significant slope, t = 1,48; $R^2$ = 0,12; p = 0,16). In their review for the French Atlantic coast, Duguy et al. (1998) evaluated that 40,7% of the leatherback individuals had ingested debris items before 1994, this frequency increasing to 68,7% only in 1995. Like for the other areas, since studies did not specify if the necropsies of the individuals found dead were systematic or not, the true zeros were not known. The sampling method to collect individuals should also have evolved from the 1980's and the observation pressure could have increased over time, e.g., due to the establishment of stranding networks and the training of observers. The protocol to collect the ingested debris, derived from the MSFD guidelines for the loggerhead turtle, was only applied since 2013 (Galgani et al., 2013).

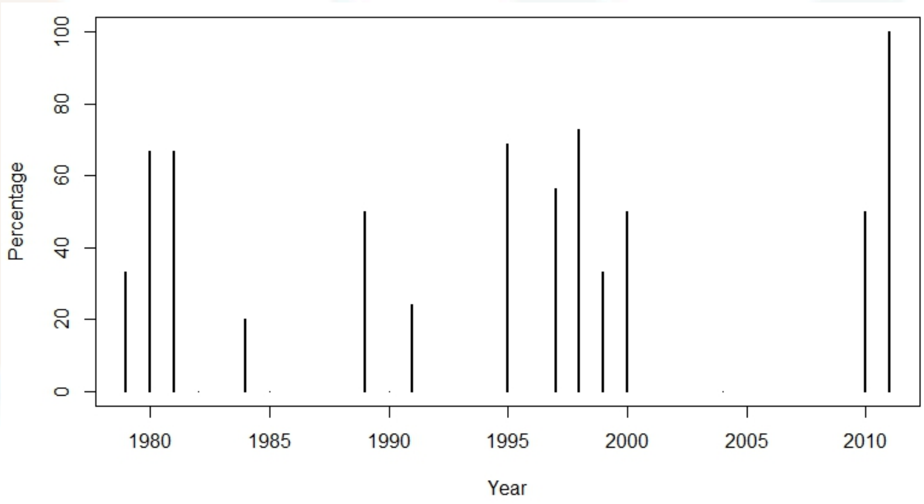


**Figure 6:** Occurrence of plastic ingestion in the leatherback turtle *Dermochelys coriacea* over years in the French Atlantic coast

### *iii. Quantity of ingested litter*

The quantity of the litter ingested by the leatherback turtles were rarely evaluated in the studied literature, but:

- Russo et al. (2003) found 2 kg of debris items in the 3 individual they necropsied. Darmon et al. (2016) noted an average of 7.27 ± 9.06 g (N = 46 individuals).

- The volume was measured in 2 references only, reaching until 5 L in one individual collected in the French Atlantic coast (Duguy et al., 1980), where Darmon et al. (2016), from the raw data or the samples available in this region, evaluated an average of 36.2 ± 46.5 mL among the individual having ingested debris.

- On the contrary, the number of ingested items was often reported in the literature (N = 12 studies, but 3 of them noted the presence of "several" pieces without specifying the exact number), and varied between 1 and 14.

- The size of the debris items was also regularly reported but not systematically for all the found pieces. There were more probably the largest items, maybe the huge ones, which were measured, but this showed leatherbacks could eat really large plastic pieces (e.g. a piece of 300 x 40 cm, Duguy et al. (1999); 17,5 cm, Den Hartog & Van Nierop (1984); a bag of 9,8 m², Katsanevakis (2008); 20 x 20 cm, Doyle (2007); a fragment of 21 x 4 cm and a plastic bag of 20 x 40 cm, Poppi et al. (2012)). The threadlike items (THR) were generally the longest ones (e.g., 23 cm, Poppi et al., 2012).

Standardized raw data are necessary to evaluate and compare the 4 parameters and test for the spatial and temporal variations in the quantities of ingested debris. Moreover, the best statistical approach to use for the definition of GES (e.g., based either only on the individuals with ingested litter or on all the collected individuals) must be evaluated.

### *iv. Description of the ingested debris items*

The authors generally noted the presence of "plastic items" without specification. When differentiated, the ingested plastic items were mostly USE SHE, FRA and THR according to the categories proposed in the MSFD guidelines (Galgani et al., 2013; Picture 2), THR associated with HOO being not considered as litter in this study. The categories PAP (Russo et al., 2003) and POTH (adhesive tape; Duguy et al., 2000) were also sometimes found.

The ingested items were rather flexible instead of hard (Duguy et al., 2000), generally white and transparent, and less often coloured (e.g., sometimes blue or orange, in Duguy et al., 1998). More data is needed to describe the litter ingested by the leatherback in the areas covered by this study and evaluate the leatherback's possible selectivity or avoidance for marine litter.

**Picture 2:** An example of debris items found in a necropsied leatherback (plastic bag of nearly 60 cm side; Darmon in collaboration with Dell'Amico (CESTM; RTMAE))

### *v. Biological constraints*

- **Individual size**

The most common measure was the straight carapace length, the authors barely specifying the method they used to assess it. The individuals' size varied from 96 cm (Duguy, 1989; France) to 256.5 cm (Eckert & Luginbuhl, 1988; Wales). On average, the straight carapace length was 158.05 cm (N = 19 studies for which the measure was provided).

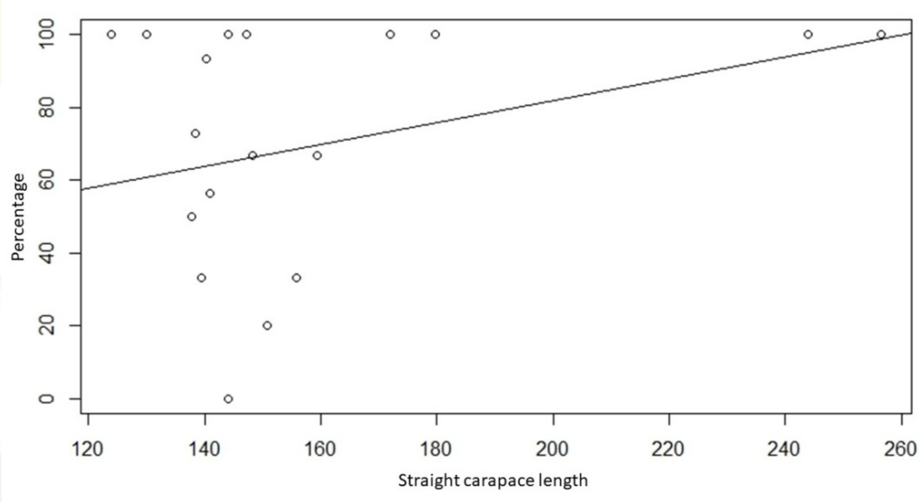


**Figure 7:** Percentage of necropsied leatherback turtles found to have ingested debris (%) according to their mean straight carapace length (cm) reported in the literature found in the RSC Barcelona and OSPAR and MSFD areas (from Appendix 1).

Removing the two most extreme data (outliers), the percentage of individuals having ingested litter did not significantly vary with the individuals' mean strength carapace length (Figure 7; F = 1.81; df = 1; adjusted $R^2 = 0.04$; p = 0.2). This is also due to the small range of lengths, the studied individuals generally measuring less than 180 cm. No correlation appeared between occurrence of litter ingestion and carapace length (F = 0.02; df = 1; adjusted $R^2 = -0.06$; p = 0.87). However, apart Duguy et al. (2001) who distinguished that the 3 smallest individuals were those who had ingested litter among the 6 tested individuals, the authors generally did not specify which, among the tested individuals had ingested debris, compromising any correct statistical test.

- **Individual sex**

Duguy (1987) observed more females than males with ingested litter and later, Duguy et al. (2000) specify that 55.3% of females (N = 54 dead individuals) compare to 29% of males (N = 31 dead individuals) had ingested debris in the French Atlantic coast in the 1980's. However, the literature prospected offered no sufficient data to test the effect of sex on litter ingestion. Standardized raw data would be necessary to evaluate the sex effect on the frequency of litter ingestion in the leatherback turtles at the entire area targeted by this study.

- **Health status**

In 1979, Duguy et al. (2000) attributed the death of one individual, because of the ingestion of 5 litters of plastic bags which caused the occlusion of its digestive tract. Authors reported various injuries caused by the ingestion of litter, such as perforation, hyperplasia of tissues, inflammatory fibrosis, occlusion at various locations of the digestive tract, bacterial enteritis, necrosis, purulent wounds, thickening of the digestive mucous, ulcers, abnormal abdominal contractions, decrease in the physical condition and thinness. Popi et al. (2012) found individuals with concentrations of heavy metals, as regularly reported by Duguy's reports in the French Atlantic coast. However, similar to the loggerhead turtle, at this stage and with the available data provided in the literature we prospected, we were not able to evaluate if the propensity to ingest debris is related to bad physical condition or inversely, if it the ingestion of litter leads to health degradation. A systematic survey of individuals' body condition associated to the collection of ingested litter could support the evaluation of such a biological constraint in the use of the indicator, i.e., assess which individuals must be retained as indicator.

- **Collection method**

The collection of dead leatherbacks was mostly performed by stranding network (13 standings, 4 bycatch and 2 offshore drifts) and the necropsies were performed by expert biologists from rescue centres, sometimes with veterinarians. Stranding was regularly associated to a consequence of bycatch, and sometimes of entanglement. However, the majority of the references did not specify which of the individuals had ingested litter according to how they had been collected. More data are thus needed to determine which individuals must be considered as indicators. In order to verify the hypothesis that stranding individuals are more prone to be in bad health condition and ingest more litter than e.g., bycaught individuals, the collecting method, the cause and the location of death on litter ingestion must be tested.

- **Spatial constraint to digestive transit duration and individual movements**

As no leatherback individual was controlled alive in a rescue centre, no experimental evaluation of the digestive transit time could be provided. Most of the authors found debris items mostly in the stomach (N = 10) more than the intestines (N = 4, and the rectum N = 1), as specified by Duguy et al. (1998)'s review. However, it is not certain all authors prospected all the digestive tract in its whole length and that the decomposition status of the animal allowed such as research. The standardized protocol used for the collection of the litter ingested by the loggerhead turtle should be disseminated to collect such standard data for the leatherback.

# III- Evaluation of the existing networks for the implementation of the indicator in the MSFD and the RSCs OSPAR and Barcelona areas

The INDICIT partners referenced the existing networks possibly available for the collection of turtles and/or data on litter ingestion, in their country. We assessed their available means and possible existing data, at the date of July 2017.

## 1. RSC OSPAR/Macaronesia

| Country | Stakeholders | Type of activity | Description of activity | Available data |
|---|---|---|---|---|
| France | Nausicaa | Transit centres | Welcome live turtles before repatriation in rescue centre; Can centralize dead individuals and samples | Centralized by Aquarium La Rochelle |
| | Musée de la Mer de Cherbourg | | | |
| | Grand Aquarium de Saint Malo | | | |
| | Océanopolis | | | |
| | Océarium du Croisic | | | |
| | Musée de la Mer de Biarritz | | | |
| | RTMAE (Aquarium La Rochelle) | Stranding network | Monitor live and dead individuals from opportunistic observations; Collect dead | Yes |

| Country | Stakeholders | Type of activity | Description of activity | Available data |
|---|---|---|---|---|
| | | | individuals, measure and describe the collected individuals (125 trained observers, April 2017) | |
| | CESTM (Aquarium La Rochelle) | Rescue centre | Offer treatment to rescue individuals (except for leatherbacks); Practice necropsies of dead individuals. | Necropsies and litter ingestion (loggerhead + leatherback turtles); MSFD protocol since 2013. |
| | MTES (Ministry in charge of Ecology) | National authorities | Relationships between stakeholders and Ministry, MSFD and OSPAR. | |
| | GTMF | Exchange information group | Coordinate all French activities related to sea turtles; Gathers French stakeholders related to sea turtles. | |
| | Pelagis observatory | Research centre | Monitor (aerial surveys) marine wildlife such as sea turtles | Yes but occasional data |
| | CEFE-CNRS | | Study sea turtles' behaviours; Elaborate standard protocols; Analyse data; regularly worked with stakeholders | Yes, in collaboration with rescue centres and stranding networks |
| Portugal | DGRM | National authorities | Relationships between stakeholders and Ministry, MSFD and OSPAR. | |
| | DRAM (Azores) | Regional authorities | | see RACA |
| | DROTA (Madeira) | | | |
| | RACAM (Madeira) | Stranding network | Monitor live and dead individuals; measure and describe individuals. | Being contacted |
| | RACA (Azores) | | | Data on standings available |
| | Flying Shark (Azores) | Rescue centre | Offer treatment to rescued individuals (except for leatherbacks); To be trained for collection of standardized data | |
| | CPRAM (Mainland) | | | Being contacted |
| | Porto d'Abrigo Zoomarine (Mainland) | | | Being contacted |
| | CCMAR (University of Algarve, Mainland) | Research centre | To be trained for collection of standardized data | Being involved |
| Spain | Rescue centres and stranding networks from the north coast (Galicia, Asturias, Cantabria and Basque Country) | Rescue centres and stranding networks | Offer treatment to rescued individuals (except for leatherbacks); Few numbers of stranded turtles. To be trained for collection of standardized data | Being contacted and involved |
| | Rescue centres and stranding networks from the South coast (Andalucía) | | | |

| Country | Stakeholders | Type of activity | Description of activity | Available data |
|---|---|---|---|---|
| | Recovery centre of Gran Canaria | Rescue centres | Being trained for standardized collection of data | Data rather on entanglement. Necropsies and litter ingestion will be analyzed in 2018 with INDICIT protocols. |
| | Recovery centre of Fuerteventura | | | |
| | Recovery centre of Tenerife | | | |
| | Stranding network of Lanzarote | Stranding networks | Being trained for standardized collection of data | |
| | Stranding network of La Palma | | | |
| | Stranding network of El Hierro | | | |
| | Stranding network of La Gomera | | | |
| | Canary islands government | Regional authorities | Red PROMAR (movil App): Citizen observers (pictures of wild animals collected by citizens are registered, dated and gps localized). | Pictures of wild animals. An "stranding network protocol for turtles" will be instaled in the PROMAR App |
| | University of Las Palmas | Research centres | Veterinarian research | Necropsies and litter ingestion. MSFD protocol since 2017 |

In France, the network of stakeholders involved in the collection of individuals is well identified, both for live and dead loggerheads and dead leatherbacks. The data are centralized by the Aquarium La Rochelle who performs the necropsies, using the MSFD protocol since 2013, in collaboration with CEFE-CNRS.

In Portugal and Spain, the networking requires more time due to the disperse areas (archipelagos) and training sessions to perform necropsies are being regularly organized. Some stakeholders are already collecting standardized data (Azores in Portugal, Gran Canarias in Spain).

Training levels are variable and stakeholders could help each other, e.g., the French Atlantic rescue centre could provide expertise in the necropsy of leatherback turtles while the Gran Canarias rescue centre can show how to collect litter ingested by alive loggerhead turtles (e.g. collection device, drainpipe, etc.).

## 2. RSC Barcelona

| Country | Stakeholders | Type of activity | Description of activity | Available data |
|---|---|---|---|---|
| Tunisia | Marine turtle rescue centre | Research centre managed by INSTM | Research, rehabilitation, data collection, awareness | Yes, starts to collect standardized data |
| | National stranding network | Research on marine turtles and | Managed by INSTM; 3 teams (North, South, | Data centralized by INSTM |

| Country | Stakeholders | Type of activity | Description of activity | Available data |
|---|---|---|---|---|
| | | cetaceans stranding | Centre) work on the issue | |
| | Fishing administrations | Local management authorities | | |
| Turkey | PAU-DEKAMER | Research and rescue Centre | Provide treatment to injured sea turtles; Performs necropsies; Collect data and samples | Yes |
| | Local network | Stranding network | Monitor and collect stranding and injured turtles | |
| | Hatay Mustafa Kemal University | Transit centres | Have convalescent pools and serves as first aid stations; transit to PAU-DEKAMER | Centralized by PAU-DEKAMER |
| | Mersin University | | | |
| | Çanakkale University | | | |
| | Istanbul Aquarium | | | |
| | Government agencies | Regional authorities | Can transport turtles to PAU-DEKAMER | |
| | Protection of Nature and Natural Parks (DKMP) | Local authorities | Transit and transport of individuals to PAU-DEKAMER | |
| | TUDAV-Turkish Marine Research Foundation | NGO | Monitoring and information to PAU-DEKAMER on nesting and stranded individuals | |
| | EKAD-Ecological research Association | | | |
| | BETUYAP-Belek Region Tourism Development Association | | | |
| | EKODOSD-Ecological Conservation Society of Friends of Kusadası | | | |
| | AKUT-Search, rescue Association Team | | | |
| France | RTMMF (Mainland + Corsica) | Stranding network | Monitor live and dead individuals from opportunistic observations; Collect dead individuals, measure and describe the collected individuals (92 trained observers, April 2017) | Yes |
| | CestMed (Mainland) | Rescue centre | Provide treatments to injured sea turtles; Performs necropsies; Collect data (ingested litter, digestive transit duration) on alive sea turtles; Collect and centralize samples. | Yes |
| | CARI (Corsica) | | Being developed; currently a transit centre | Yes |

| Country | Stakeholders | Type of activity | Description of activity | Available data |
|---|---|---|---|---|
| | | | to Sardinia's rescue centre | |
| | CEFE-CNRS | Trained and regularly worked with the stakeholders | Elaborate standard protocols; Collect and analyse data; regularly worked with stakeholders | Yes, in collaboration with other stakeholders |
| | MTES (Ministry in charge of Ecology) | National authorities | Relationships between stakeholders and Ministry, MSFD and Barcelona RSC. Advisory board. | |
| Spain | Stranding networks | Stranding networkds | Perform necropsies; can have veterinary assistance | |
| | 2 rescue centres from the Andalusia region<br>1 rescue centre from the Murcia region<br>2 rescue centres from the Balearic Islands<br>1 rescue centre from Catalonia | Rescue centre | Provide treatments to injured sea turtles; Performs necropsies; Collect and centralize samples | Yes, starting to collect standardized data |
| | Environmental office of regional governments of Andalusia (South Spain) and the Balearic Islands | Regional authorities | Relationships between stakeholders and Ministry, MSFD and Barcelona RSC | Yes, starting to collect standardized data |
| | University of Valencia | Research centre | Elaborate protocols, collect and analyse data | Yes |
| | Spanish Ministry of Environment | National authorities | Relationships between stakeholders and Ministry, MSFD and Barcelona RSC | |
| Italy | ISPRA | Research centre | Elaborate standard protocols; Collect and analyse data; regularly worked with stakeholders | Yes, since 2011, in collaboration with other stakeholders |
| | IAMC-CNR (Sardinia island) | Research and rescue centre | Elaborate standard protocols; Collect and analyse data; regularly worked with stakeholders | Yes, data from Sardinia island since 2008 |
| | Stazione Zoologica Napoli Rescue Centre | Rescue centre | Collect and analyse data from Western Mediterranean Sea sub-Region | Charged by ISPRA to collect new data |
| | Istituto Zooprofilattico Sicilia | Veterinary Medical Research Institute Sicily | Collect and analyse data from Central Mediterranean Sea sub-Region | Trained and charged by ISPRA to collect standardized data |

| Country | Stakeholders | Type of activity | Description of activity | Available data |
|---|---|---|---|---|
| | Istituto Zooprofilattico Abruzzo/Molise | Veterinary Medical Research Institute | Collect and analyse data from Adriatic Sea sub-Region | Trained and charged by ISPRA to collect new data |
| | Italian Ministry of Environment | National authorities | Advisory board | |
| Greece | HCMR | Research centre | Elaborate standard protocols; Collect and analyse data | Yes, are starting collecting standardized data in collaboration with other stakeholders |
| | Archelon | Rescue centre | Provide treatments to injured sea turtles; Performs necropsies; Collect and centralize samples. | Yes, Observations on injured/dead sea turtles |
| | MEDASSET | Advocacy and awareness, networking | Can monitor and inform on the presence of sea turtles; networking and dissemination of information. | |
| | National Park Zakynthos | National park | Can participate in monitoring, observation at sea and stranding, collect samples | Observations on injured/dead sea turtles |
| | Amvrakikos Wetlands National Park | | | |
| | Archipelagos Institute of Marine Conservation | Research and conservation centre | Observations at sea, provides first aid to injured turtles and performs necropsies | |
| | School of Biology A.U. Th. | Research centre | Involved in relevant networks | |
| | School of Veterinary Medicine, Aristotle University of Thessaloniki | | Performs necropsies; Coordinates network for collecting dead individuals and distributes samples | Observations on dead sea turtles |

Training levels are variable among the stakeholders, the regular workshops organized locally will progressively regularize the differences and allow to be able to collect individuals and data on ingested litter in a standardized manner. This will also allow to better assess the existing and needed material means.

The network is being enlarged to cover most of the Mediterranean basin, simultaneously by: 1) inviting experts to training sessions, such as the one organized by ISPRA in Italy in July 2017 (Portici, Naples) where representatives of MED POL-UNEP MAP, ministries, rescue centres and research centres were invited, 2) directly contacting experts from other countries; 3) participating in local and international conferences and, 4) proposing a dissemination meeting, which is planned at the end of the INDICIT project.

# IV- Recommendations for the implementation of the indicator “Litter ingested by sea turtles”

The following recommendations are based on the results of the literature review presented above, and considering the feedback and advice from stakeholders and the INDICIT’s External Advisory Board. This preliminary study had aimed to bring out the current knowledge on litter ingestion in sea turtles in the Barcelona and the OSPAR RSCs, the Macaronesia, and the MSFD areas, and the factors which may be taken into account as biological constraints for the definition of the indicator GES and criteria. The literature review, as well as the listing of the existing networks and available means, allowed to identify the knowledge still needed to acquire, and which aims to be completed thanks to the data that the INDICIT program had started to collect in the same time with the network trained for using standard protocols from necropsies and/or observations of live turtles. The grand means which are presented here, can give an overview on the occurrence and amount of litter ingestion in the loggerhead and the leatherback, as a starting point for the discussion and next analyses which will be performed from the raw data being gathered by the INDICIT consortium.

## 1. Sampling collection and turtle handling

### a) Considering both loggerhead and leatherback turtles

At a global scale, all marine turtle species have been shown to ingest litter (Carr, 1987; Bjorndal et al., 1994; Dell’Amico & Gambaiani, 2013; Hoarau et al., 2014), with maybe a higher prevalence in the hawksbills *Eretmochelys imbricata* according to Plotkin & Amos (1990) and Schuyler et al. (2012), or in green *Chelonia mydas* and leatherback turtles according to Schuyler et al. (2014) with a larger sample size compared to the previous study. Balazs (1985) reported the green turtle as the most likely species to ingest litter, followed by the loggerhead, the leatherback and lastly the hawksbill turtle. The observed differences in the probability of litter ingestion may be related to species’ diet, its possibility to differentiate natural food from litter and the retention time in its digestive tract (not enough known at this time; see below). This also depends on the species’ spatial and feeding behaviour and the risks to encounter high quantity of debris at sea, which vary locally (Schuyler et al., 2014; Darmon et al. 2016; see below).

In the framework of the monitoring of litter abundance and impact, two qualities for an indicator should be retained: 1) the selected species must have a large distribution and abundance, so that the individuals could be easily collected (see below) and the sub-regions could be compared; and 2) the spatial and temporal tendencies in litter ingested by the collected individuals must be comparable within and between species (see below). The loggerhead turtle was suggested as indicator (ECAP pilot indicator 18, UNEP-MAP MEDPOL, candidate for OSPAR IV), and the leatherback turtle, being possibly more abundant in the northern Atlantic side of the targeted area, was also proposed to be considered (Claro et al., 2014). In the first part of this study, our state of the art in the prevalence (number of case in the tested population) and the quantity of ingested litter specifically in the areas targeted by the OSPAR and Barcelona RSCs as well as the MSFD areas, showed indeed that both the loggerhead and the leatherback turtles are highly concerned (from respectively 63 and 48 found records): The first evaluations from the mean numbers presented in literature, and considering studies’ sample size, showed an average of 46.82 ±29.27%; weighted mean of 39.34 ±22.18%; N = 30 studies) in the loggerhead (from both dead and alive individuals combined) and 77.33 ±28.65%; weighted mean of 87.13 ±7.63%; N = 6 studies) in the leatherback (from dead individuals).

It is likely that the necropsies of individuals found dead were not systematically practiced, especially in the oldest studies, and the use of a standard protocol appeared quite belatedly. Camedda et al. (2014) was the first published study using the MSFD protocol for alive turtles (Galgani et al., 2013). At this time, the occurrences of litter ingestion are badly estimated. This is even more the case for the quantities of ingested debris (see below), which were rarely and differently assessed among authors, making impossible a general description and a comparison between studies' results. Raw and standardized data are needed to provide reliable estimations and compare species with each other.

### b) Collection of individuals

The acquisition of knowledge, thanks to the collection of more data, is needed to compare litter ingestion between the provenance of the individuals, either stranded or bycaught, and the modes of capture (fishing gears) in the latter case. It has been hypothesized that bycaught individuals were more representative of the litter abundance at sea than stranded individuals, who could be in worst health condition compared to the former (Claro et al., 2014). However, at this time, there is clearly no proof in that sense, bycaught individuals could also have less swimming abilities, or they could tend to be recaptured ("trap-happy" behaviour). The decision for the stratification of the data according to the provenance of individuals should be complemented by further research on: 1) the difference of litter ingestion between stranded and bycaught individuals; and 2) capture-recapture history for identified individuals in order to evaluate if captures are random.

Our literature review indicated that the leatherback individuals were mostly found stranded, while the loggerheads were either found stranded or bycaught. However, the collection method may vary among countries according for example, to the development of the observation networks and their experience, to the local turtle densities and to the configuration of the coastal environment which influences both the accessibility and the detectability, and the local different fishing effort. The dissemination of tool kits and regular training sessions are necessary to improve or maintain, and homogenise the expertise of observers/collectors. The fishing activities (e.g. kind of net, duration of a set of fishing net, distance to the coast, etc.), which may also vary among countries (Hernandez, 2011; Sacchi, 2015), are also likely to influence the probability to capture sea turtles. Working closely with fishermen is necessary to collect bycaught individuals. Building a relationship of trust is important in order for fishermen to return all bycaught turtles to the rescue centres or the authorities in charge of the collection. Turtles seeming in good physical condition should also be recovered, so that the presence of debris items in the faeces (see below) and the absence of injury can be verified before release. Finally, it is important to disseminate the local procedures to alert and collect the individuals.

The question of the storage of individuals and samples is also raised. Dead individuals should ideally be necropsied upon their discovery, because freezing may interfere the characterization of the health status. If this is not possible, freezers must be large enough to store individuals. For the observation of live individuals, large pools are necessary to anticipate a monitoring for a long duration.

### c) Enlarging the data sets to better assess if a stratification is needed

Another question is the origin and health before the death of the tested individuals. Since it is necessary to have a good representation of the litter in the environment, the collection of individuals should be as random as possible. Both dead and alive individuals should not have ingested litter due to a bad health condition preventing them to eat normally, which could over-estimate litter ingestion.

The literature we found clearly shows negative impacts of the ingested litter and body condition, both in alive and dead turtles, but it is impossible from the current state of knowledge, to determine if the bad condition was the cause or the consequence of litter ingestion (see below). According to Matiddi et al., (2017), in order to stratify the sample, it is better to exclude from the analysis animals unable to feed from a long time before sampled. For this reason, turtles with a completely empty digestive tract, with neither litter nor food or natural remain, should not be considered.

Moreover, the recovery mode of the individuals may interfere in the evaluation of litter ingestion if e.g. stranded and bycaught individuals do not have the same probability to ingest litter. On average in the available literature for RSC and MSFD areas, the occurrence of litter ingestion in loggerheads was higher in stranded individuals (54.77 ±31.47%; N = 9 records) than in bycaught individuals (17.43 ±4.62%; N = 3 records). Contrary to fulmars (OSPAR indicator), which can strand massively following storms, sample sizes will probably be lower with sea turtles in a short duration.

### d) Working with both alive and dead loggerhead turtles

Among the literature found, the collection of litter in live rescued individuals occurred in 11 studies (Appendix 1). Casale et al. (2016) found a lower occurrence of debris ingestion in dead (N = 29 individuals) compared to live individuals (N = 538 individuals, observed from 1 to 534 days) collected around the Lampedusa island, and decided to work only with live individuals due to the larger sample size. In this study, we detected no significant difference in the occurrence of litter ingestion between dead and alive turtles, on the basis of the literature we found and targeting the RSCs and MSFD scales (grand mean of 51.55 ±29.66%; weighted mean of 37.96 ±5.36; N = 20 studies) from necropsies and 36.27 ±31.94%; weighted mean of 32.25 ±3.66; N = 6 studies) from faeces). We thus combined the data collected from the two protocols.

Collecting data from necropsy allows the prospection of the entire digestive tract and find all the ingested items with almost certainty. To this end, necropsies would provide more accurate data than observation of the excretions. Schuyler et al. (2014) indeed found necropsy to be the most effective method. For the observation of alive individuals, several biological and environmental factors may influence the individual's possibility and duration of excretion of the ingested litter, such as the quantity and shape of the items, its health status and stress level, as well as the administered food and the water temperature, which can influence the digestive transit process, but which can be controlled. However, recovering the faeces of the individuals requires an equipment for filtering the entire tank water in a 1 mm mesh filter at regular intervals.

In the framework of the indicator, all individuals should be collected and tested. The acquisition of more data is indeed necessary for a more powerful statistical test based on individuals (raw data) and confirm that the data can be combined, or inversely that they should be treated separately. At this time, every opportunity to examine bycaught and stranded individuals should be used (Bjorndal et al., 1994). Alongside gut contents from necropsied turtles, faecal samples from live specimens should also be analysed. This may also offer insights into survival, partial or total digestion, and comparisons with dead turtles with plastic loads (Witherington, 2002; Hoarau et al., 2014).

### e) Sanitary precautions

A complete waterproof lab coat, covering the entire body (e.g., one-piece suit), is ideal for necropsies in order to protect from projections, because contamination, even rare, could be dangerous for the manipulator. Two pairs of gloves above a cut-resistant glove is also more secure, the first glove could be removed if dirty, e.g. for filling the necropsy report (J. Befort, veterinarian in a French veterinary laboratory, personal communication). If necropsy must be done on beaches, especially for the leatherback turtle, it is also necessary to protect bystanders (e.g. the tourists). Practicing a necropsy of a sea turtle can be difficult, training is necessary and working with veterinarians can facilitate the manipulations. The work with live individuals also requires a protection with a lab coat and gloves, necessary both for the manipulator and for the turtle, which also might be not protected from human disease.

## 2. Indicator criteria

### a) Parameters to measure the Good Environmental Status

The GES must be evaluated through both the occurrence of ingested litter and the quantity of debris items ingested by the individuals. The quantity should be systematically and homogenously assessed by the authors in order to compare and decide for a parameter, among the dry mass, the volume, the number of items or their size. The former, probably correlated with the other (Darmon & Miaud, 2016), seems easier to collect. Indeed, evaluating the volume can be hard depending on the type of litter. A methodology would also be necessary to consider the size of the items, which depends on its shape. While in its definition (JRC, 2013), the indicator consider both micro (1-5mm) and macro 💿5mm) items, The INDICIT consortium proposed to classify items in size classes (micro (1-5 mm), meso (5 mm to 2.5 cm) and macro (>2.5 cm)). The collection of such information is proposed as an optional parameter in the INDICIT standardized database, in order to evaluate the specific impact of each of these class sizes.

Acquiring more standardized data will allow comparing the parameters and discuss the statistical approaches, e.g., calculating the averages based on all the tested individuals (population level) or only on the individuals having ingested debris, and defining threshold or tendencies. The discussion should also deal with the definition of the GES. Matiddi et al. (2017) proposed a definition of a GES for litter ingestion in sea turtles in the Mediterranean Sea, on the basis of data collected along the Italian coast between 2011 and 2014. Following the Fulmar EcoQO definition, a first scenario was elaborated as: "There should be less than X% of loggerheads having Y g or more plastic in the stomach in samples of 50-100 stranded loggerheads from each sub-region", where Y is the average value of ingested plastics and X the percentage of sea turtles with more grams of plastic than Y. In the second scenario, harm on turtles caused by litter was evaluated, considering that an animal with more plastic than food remain is in a really bad health condition. The second scenario was elaborated as follow: "There should be less than X% of loggerheads having more plastic grams than food remains (FOO) in the stomach in samples of 50-100 stranded loggerheads from each sub-region", with no fixed value, and plastic grams were compared with food remain for each loggerhead. Dell'Amico and Gambaiani (2013) also proposed three scenarios, the first, which the authors themselves consider as non-realist, targeting an absence of marine litter among 50 necropsied turtles; the second, also non-realist, with no necropsied turtle with more than 0.1g of debris items in their digestive tract among 50 individuals; and the third based on the minimum occurrence and quantity of ingested litter found in all the prospected area (i.e., "there should be less than 35% (the minimum observed in literature at the time of Dell'Amico and Gambaiani's study) with more than 0.1 g of debris in their digestive tract among 50 individuals"), as could be expected in the less polluted areas.

At the present time, the minimum occurrence was found in Sardinia by Camedda et al. (2014) who observed 14.04% of litter ingestion on both dead and live loggerheads (N =

121 individuals), and the minimum mass of ingested litter was found by Lazar & Gracan (2011; 0.08 ± 0.18 g, N = 54 necropsied individuals). The UNEP's report in 2015, gave a first proposal for a maximum threshold between 40 and 60% occurrence of litter ingestion among the necropsied loggerhead turtles, and between 1 to 3 g in terms of dry mass, at the scale of the whole Mediterranean basin. The first evaluations of 46.8 % found by averaging the results provided in the literature we found for the dead and alive loggerheads respect this threshold. However, the evaluated standard deviation of 30.1% demonstrates a high variability within the data and highlights the need of more data at a large scale to provide more accurate results, propose a threshold, also for the leatherback, and select a GES, for example based on the proposal of Dell'Amico and Gambaiani (2013) or Matiddi et al. (2017), or on a percentage of decrease (tendency) of the occurrence and the grams of ingested litter during a determined time period.

This must also allow to verify if and what biological constraints to consider for the stratification of the data (e.g. between species, between individuals' developmental stage, etc.).

## b) Biological constraints

Van Franeker and Meijboom (2002) proved that all data can be combined to employ the OSPAR indicator "Litter ingested by fulmar". Indeed, despite juveniles ingest more litter, both in terms of frequency and quantity, than adults, the temporal and spatial variations in the frequency and quantity of litter ingestion are the same for the two age classes. Therefore, the data do not need to be stratified, the data from both juveniles and adults can be combined.

For the sea turtle indicator, literature on the factors which could influence litter ingestion is scarce and results highly vary among studies, highlighting that raw standardized data are necessary for the acquisition of further knowledge and powerful statistical tests. Regional differences in individuals' behaviour are also important to consider, e.g. related to oceanography and availability of resources. These processes are likely to differ among the ocean and the Mediterranean Sea, a comparatively small and almost closed area, which individuals could possibly cross more quickly and in which neritic habitats could be more accessible to juveniles than in the ocean. Although further knowledge must be acquired, meanwhile, the available literature showed no influence of the factors we tested, in the RSC and MSFD area:

- **Individuals' like history stage**

The life history stage (or developmental stage) is likely related to individual size and swimming abilities, which influences the feeding behaviour. Anthropogenic debris accumulates in oceanic gyres (Lebreton et al., 2012) in which one might expect oceanic-phase turtles to be more vulnerable to ingest litter than coastal foragers. At a global level, studies reported 49.2% of oceanic turtles' ingested litter (Parker et al., 2005; Boyle & Limpus, 2008; Frick et al., 2010; Parker et al., 2011). Casale et al. (2008) investigated loggerhead turtles bycaught in oceanic waters in longlines and those bycaught in nearby benthic waters by trawls around Lampedusa Island, and found respectively 64% and 22% with ingested litter. On the other hand, the comparison of 2 populations of similarly sized juvenile loggerhead turtles with different foraging strategies showed that 35% of animals that foraged in the open ocean had ingested litter (Parker et al., 2005), whereas none of the coastal benthic-feeding turtles (Peckham et al., 2011). Other studies involving stranded turtles reported that smaller oceanic turtles are more vulnerable to ingest litter than larger ones (Plotkin & Amos, 1990; Schuyler et al. 2012). Balazs (1985) presented similar results: 69% of immature turtles ingested litter, whereas 31% of adult turtles ingested debris. This raises the question of the highest vulnerability to litter ingestion of young oceanic individuals compared to older benthic-feeding ones, with possibly more risks of occlusion and perforation caused by the ingested debris items due to their relatively smaller and thinner digestive system (Schuyler et al., 2012, Nelms et al., 2016). However, the available literature at the RSC and MSFD areas in which the indicator would be applied, sometimes showed a correlation between turtles' size and the number of ingested debris items (e.g., Campani et al., 2012) or length of items (Pham et al., 2017). On the contrary, Matiddi et al. (2017) observed no correlation between CCL vs grams (Spearman correlation = 0.08) or CCL vs number of items (Spearman correlation = 0.04). Furthermore, the turtles' mass does not influence the occurrence of litter ingestion (Lazar & Gracan, 2011; Camedda et al., 2014; Darmon et al., 2016).

As postulated above, a hypothesis is that possible differences in diet and litter ingestion between age classes may be not significant in the Mediterranean Sea compared to the ocean, the shift between oceanic/pelagic stages being probably less strict in this area compared to the ocean (Casale et al., 2008). Accessing to rawer data, considering the size and the capture mode of the individuals and the presence/absence of ingested litter, will allow to verify this hypothesis and compare variations in developmental stage and habitat use. Moreover, as shown for the fulmar indicator, comparing the variations in litter ingestion between the stages is important to take decision for the development of the indicator. In this respect, a standard method to measure individual's size and a standard typology of collection method, especially for bycatch gears, is necessary (see below).

- **Sex**

There is no sufficient data to test for the effect of sex on litter ingestion. Bjorndal et al. (1994) found that females ingest litter more frequently than males but as much in terms of mass and volume, their results being mostly based on the green turtle. On the contrary, Lazar and Gracan (2011) and Darmon and Miaud (2016) noticed no effect of sex on litter ingestion. In order to confirm that data on both males and females can be combined for the use of the indicator, further studies are needed to investigate sex-based differences and variations in plastic ingestion.

- **Health status**

As at a global level (Nelms et al., 2016), many of the turtles examined in the available literature did not ingest large quantities of debris items. However, for several, if not most of them, this resulted rarely to direct mortality, but in negative consequences for body condition due to gut perforation, obstruction or infection, leading to troubles in feeding behaviour and emaciation. This impact for health condition is also caused by the ingestion of plasticizers associated with plastics, the most common type of litter consumed by both loggerhead and leatherback turtles, such as bisphenol-A (BPA) and phthalate, (Oehlmann et al., 2009). Plasticizers might indeed function as endocrine disruptors (Krishnan et al., 1993) and thus have population-level effects as hypothesized for seabirds (van Franeker & SNS Fulmar Study Group, 2011). Floating plastics also readily absorb heavy metals and other toxins and can release them into the animal's tissues upon ingestion (Teuten et al., 2009).

Some authors found more litter in individuals in bad condition compared to healthy individuals (e.g., Travaglini et al., 2013, in Dell'Amico & Gambaiani, 2013), but considering the entire available literature at the RSC and MSFD areas, no clear relationship between individuals' health condition and ingested litter appeared. It was not possible to test the effect of health status on litter ingestion, either measured from observed injuries, carapace length or body mass. Therefore, raw data shall be analysed to test the influence of body condition (e.g. carapace lengths, mass, body condition indices; see below) on litter ingestion.

## c) Spatial and temporal scale of use

Two major criteria for the implementation of the indicator "Litter ingested by sea turtle" are the unit scale at which the GES must be defined and compared. The spatial management unit for the application of the indicator and the definition of GES is determined by the spatial scale at which no difference in litter ingestion does significantly appear. The state of the art in litter ingestion underlined no spatial differences within the RSCs and MSFD scale for the loggerhead turtle, both between the Atlantic and the Mediterranean (respectively 53.6% and 42.3%, with a mean dry mass of 13 g and 4.8 g) as well as among countries. However, our literature review clearly highlighted the need for more data for a powerful statistical test, both for the loggerhead and for the leatherback turtles.

At a global level, Balazs (1985) reported no correlation between high proportions of litter ingestion at locations where stranded turtles were found and areas of high litter concentrations as determined from ocean-current modelling. Nonetheless, turtles surveyed in undeveloped areas could also ingest litter (e.g. Tourinho et al., 2010). In all regions studied by Schuyler et al. (2014), aside from the Persian Gulf, there was no discernible geographic pattern of litter ingestion relative to global models of debris distribution.

Given that most turtles migrate long distances during their post-hatchling pelagic phase and during breeding migrations (Musick & Limpus, 1997; Luschi et al., 2003), they are highly likely to encounter ocean-borne litter during their life history. Turtles may encounter and ingest the debris far from where they are found stranded. Since Balazs (1985)'s study, the plenty of data which have been acquired on litter ingestion and litter abundance and distribution should be compared, even if they are non-standard. In a second instance, the homogenised approaches disseminated thanks to the MSFD programs in the framework of the Descriptor 10 shall allow the use of standard and thus comparable data. Several approaches can be combined to help define the indicator management unit, such as modelling the spatial distribution of risks for turtles to encounter litter based on either simulation (Schuyler et al., 2013) or empirical data (Darmon et al., 2017). The acquisition of large data set of raw data at large spatial scale shall allow to determine the indicator spatial units by comparing litter ingestion with these predicted risky areas and maps of major oceanographic process (Darmon et al., 2016).

The temporal unit of the indicator is the temporal window at which tendencies should be measured and thus at which restauration measures could have a significant impact on litter ingestion in sea turtles. For example, in the framework of the OSPAR Fulmar indicator, temporal trends are considered according to the situation over the most recent 5-year period. During this period, averages refer to population, calculated over all individuals including the birds that do not have ingested plastics. In sea turtles, Schuyler et al. (2014) showed, at the global level, different temporal tendencies in the probability of litter ingestion among species from 1985 to 2012, with an increase of approximately 30% of the probability to ingest litter for a green turtle in 1985, to nearly 50% in 2012. These authors showed a not significant temporal increase for the loggerhead, and for the leatherback, a significant increase when considering historical data in their analyses, but a stabilisation after 1985, which suggested a saturation. These results are consistent with those of Mrosovsky et al. (2009), who also found that litter ingestion by leatherback turtles stabilized in the 1980s. In the available literature, the first reports on litter ingestion date from 1967 for the loggerhead and 1968 for the leatherback. However, since then, the collection of data was not systematic and barely comparable among studies, preventing to test for temporal tendencies. The progressive collection of standardized data will allow evaluating temporal trends, e.g., as for the Fulmar, by assessing the linear regression between individuals on the year of collection over the past e.g. 1, 5 or 10 years ("recent trend"; Van Franeker and Meijboom, 2002).

### d) Sampling size

The sampling size is highly variable among authors. Data is necessary to evaluate the plateau at which no more occurrence and no more quantity of ingested litter can be detected. It could be reached thanks to simulations based on reliable estimations based on larger datasets than those available today.

## 3. Through the necessary acquisition and analyse of standardized data

Monitoring the impacts of marine litter on biota needs a strategy to be developed, which not only includes the collection of data on occurrence, quantity and constraints on litter ingestion, but also research for the acquisition of new knowledge to support the monitoring efforts, taking into account all factors before defining targets and acceptable levels of harm. In a management perspective, as much data as possible must be collected before evaluating if stratification is needed. Moreover, the cases of absence of interaction between marine turtle and plastic litter are barely reported in literature (only two studies to our knowledge, Flint et al., 2010 and Reinhold, 2015), whereas important to consider for a better understanding of the spatio-temporal trends in rates of interactions.

Characterizing the collected individuals is important to evaluate if body size and health status must be considered in the use of the indicator. Various parameters and different measure approaches were found in the available literature. A common methodology is important, for example to measure the curved carapace length in the loggerhead or the straight carapace length is the leatherback. Establishing on a common typology for identifying the health status or the possible cause of death, especially in case of bycaught or entanglement (see feasibility study for Indicator "Entanglement of marine fauna in litter"), is also needed. Various measures such as on carapace lengths, body mass (in loggerhead), sex, fat reserves and description of possible injuries should ideally be collected. Integrating body condition indices into necropsy practices will generate a better understanding of the sublethal impacts of plastic ingestion, such as malnutrition and the absorption of toxins (Bjorndal et al., 1994; Gregory, 2009; Labrada-Martagón et al., 2010). It may also be useful to record conditions such as the presence of fibropapillomatosis or epibiotic loads (such as barnacles) as they are also often used as indicators of health (Aguirre and Lutz, 2004; Stamper et al., 2005).

Various measures of the quantity and the description of the ingested litter are required in order to compare them and decide among stakeholders which parameters must be retained according to their sensitivity (significant spatio-temporal tendencies) and feasibility in the field and laboratory. This is also important to be sure of what the indicator indicates. While most of the authors observed "plastic" as the main type of ingested litter, the MSFD guidelines (Galgani et al., 2013)'s categories SHE (sheet like items), THR (threadlike materials) and FRA (fragments) are the main plastics found. This may suggest for example that the indicator could be good to monitor policy aiming to reduce plastic bags. All these different parameters were discussed within the INDICIT consortium, which proposed and started to disseminate standardized measures (see Appendices 2 and 3, see IV.3.2.). A protocol based on the MSFD guidelines was elaborated, with basic and optional parameters, the former being the minimum necessary to define the indicator criteria, the latter providing further knowledge for more accuracy (Appendix 3). Training sessions are organized locally. Toolkits with e.g., video-tutorials, are being elaborated. National workshops could also be organized so that stakeholders could share their expertise with practical advice (e.g., monitor live individuals, provide treatments, necropsy leatherback individuals, etc.).

## V – Summary of the main results

### From literature:

- ***Litter ingestion:***

  - Studies are barely comparable, standard data being rarely collected;
  - The occurrence of litter ingestion in the MSFD and RSCs OSPAR and Barcelona areas is high. The average weighted mean (grand mean from literature) for *Dermochelys coriacea* is 87.1% and 39.3% for *Caretta Caretta*;
  - The quantity of ingested litter has rarely been measured, and authors rarely used the same parameter.

- ***Sample and data collection:***

  - There is no observed difference between the occurrence of litter ingestion between live and dead loggerhead turtles (respectively 32.2% and 37.9%), but few studies worked with live individuals and even less used the two protocols;
  - The mean occurrence is found to be higher in stranded (54.7%) compared to bycaught (17.4%) individuals, but further analyses are necessary to evaluate the differences in litter ingestion according to individuals' origin and health condition, by considering sampling and fishing effort;

- ***Biological constraints:***

  - The variation of litter ingestion occurrence according to the individual's size or health status depends on the considered parameter, highlighting the importance to develop specific typologies/protocols to evaluate body condition and that raw data are necessary to better test these effects.

- ***Units:***

  - The GES spatial unit cannot be assessed with the available data. The acquisition of raw data will allow to test the significant difference in litter ingestion at various scales (significant differences would imply to define different GES for each area);
  - The temporal unit cannot be assessed with the available knowledge. Raw data are needed to perform linear regressions at various temporal scales and assess if temporal tendencies are significant.

### Strategy to develop the indicator:

- A large standard dataset is necessary for further and powerful statistical tests in order to identify possible biological constraints to use as criteria;

- All data must be considered (both loggerhead and leatherback turtles, both bycaught and stranded individuals, both live and dead loggerheads, individuals in bad condition and healthy individuals…) for further analyses with powerful tests and then define if stratification is necessary;

- Several GES scenarios are proposed in the literature, but rarely considered individual health and digestive capacity. These scenarios will be discussed and tested by the INDICIT consortium.

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# Appendix 1. Literature review on litter ingestion in the Regional Sea Conventions OSPAR, HELCOM and Barcelona and the Marine Strategy Framework Directive areas

The two following tables presents all the literature reviewed respectively for the loggerhead turtle and the leatherback turtle according to the Area (Atlantic then Mediterranean), the country and the authors of the studies, successively for the loggerhead and the leatherback turtles. The columns specify: the region within the country (column "Region"); the number of individuals tested in the corresponding study ("N"); the percentage of individuals having been found with ingested debris among all the tested individuals ("%"); the type of ingested debris items, according to the MSFD classification (Galgani et al., 2013; "Type"); the amount of ingested litter, in terms of dry mass in grams, volume in millilitres, number of items, and size in cm, $cm^2$ and sometimes $cm^3$ (respectively columns "Mass", "volume", "Number", "Size"); the range of years considered in the corresponding study ("Year Min" and "Year Max"); the individuals' mean curved carapace length in centimetres ("CCL"), the protocol used by the authors to measure litter ingestion ("Protocol") and the way the individuals have been collected ("Collection mode"). The empty boxes correspond to a non-available information. The records in grey and italic were not considered to calculate the averages (see details explanations in the text).

## Loggerhead turtle *Caretta caretta*

| N° | Area | Country | Region | N | % | Type | Mass | Volume | Number | Size | Year Min | Year Max | CCL | Protocol | Collection mode | Authors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Atl | France | East coast | 25 | 88 | Plastics, SHE, THR, FRA | 8,06 ±23.96 | 6.88 ±17 ml | 4.32 ±3.98 | 3.98 ±2.61 | 1995 | 2016 | | Necropsy | Stranding | Darmon & Miaud, 2016; Darmon et al., 2014a, b, c; Darmon et al., 2016; Darmon et al., 2015 |
| *2* | *Atl* | *France* | *South West* | *6* | *33.33* | *Plastics, THR* | | | | | *1998* | *1998* | | *Necropsy* | | *Duguy et al., 1999* |
| *3* | *Atl* | *France* | *South West* | *20* | *5* | *SHE, FOA, other* | | | *2* | | *1988* | *1995* | *134-151* | *Necropsy* | | *Duguy et al., 1998* |
| *4* | *Atl* | *France* | *South West* | *4* | *25* | *Plastics* | | | | | *2002* | *2002* | *24-148* | Necropsy | | *Duguy et al., 2002* |
| 5 | *Atl* | *France* | *South West* | | *15.7* | | | | | | 1998 | 2008 | | | | *Morinière & Dell'Amico, 2011* |
| *6* | *Atl* | *Portugal* | *Azores* | *3* | *33* | *Plastics, HOO, THR* | | | | | *2004* | *2008* | *64,1; 53; 37.3* | *Necropsy, Visual observation* | *Stranding following bycatch entanglement* | *Barreiros & Raykov, 2014* |
| 7 | Atl | Portugal | Azores | 4 | 18.75 | FRA, POTH, FOAM | | | | | 1989 | 1989 | 13.6-29.8; 18.2 ± 7.8 | Faeces | Stranding, bycatch | Frick et al., 2009 |
| 8 | Atl | Portugal | Azores | 12 | | | | | | | 1986 | 2001 | 9.3-56; 28.3 ± 18.0 | Necropsy | Stranding, bycatch | Frick et al., 2009 |
| 9 | Atl | Portugal | Mainland | 95 | 58.95 | Plastics (56.8%) (SHE: 45.3%; THR, IND, FOA, FRA), Other than plastics, NFO | 1.35 ± 4.40 g; max 78; 96.8% individuals with less than 5 g | | 9.68±16.79 items; 76.8% individuals with less than 10 items | From 1.4 cm±0.83 to 16.9 cm±2.98 | 2010 | 2013 | 25.4 -75.5; 49.8±9.3 | Necropsy | Stranding | Nicolau et al., 2013; Nicolau et al., 2016 |

| N° | Area | Country | Region | N | % | Type | Mass | Volume | Number | Size | Year Min | Year Max | CCL | Protocol | Collection mode | Authors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | Atl | Portugal | Azores | 24 | 83.33 | SHE, FOA, THR, IND | 1.07 ± 0.41 g | | 15.83 ± 6.09 (± SE) | 1 mm-3,1 cm; 95% <80mm; mean 20,3 ±1,6 mm (decreasing length from Oesophagus, stomach, intestines). | 1996 | 2016 | 9.4-71; 32.35±20.16 | Necropsy | Stranding, Bycatch | Pham et al., 2017 |
| 11 | Atl | Portugal | Madeira | 3 | 33 | FRA | | | 1 | | 1968 | 1968 | | Necropsy | | Brongersma, 1972 |
| 12 | Atl | Portugal | Madeira | 1 | 1 | Plastics (FRA) | | | | | 1967 | 1967 | | | | Brongserma, 1968, 1969 in Balazs, 1985 |
| 13 | Atl | Portugal | Madeira | 5 | 100 | plastics (FRA, THR, IND), PAP, TAR, RVA (glass) | | | | Glass up to 4 cm long, nylon 5 cm long, FRA up to 1 cm long | 1979 | 1981 | 21.5-52 (SCL) | Necropsy | Purchase to fishermen, bycatch | Van Nierop & Den Hartog, 1984 |
| 14 | Atl | Portugal | Azores | 5 | 100 | PAP, THR, FRA, TAR | | | 1 PAP, 5 THR, 6 FRA | 3 x 3 cm (PAP), 1-3 cm (THR), 4x1x1 (THR (ball)), 1 x 0,5 cm (FRA) | 1979 | 1981 | 26 (SCL) | Necropsy | Purchased | Van Nierop & Den Hartog, 1984 |
| 15 | Atl | Spain | Balearic islands | 1 | 1 | FRA, THR, TAR, RUB KIT (onion) | | | | | 1975 | 1975 | 40 | | | Salvador, 1978, in Balazs, 1985 |
| 16 | Atl | Spain | Canary islands | 128 | | TAR, THR, HOO (25,7%) | | | | | 1993 | 2001 | | Necropsy | Stranding | Oros et al., 2004 |
| 17 | Atl | Spain | Canary islands | 58 | 0 | HOO, THR (15,51%) | | | | | 1998 | 2000 | 20-75 | Necropsy | Stranding | Oros et al., 2003 |
| 18 | Atl | Spain | Canary islands | 906 | 1.30 | SHE, FRA, THR | | | | | 1998 | 2003 | 37.1 | Faeces; Necropsy | Stranding | Calabuig et al., 2004 |
| 19 | Atl | Spain | Canary islands | 93 | 0 | THR, HOO, TAR | | | | | 1998 | 2001 | Juveniles | Necropsy | Stranding | Oros et al., 2005 |
| 20 | Atl | Spain | Canary islands | 32 | | THR, HOO(37,5%) | | | | | 2002 | 2005 | Cc (41±11.7), Cm (52cm), Dc (231kg) | Necropsy | Stranding | Oros et al., 2009 |
| 21 | Atl | Spain | Canary islands | 2 | 0 | Empty | | | | | 1994 | 1997 | 31; 33 | Necropsy | | Oros et al., 2004 |
| 22 | Med | Croatia-Slovenia | Adriatic | 54 | 35.2 | Plastics (69,4%), | 0.08 ± 0,18 g | | 4.3 ± 6,6 | 1–16; 3,7 ± 1.8 cm | 2001 | 2004 | 25-79.2 | Necropsy | Stranding, bycatch | Lazar & Gracan, 2011 |

| N° | Area | Country | Region | N | % | Type | Mass | Volume | Number | Size | Year Min | Year Max | CCL | Protocol | Collection mode | Authors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | SHE, THR, FOAM | | | | | | | | | | |
| 23 | *Med* | *France* | *South coast* | *20* | 35 | *Plastics, THR, HOO* | | | | | *2003* | *2008* | | *Necropsy* | *Stranding, bycatch* | *Claro & Hubert, 2011; Dell'Amico and Gambaiani, 2013* |
| 24 | Med | France | South coast | 25 | 76 | USE SHE, FRA, THR | 1,82 ±2,47 g | 4,84 ±9.02 mL | 10.05 ±13.34 | 7.74 ±21.7 | 1995 | 2016 | 30.6-77.5; 46.51±13.07 | Necropsy | Stranding, bycatch | Darmon & Miaud, 2016; Darmon et al., 2014a, b, c; Darmon et al., 2016; Darmon et al., 2015 |
| 25 | Med | France | Corsica | 2 | 100 | | | | | | 1995 | 2016 | | | Stranding, bycatch | Darmon & Miaud, 2016; Darmon et al., 2014a, b, c; Darmon et al., 2016; Darmon et al., 2015 |
| 26 | Med | France | South coast | 56 | 48.21 | | 2,02 ±2.28 g | 5,39 ±10.22 | 3.92 ±6.76 | 4.27±3.05 cm (mean of max size) | 2006 | 2016 | 25-51.5; 32±8.05 | Faeces | Stranding, bycatch | Darmon & Miaud, 2016; Darmon et al., 2014a, b, c; Darmon et al., 2016; Darmon et al., 2015 |
| 27 | *Med* | *Greece* | *Ionian see, Kefalonia islands* | *1* | *100* | *USE SHE* | | | *1* | *15* | *2000* | *2000* | *55* | *Necropsy* | *Stranding* | *White, 2004* |
| 28 | *Med* | *Italy* | *Sicily* | *22* | *91* | *Plastics (soft, 91%)* | | | | | *2007* | *2007* | *27-63* | *Faeces* | | *Armanasco et al., 2010* |
| 29 | *Med* | *Italy* | *Gulf of Naples* | | | *Plastics, TAR, NFO* | | | | | *1993* | *1996* | *≤70 cm* | *Faeces; Necropsies* | *Bycatch* | *Bentivegna & Paglialonga, 1998* |
| 30 | Med | Italy | Sicily | 32 | 93.75 | FRA, SHE | | | | | 2006 | 2007 | | | Faeces | Botteon et al., 2012 |
| 31 | Med | Italy | Sardinia | 30 | 20 | SHE, FRA, THR, FOA, POL, IND | 1,63 ± 1,02 g | | 19,58 ± 10,97 (SE) | | 2008 | 2012 | 21-73; 51.38±1.13(SE) | Necropsy | Bycatch, stranding, found in buoyancy status | De Lucia et al., 2012; Camedda et al., 2012; Camedda et al., 2014 ; Camedda et al., 2015 |
| 32 | Med | Italy | Sardinia | 91 | 12.09 | | | | | | | | | Faeces | | De Lucia et al., 2012; Camedda et al., 2012; Camedda et al., 2014 ; Camedda et al., 2015 |
| 33 | Med | Italy | Tyrrhenian Sea | 31 | 71 | Plastics (91,7%, mostly SHE) | | | 19 ± 23.84 in juveniles (N=13); 26.87 ± 35.85 in adults (N=18) | | 2010 | 2011 | 29-73 | Necropsy | Stranding, Bycatch | Campani et al., 2012; Campani et al., 2013 |
| 34 | *Med* | *Italy* | *Lampedusa island* | *79* | *51.4* | *Plastics (THR, SHE, FRA), TAR, HOO, NFO* | *(Max 47.5 g for 1 individual)* | | | | *2001* | *2005* | *24-80.3* | *Necropsy* | *Bycatch* | *Casale et al., 2008* |
| 35 | *Med* | *Italy* | *Lampedusa island* | | *44,7* | | | | | | | | | *Faeces* | | *Casale et al., 2008* |

| N° | Area | Country | Region | N | % | Type | Mass | Volume | Number | Size | Year Min | Year Max | CCL | Protocol | Collection mode | Authors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 36 | Med | Italy | Lampedusa, Sicily | 538 | 36.,4 | SHE, FRA, mostly | 0.1-9.6; median 0.7 g | | | 0.26-53; median 2 cm | 2005 | 2015 | 18.2-82; 50.8±12.3 | Faeces | Stranding, bycatch, capture | Casale et al., 2016 |
| 37 | Med | Italy | | 29 | 17.24 | | | | | | | | | Necropsy | Stranding, bycatch, capture | Casale et al., 2016 |
| 38 | Med | Italy | Lampedusa | 400 | 75 | Plastics | | | | | 2005 | 2009 | | Faeces | | Freggi et al., 2013 |
| 39 | Med | Italy | Sicily | 763 | 28 | | | | | | 2001 | 2011 | | Necropsy | | Insacco et al., 2011 |
| 40 | Med | Italy | Lazio | 23 | 47,83 | Plastics | | | | | 2015 | 2016 | | Necropsy | | ISPRA (Matiddi), 2017 |
| 41 | Med | Italy | Sardinia, Tuscany, Lazio, Campania | 120 | 85 | SHE, FRA, THR, mostly | 1,3 ± 0,2 g | | 16 ± 3 | | 2011 | 2014 | 21 – 82.7 ; 60.6 | Necropsy | Stranding | Matiddi et al., 2017 |
| 42 | Med | Italy | Sicily | 121 | 18.2 | FRA, THR, SHE, PAP, TAR | | | | | 1994 | 2003 | <70 | Faeces | Capture | Russo et al., 2003 |
| 43 | Med | Italy | Sicily | 44 | 15.9 | Plastics, PAP, TAR | | | | | | | | Necropsy | | Russo et al., 2003 |
| 44 | Med | Italy | Central and Southern waters | 341 | 67 | Plastics (76%), SHE, FRA | | | | | 1996 | 2012 | 9.4-88 | Faeces; Necropsy | Stranding, capture | Travaglini et al., 2013, in Dell'Amico & Gambaiani, 2013 |
| 45 | Med | Italy, Spain | | 155 | 50 | | | | | | 2001 | 2011 | | | | Casini et al., 2012, in Dell'Amico & Gambaiani, 2013 |
| 46 | Med | Malta | | 99 | 20.2 | Plastics, RVA, TAR | | | 1-3 items | 1mm -17 cm² | 1986 | 1986 | 20-69.5 | Necropsy; Faeces | Bycatch | Gramentz, 1988 |
| 47 | Med | Morocco | North West | 7 | 71.4 | Plastics, NFO, HOO, THR, TAR | 11,8 g | | | | 2002 | 2007 | 24-80.6; 60 | Necropsy | Stranding | Benhardouze, 2009; Benhardouze et al. 2006; 2008; 2012; 2014 |
| 48 | Atl | Morocco | North West | 13 | 53.8 | | 41,7 g | | | | | | | Necropsy | | Benhardouze, 2009; Benhardouze et al. 2006; 2008; 2012; 2014 |
| 49 | Med | Spain | Valencia | 84 | 0 | FOO | | | | | 1995 | 2013 | | Necropsy | Stranding | Domenech, 2014 |
| 50 | Med | Spain | Valencia | 64 | 83.4 | Plastics, HOO, THR, TAR, NFO, other | | | | | 1995 | 2006 | | Necropsy | Stranding | Maison, 2006 |
| 51 | Med | Spain | Valencia | 1 | | | | | | | 2004 | 2004 | | Necropsy | | Mifsud et al., 2009 |
| 52 | Med | Spain | Balearic archopelago (Majorca, Minorca) | 54 | 37.5 | Plastics, TAR, PAP, FOAM, THR, HOO, NFO (wood) | | | | 3.98 ± 8.92 cm² | 2002 | 2004 | 46.5±10.8 (continental); 50.6±5.2 (pelagic) (SCL; no significant difference) | Necropsy | Stranding | Revelles et al., 2007 |

| N° | Area | Country | Region | N | % | Type | Mass | Volume | Number | Size | Year Min | Year Max | CCL | Protocol | Collection mode | Authors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 53 | Med | Spain | East | 64 | 83.4 | Plastics (63%), FOAM, HOO, TAR, PAP | | | | | 1995 | 2006 | 32-79 | Necropsy | Stranding | Tomas et al. 2008 |
| 54 | Med | Spain | North-East | 54 | 79.6 | SHE (mostly), TAR, PAP, FOA, NFO, THR, HOO | | Mostly <20 mL | 62.8% of individuals with >1 type of debris item | | | 2002 | 34–69; 49.4±8.98 | Necropsy | Bycatch (seized by the Police) | Tomas et al., 2002 |
| 55 | Atl | *Spain* | *Canary islands* | *1* | *100* | *Plastics, TAR, THR* | | | | | *1998* | *1998* | *50* | *Necropsy* | *Stranding* | *Torrent et al., 2002* |
| *56* | *Med* | *Tunisia* | *Tunisian coasts* | *19* | *21* | *Plastics and solid waste* | | | | | *2004* | *2007* | | *Observation* | *Bycatch* | *Elouaer et al., 2008* |
| 57 | Med | Tunisia | South (Gabès) | 132 | 9.85 | Plastics, PAP, THR, HOO (5 with only hook excluded) | 0,13 - 38; 15 ± 11.45 g | | 1-3 | | 2004 | 2009 | 50-72; 59.47±6.52 | Necropsy | Stranding | Unpublished data from Bradai et al., INSTM, 2017 |
| 58 | Med | Tunisia | Centre | 41 | 41.5 | Plastics and plastics with hook and fishing line | | | | | 2006 | 2016 | 21-77; 52.5±17.36 | Necropsy | | Unpublished data from Bradai et al., INSTM, 2017 |
| 59 | Med | Tunisia | North | 14 | 50 | Plastics, PAP, and sometime also HOO and THR | | | | | 2002 | 2014 | 41-91; 73±17.46 | Necropsy | | Unpublished data from Bradai et al., INSTM, 2017 |
| 60 | Med | Tunisia | Centre + North | 67 | 9 | Plastics | | | | Up to 20 cm | 2007 | 2016 | 53-72; 62.42, ± 7.93 | Faeces | | Unpublished data from Bradai et al., INSTM, 2017 |
| 61 | Med | Turkey | | 65 | 5 | SHE, HOO | | | 1 | | 2001 | 2001 | 47-80; 67.43±9.90 | Necropsy | Stranding | Kaska et al., 2004 |

## Leatherback turtle *Dermochelys coriacea*

| N° | Area | Country | Region | N | % | Mass | Volume | Number | Size | Year Min | Year Max | SCL | Collection mode | Authors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Atl | England, Netherlands | North sea | 6 | 50 | | | | 4-17.5 cm; nylon 1-6 cm | 1969 | 1981 | 168-244 | | Den Hartog & Van Nierop, 1984 |
| 2 | Atl | France | West coast | 46 | 93.48 | 7.27±9.06 | 36.2±46.55 | 1.73±1.94 | 31.03±34.02 | 1988 | 2016 | 140.3; 93-203 | Stranding | Darmon & Miaud, 2016; Darmon et al., 2014a, b, c; Darmon et al., 2015; Darmon et al., 2016 |
| 3 | Atl | France | South West | 8 | 87.5 | | | | | | | | | Duron & Duron, 1980, in Balazs, 1985 |
| 4 | Atl | Portugal | Azores | 1 | 100 | | | 8 | | 2000 | 2000 | 144 | Bycatch | Barreiros & Barcelos, 2001 |
| 5 | Med | Italy | Tyrrhenian Sea | 4 | 100 | | | | | 1995 | 2005 | | | Travaglini et al., 2006 |
| 6 | Med | Tunisia | North | 3 | 100 | | | | | 1986 | 2011 | 191,172, 176 | Stranding | Bradai, 2017 |
| 7 | Atl | Morocco | North-West | 3 | 33 | | | | | | | 102-115 | Stranding ; Bycatch | Benharoudouze et al., 2014 |
| *8* | *Atl* | *Spain* | *Canary islands* | | | | | | | *2002* | *2005* | *Cc (41±11.,7), Cm (52cm), Dc (231kg)* | *Stranding* | *Oros et al., 2009* |
| *9* | *Atl* | *England* | *South West* | *1* | *100* | | | | | *1971* | *1971* | *172* | *Stranding, entanglement* | *Brongserma, 1972* |
| *10* | *Atl* | *France* | *South West* | *2* | *50* | | | | | *2010* | *2010* | | | *Dell'Amico & Morinière, 2011* |
| *11* | *Atl* | *France* | *South West* | *2* | *100* | | | *1, several* | | *2011* | *2011* | *154, 140.5* | | *Dell'Amico & Morinière, 2012* |
| *12* | *Atl* | *France* | *South West* | *12* | *33.33* | | | | | *1999* | *1999* | *108-159; 139.36±14.8* | | *Dell'Amico et al., 2000* |
| *13* | *Atl* | *France* | *South West* | *3* | *66.67* | | | *2, several* | | *1980* | *1980* | *148, 165, 165* | *Floating dead; Stranding, bycatch* | *Duguy & Duron, 1981* |
| *14* | *Atl* | *France* | *South West* | *3* | *0* | | | | | *1982* | *1982* | *165, 152, 115* | *Stranding, bycatch* | *Duguy & Duron, 1983* |
| *15* | *Atl* | *France* | *South West* | *3* | *66.67* | | | | | *1981* | *1981* | *133, 160, 152* | *Stranding, bycatch* | *Duguy & Duron, 1983* |
| *16* | *Atl* | *France* | *South West* | *5* | *20* | | | *1* | | *1984* | *1984* | *150.7±15.7* | | *Duguy & Duron, 1984* |
| *17* | *Atl* | *France* | *South West* | *5* | *20* | | | *1* | | *1984* | *1984* | *130, 159, 163, 142, 170, 140* | | *Duguy & Duron, 1985* |
| *18* | *Atl* | *France* | *South West* | *3* | *33.33* | | *5 L* | | | *1979* | *1979* | *150, 160, 157* | | *Duguy et al., 1980* |

| N° | Area | Country | Region | N | % | Mass | Volume | Number | Size | Year Min | Year Max | SCL | Collection mode | Authors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | *Atl* | *France* | South West | *43* | *51,1* | | *5L in 1 individual* | | *about 40 x 70 cm* | *1978* | *1999* | *112-176* | *Stranding* | *Duguy et al., 1998* |
| 20 | *Atl* | *France* | South West | *16* | *56,2* | | | | | *1997* | *1997* | *110-165; 141±11,8* | | *Duguy et al., 1998* |
| 21 | *Atl* | *France* | South West | *11* | *72,7* | | | | *Max 300 x 40 cm* | *1998* | *1998* | *108-164; 138.45±16.54* | *Stranding mostly, bycatch* | *Duguy et al., 1999* |
| 22 | *Atl* | *France* | South West | *87* | *55,1* | | *Up to 5 L* | | *Up to 300 x 40 cm* | *1979* | *1999* | | | *Duguy et al., 2000* |
| 23 | *Atl* | *France* | South West | *6* | *50* | | | | | *2000* | *2000* | *121, 129, 135, 138, 144, 160* | | *Duguy et al., 2001* |
| 24 | *Atl* | *France* | South West | *1* | *100* | | | | | *2002* | *2002* | | | *Duguy et al., 2002* |
| 25 | *Atl* | *France* | South West | *1* | *100* | | | *1* | | *2003* | *2003* | | *Stranding* | *Duguy et al., 2004* |
| 26 | *Atl* | *France* | South West | *2* | *0* | | | | | *2004* | *2004* | | *Drift, stranding* | *Duguy et al., 2005* |
| 27 | *Atl* | *France* | South West | | *0* | | | | | *1754* | *1826* | | | *Duguy, 1968* |
| 28 | *Atl* | *France* | South West | *2* | *0* | | | | | *1985* | *1985* | | | *Duguy, 1986* |
| 29 | *Atl* | *France* | South West | *2* | *50* | | | *Several* | | *1989* | *1989* | *96, NI* | | *Duguy, 1989* |
| 30 | *Atl* | *France* | South West | *2* | *0* | | | | | *1990* | *1990* | *154, NI* | | *Duguy, 1990* |
| 31 | *Atl* | *France* | South West | *4* | *24* | | | | | *1991* | *1991* | | | *Duguy, 1992* |
| 32 | *Atl* | *France* | South West | | *(1 individual)* | | | | | *1993* | *1993* | *120-167* | | *Duguy, 1994* |
| 33 | *Atl* | *France* | South West | *1* | *0* | | | | | *1994* | *1994* | | | *Duguy, 1995* |
| 34 | *Atl* | *France* | South West | *16* | *68,75* | | | | | *1995* | *1995* | | | *Duguy, 1996* |
| 35 | *Atl* | *France* | South West | *93* | *46,2* | | | | | *1998* | *2008* | | | *Morinière & Dell'Amico, 2011* |
| 36 | *Atl* | *Ireland* | | *1* | *100* | | | | *20x20cm* | | | | | *Doyle, 2007* |

| N° | Area | Country | Region | N | % | Mass | Volume | Number | Size | Year Min | Year Max | SCL | Collection mode | Authors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 37 | *Atl* | *Netherlands* | *Friesland, Wadden sea, North sea* | *1* | *100* | | | *1* | | *1968* | *1968* | *244* | *Stranding* | *Brongserma, 1972* |
| 38 | *Atl* | *Spain* | *Canary islands* | *4* | | | | | | *1993* | *2001* | | | *Oros et al., 2004* |
| 39 | *Atl* | *Wales* | *North West* | *1* | *100* | | | *1* | *23 cm x 15 cm* | *1988* | *1988* | *256.5* | *Bycatch* | *Eckert & Luginbuhl, 1988; Morgan, 1989* |
| 40 | *Med* | *France* | *South* | *1* | *100* | | | *2* | | *1985* | *1985* | *130* | | *Duguy, 1986* |
| 41 | *Med* | *Greece* | *Saronikos gulf, Aegean sea* | *1* | *100* | | | | *9,8m²* | | | | | *Katsanevakis, 2008* |
| 42 | *Med* | *Italy* | | *1* | *100* | | | | *Wood 15 cm* | *1995* | *1995* | *124* | | *Bentivegna et al., 1996, in Dell'Amico and Gambaiani, 2013* |
| 43 | *Med* | *Italy* | *Sicily* | *1* | *100* | | | | | | | | | *Jereb & Ragones, 1990, in Dell'Amico and Gambaiani, 2013* |
| 44 | *Med* | *Italy* | *North Adriatic* | *1* | *100* | | | *14* | *Min 4.5 x 2; Max 40 x 20 (SHE), 21 x 4 (FRA), 23 (THR); presence of an hygienic pad (RVA) of 15 x 5 cm* | *2009* | *2009* | *138 (CCL)* | *Stranding* | *Poppi et al., 2012* |
| 45 | *Med* | *Italy* | *Lampedusa* | *1* | *100* | *2 kg* | | | | | | | | *Russo et al., 2003* |

# Appendix 2. Example of the dissemination of the INDICIT standardized approach in the Canaries Islands

# Appendix 3. Standard basic and optionnal parameters to collect proposed by the INDICIT consortium

| General data: description of the individual and the discovery | Protocol related to litter ingestion : cells to classify by digestive section (oesophagus / stomach / intestines) for dead individuals or faeces for alive individuals | Mandatory (MSFD) parameters | **Optional parameters** |
|---|---|---|---|

| | | TITLE COLUMN | OPTIONS | OBSERVATIONS |
|---|---|---|---|---|
| **GENERAL DATA** | | **Species** | Cc / Dc | Loggerhead = Cc / Leatherback = Dc |
| | | **Tag/ chip** | NO / XXXXXXXXXXXX | If present on the flipper, report the code. If not, insert NO |
| | | **ID Code** | Local ID given by the institution / stakeholder | Ex en Italy : IT-La-01 = First turtle collected in Italy at Lazio locality ; Ex in France : 2 letters for the location/Institution _ Year _ Month_Day_N° turtle _Type of sample (Faeces/Oeso/Stom/Intest) |
| | | **Date of discovery** | XX/XX/XX | Format Day/Month/Year |
| | | **X coord** | XXXXX,XXX | X coordinate in decimal degrees of the finding location |
| | | **Y coord** | XXXXX,XXX | Y coordinate in decimal degrees of the finding location |
| | | **Status** | 1 / 2 / 3 / 4 / 5 | 1: Alive individual. From 2-5: For dead individuals, visual observation of decomposition status of the animal (See pictures in the tab "Legend Decomposition status") |
| | | **Circumstances** | Stranding / by-catch / Death at Rescue Centre / At sea finding | Stranding includes all the turtles found on the beach ; By-catch includes only animals collected by fishermen. |

| | | | | |
|---|---|---|---|---|
| | | **Cause of death** | *Bycatch related / Entanglement in debris / Ingestion of litter / Antrhopogenic trauma / Natural trauma / Disease / Oils / Healthy / Unidentified / other* | See the tab " Legend Cause of death" for precisions. |
| | | **Health status** | Poor/Fair/Good | See tab "Legend Health status" for precisions |
| | | **By-catch engine cause** | *Straight thread / trawl / drift net / fishing rod / Non identified / Other* | Only for individuals having been by-caught. Please specify in the column "Notes" the distance from the coast and the duration of the nests deployement before nests brougt aboard. |
| | | **Main Injuries** | *No/ Fracture / Amputation / Abrasion / Disease / Other* | See the tab " Legend Main injuries" for precisions (No : if good health or no visible injury). |
| | | **Affected body part** | *RFF / LFF / RRF / LRF / Neck / Carapace / Plastron / Head / Several / Other* | If the animal presents injuries, identify the affected body part. For Several or Other, specify in the column "Notes". See the tab "Legend Affected body part" for precisions. |
| | | **Entanglement type** | *Active / Passive / Undetermined* | Only if individuals have been found entangled in marine debris. Active : from active fishing gear (individual released by a fisherman, or nest cut after entanglement) ; Passive : Individual entangled. Note that presence of hook is considered as active entanglement. |
| | | **Litter causing entanglement** | *Ghost nets / Plastic debris / Nylon / Undetermined / Other* | Only if individuals have been found entangled in marine debris. See the tab "Legend Litter category causing entanglement » for explanations on categories. For Undetermined or Other, please describe in the column "Notes". |
| | | **Photo at finding** | Yes / No | Photo taken on board or on the beach before any intervention on the place of discovery |
| | | **Freezing** | Yes / No | Speciy if the dead individual has been frozen before necropsy |
| | | **Date of necropsy** | XX/XX/XX | Format Day/Month/Year ; NA for alive individual |
| | | **Standard Curved Carapace Length (CCL)** | XXX,XX | Measure (cm) of the standard curved carapace Length (measured with a tape measure above the spines), see the tab "Legend Biometry" for explanations. |
| | | **Max Curved carapace length** | XXX,XX | in centimeters, see the tab "Legend Biometry" for explanations. |

| | | | | |
|---|---|---|---|---|
| | | **Min carapace length (CCL min)** | XXX,XX | in centimeters, see the tab "Legend Biometry" for explanations. |
| | | **Straight carapace length (SCL)** | XXX,XX | in centimeters, see the tab "Legend Biometry" for explanations. |
| | | **Curved plastron length** | XXX,XX | in centimeters, see the tab "Legend Biometry" for explanations. |
| | | **Straight plastron length** | XXX,XX | in centimeters, see the tab "Legend Biometry" for explanations. |
| | | **Fat reserves** | Thin / Normal / Fat | Fat reserves are evaluated visually at the neck (e.g., a thin animal has a sunken neck, inversely to a fat animal) and with the fat abundance observed when opeing the plastron in autopsie of dead individuals |
| | | **Weight** | XXX,XX | In kilograms (2 decimals) |
| | | **Stage** | <40 cm / > 40 cm | <40 cm : young individual in an oceanic stage ; >40 cm : sub-adult or adult in a neritic stage |
| | | **Sex** | Male / Female / NI | NI=No identified, see the tab "Legend Biometry" for explanations. |
| **Litter ingestion** | **Total for the entire digestive tract** | **Occurrence ingested debris items** | Y / N | Presence of litter in the GI |
| | | **Plastic volume (ml)** | XXX,XXml | Volume (ml) of the cumulated plastic items found in the 3 sections of the digestive tract, using a measuring cylinder |
| | | **Total dry mass (mg)** | XXX mg | Dry mass (mg) of the cumulated plastic items found in the 3 sections of the digestive tract |
| | | **Total nber of plastic items** | XX | Number of all the plastic items found in the 3 sections of the digestive tract |
| | | **Total nber of micro-plastic items** | XX | Number of 1-5 mm plastic items found in the 3 sections of the digestive tract |
| | | **Total nber of meso-plastic items** | XX | Number of 5 -25 mm plastic items found in the 3 sections of the digestive tract |
| | | **Total nber of macro-plastic items** | XX | Number of >25 mm plastic items found in the 3 sections of the digestive tract |
| | | **White-transparent debris** | XX | Number of white or transparent plastic items found in the 3 sections of the digestive tract |
| | | **Dark coloured debris** | XX | Number of dark (black, brown, dark green, etc.) coloured plastic items found in the 3 sections of the digestive tract |
| | | **Light coloured debris** | XX | Number of light (yellow, pink, light green, light grey, etc.) coloured plastic items found in the 3 sections of the digestive tract |

| | | | | |
|---|---|---|---|---|
| | **Dry mass per category AND digestive section (0,01 g) TO BE DONE SEPARATELY FOR OESOPHAGUS, STOMACH AND INTESTINES (for dead individuals) OR ONLY FOR FAECES (for alive individuals)** | **Ind. Plastic** | XXXmg | Industrial plastic granules (usually cylindrical but also oval spherical or cubical shapes exist) or suspected industrial item, used for the tiny spheres (glassy, milky, ….) |
| | | **Use she** | XXXmg | Remains of sheet, eg from bag, cling-foil, agricultural sheets, rubbish bags etc |
| | | **Use thr** | XXXmg | Threadlike materials, eg pieces of nylon wire, net-fragments, woven clothing |
| | | **Use foa** | XXXmg | All foamed plastics eg polystyrene foam, foamed soft rubber (as in matras filling), PUR used in construction etc |
| | | **Use frag** | XXXmg | Fragments, broken pieces of thicker type plastics, can be a bit flexible, but not like sheetlike materials |
| | | **Other (Use Poth)** | XXXmg | Any other, including elastics, dense rubber, cigarette filters, balloon pieces, soft airgun bullets. |
| | | **Other Litter (non plastic)** | XXXmg | All the non plastic rubbish and pollutant |
| | | **Natural Food (Foo)** | XXXmg | Natural food of the animal |
| | | **Natural No Food (Nfo)** | XXXmg | Anything natural, but which cannot be considered as normal nutritious FOOD (stone, wood, pumice, etc.) |
| | **Number of items per category AND digestive section (0,01 g) TO BE DONE SEPARATELY FOR OESOPHAGUS, STOMACH AND INTESTINES (for dead individuals) OR ONLY FOR FAECES (for alive individuals)** | **Ind. Plastic** | XX | Industrial plastic granules (usually cylindrical but also oval spherical or cubical shapes exist) or suspected industrial item, used for the tiny spheres (glassy, milky, ….) |
| | | **Use she** | XX | Remains of sheet, eg from bag, cling-foil, agricultural sheets, rubbish bags etc |
| | | **Use thr** | XX | Threadlike materials, eg pieces of nylon wire, net-fragments, woven clothing |
| | | **Use foa** | XX | All foamed plastics eg polystyrene foam, foamed soft rubber (as in matras filling), PUR used in construction etc |
| | | **Use frag** | XX | Fragments, broken pieces of thicker type plastics, can be a bit flexible, but not like sheetlike materials |
| | | **Other (Use Poth)** | XX | Any other, including elastics, dense rubber, cigarette filters, balloon pieces, soft airgun bullets. |
| | | **Other Litter (non plastic)** | XX | All the non plastic rubbish and pollutant |

| | | | | |
|---|---|---|---|---|
| | | **Natural Food (Foo)** | XX | Natural food of the animal |
| | | **Natural No Food (Nfo)** | XX | Anything natural, but which can not be considered as normal nutritious FOOD (stone, wood, pumice, etc.) |
| | | **Mass of full oesophagus** | | Mass of the clamped section, with its content, before emptying it |
| | | **Volume of full oesophagus** | | Volume of the clamped section, with its content, before emptying it |
| | | **Mass of full stomach** | | Mass of the clamped section, with its content, before emptying it |
| | | **Volume of full stomach** | | Volume of the clamped section, with its content, before emptying it |
| | | **Mass of full intestines** | | Mass of the clamped section, with its content, before emptying it |
| | | **Volume of full intestines** | | Volume of the clamped section, with its content, before emptying it |
| | | **Mass of empty oesophagus** | | Mass of the emptied section |
| | | **Volume of empty oesophagus** | | Volume of the emptied section |
| | | **Mass of empty stomach** | | Mass of the emptied section |
| | | **Volume of empty stomach** | | Volume of the emptied section |
| | | **Mass of empty intestines** | | Mass of the emptied section |
| | | **Volume of empty intestines** | | Volume of the emptied section |
| | | **Notes** | | Personal remarks |

STATE OF THE ART AND FEASIBILITY STUDY
FOR THE IMPLEMENTATION OF 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**

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European Commission

INDICIT
INDICATOR • IMPACT • TURTLE

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# INDEX

# I – CONTEXT

This report is a deliverable of INDICIT Activity 2 (« Acquiring and using scientific knowledge to develop the indicators of litter impact at the (sub)regional and the whole MSFD spatial scales »), which aims to fill in the gaps of knowledge in order to evaluate the relevance of Indicator "Entanglement of marine biota in debris" (entitled "Indicator 2" for the INDICIT project, targeted species to be evaluated) and the feasibility of the potential implementation of Indicator 2, by considering the whole MSFD area, and after having selected one or several pertinent taxa (among e.g. cetaceans, birds, turtles, etc.).

The objective of this report is to provide:

1) the state of the art regarding the biological constraints, which can influence the criteria of indicator 2

   This synthesis is part of Task 2.1. "Establishment of a state-of-the-art on the biological constraints which can influence the indicators' criteria". This task aims to provide a literature synthesis, based on available grey and published literature shared by the participants or listed online, on the biological constraints, which can influence the criteria of: indicator (1) "Macro-debris ingested by sea turtle" (coordinated by CNRS); and of the 2 new indicators (2) "Entanglement of marine biota (cetaceans, fishes, birds, turtles) in debris (coordinated by MNHN), and (3) "Micro-debris ingested by fish/other biota" (co-coordinated by ISPRA and ULPGC).

2) a feasibility study of the indicator

   This study is part of Task 2.2. "Pilot study for the Indicator (2) "Entanglement with debris by marine biota". Considering the results and orientations provided by the literature review performed in Task 2.1., this Task aims to conduct a pilot study in order to evaluate the feasibility of the indicator and a long term continuous monitoring.

# II – ANALYSIS OF LITERATURE

## 1. Methods

All partners participated in the collection of literature (grey and published) and data (shared on the private area), under the coordination of MNHN, which prepared the report in collaboration with IMAR (invertebrates). Since, the objective was to evaluate which indicator is pertinent, no species was nominated specifically and the literature review focused on all the taxa relevant at the MSFD and RSC scales and on several reviews, which were used for a general overview about the entanglement at a global scale.

Literature was found online (Web of Science, Google Scholar, Mendeley, ResearchGate) using the following keywords: "entanglement", "marine debris", "megafauna", "stranding network", "rescue center", "cetacean", "sea turtle", "sea bird", "fish", "shark", "marine biota", "invertebrate", "coral", "monitoring" and "impact");

The report was reviewed by the partners and will be submitted to the External Advisory Board at the next INDICIT meeting.

## 2. Results

In order to improve the understanding within the feasibility study, we present hereafter only the global results, while specific results will be reported in the feasibility study chapters corresponding to the biota species. Six articles were examined for their global approach on entanglement (Duncan et al., 2017; Gall & Thompson, 2015; Kühn et al., 2015; Nelms et al, 2016; RAC SPA, 2017; Werner et al., 2016). Twenty-seven publications and 15 reports were selected according to the geographical range of the study.

The analysis of the literature showed that 252 species of Marine mammals, birds, fish and reptiles have been recorded to suffer from entanglement in the world (Kühn et al 2015). Twenty-six Megafauna' species were documented to be entangled by marine litter within the study area. These species are mainly Megafauna observed at sea or stranded, and invertebrates on the sea floor.

At a global scale, animals were found entangled in several kinds of litter. According to RAC SPA (2017), abandoned fishing gear fragments (nets, fishing lines, ropes, dahn lines, traps and pots) represent 72% of all observations of entanglement. The second main source of entanglement is human product packages. Based on 106 experts' opinions from 43 countries, Duncan et al. (2017) reported that in most cases, entanglement is caused by material from fishery origin, the number of entanglement in ghost gears would be slightly higher than active bycatch. However, data reporting entangled species in fishing gears rarely specify if entanglement was caused by an active gear, cut and took away by the individual, during fishing activities, or if it is due to ghost nets/gears, which were abandoned or lost, then drifting or sinking in the marine environment. In the first scenario, animals may have been first caught, and drown or chocked during active fishing activities, then be released by fishermen with a part of the gear they may have cut for gaining time. In the second scenario, animals were entangled by abandoned pieces of gears. No typology was found in the literature, which would permit to discriminate these two cases. Likewise, the size of the debris causing entanglement was not specified.

Different reasons why biota is entangled are proposed in the literature. Depending on the species, animals could be attracted by the debris, due to their specific feeding behaviour (opportunistic diet), exploratory and playful behaviour in immature individuals (Marine Mammals), anatomic features (vision/ perception abilities, which do not permit to discriminate feeding resources from debris), or other biological factors (immature sea turtles using currents are more exposed to debris aggregations in gyres). For benthic species, the entanglement occurs when debris sink towards the bottom of the sea, and some authors distinguish "smothering" from "entanglement", in particular for corals when they are covered by debris (see Kühn et al 2015 for review).

Entangled specimens of Megafauna were mainly observed stranded on beaches, during regular surveys by stranding networks or naturalists. In some cases, observations occurred during sea observation campaigns, on the sea surface or in the water column (fisheries, oceanographic sampling operations, participative campaigns). Observations on the sea bed occurred through photographic sampling during sea campaigns, diving explorations with Remotely Operated Vehicles or submarines working for scientific purposes or monitoring.

Different kinds of impact on biota entangled by marine debris are reported in the literature; these result in direct or indirect effects (see for review Gall & Thompson, 2015; Kühn et al., 2015; Nelms et al., 2016; RAC SPA, 2017; Werner et al. 2016), which can reduce the chances of survival at the individual and population levels.

Two types of effects are distinguished:

a) direct effects,

which in Megafauna consist in external injuries, limitation of mobility or immobility (entrapment), constriction and severance of arteries, suffocation or asphyxia (ligature around the neck or occlusion of the blowhole), and consecutive/or immediate death (drowning, predation, ship strikes). In the case of benthic invertebrates, direct effects consist in reduced mobility and damages (e.g. broken branches in corals);

b) indirect effects,

with reduce chances of individual survival, such as a lower ability to ingest and digest food, leading to reduced fitness, reproduction and mobility performance needed to avoid predators or for migration; necrosis and secondary infection of tissues when they have been damaged; loss of flippers; bone deformation and constrictions during immature individuals' growing process. In nesting sea turtles, females with partial or total rear flipper amputations were not able to achieve the nest successfully, which may have consequences on the population (Sanchez-Sierra, 2017). In the case of benthic invertebrates, debris may provide new substrate for their development, in particular in sponges.

In the prospected literature, we found very few information about practical operations and feasibility for monitoring entanglement. In the Mediterranean Sea, RAC SPA (2017) is the most comprehensive publication about monitoring. It provides useful information and recommendations in the perspective of choosing new indicators and monitoring procedures, and describes all the constraints (biological, methodological, environmental, logistical, conservation and regulatory), which have to be taken in account. These lists and descriptions of constraints were used below for the structuration of our feasibility study report. Specific studies, since they were based on data sets, provided at least some information about data producers, specimens/data accessibility and abundance, and the biological traits, which may be sources of bias for candidate indicator species. However, the available published data could not provide a complete view representative of the whole project area. Methods were designed according to the objective/problematic of each research work. The only existing document related to a common approach for monitoring entanglement is the guidance published in the frame of MSFD by JRC (TGML 2013).

# III – Feasibility study

## 1. Methods

All partners participated in this report and MNHN (France) was responsible for its coordination.

All types and size of debris were considered for the evaluation of the Entanglement indicator.

The pilot study consisted in: (i) Identifying existing networks (e.g. among NGOs, rescue centres, fishermen/sailors and other sea users) or the human and logistical resources, which should be necessary for the implementation of such indicators; (ii) Collecting available data (e.g. thanks to the databases shared by the participants through the project Intranet); iii) Collecting new data, following the establishment of dedicated protocols and sharing thanks to the common databases of the project Intranet.

In order to collect the information and data, a questionnaire (Appendix 1) was prepared by MNHN, revised, translated and disseminated by the other partners in their specific countries/regions.

## 2. Results

Twenty-one questionnaires were received by the INDICIT partners. Seven data sets were collected for sea turtles and birds from Greece, France, Spain, Turkey and Italy. Information extracted from these questionnaires and data, as well as from the literature, is presented hereafter by taxon.

### *Marine mammals*

#### Sensitive taxa, occurrence and circumstances

The State of the Art showed that entanglement was reported in 51 of the 123 marine mammal species at a global scale (for review see Kühn et al 2015). Furthermore, according to Baulch & Perry (2014), most part of entanglements were caused by abandoned fishing gears (97%), compared to other types of debris.

In Spain, entanglement was reported in three species (*Globicephala macrorhynchus, Balaenoptera acurostrata, Stenella coeruleoalba*) by Diaz-Delgado (2015). In the Canary Islands, Arbelo et al. (2013) described that 4.35% of cetaceans stranded (N=138) were affected by marine debris (ingestion and/or entanglement). This prevalence was slightly higher than the 1.81% observed by Diaz-Delgado (2015) in cetaceans stranded in the same area (N=320), mainly in fishing material (Figure 1).

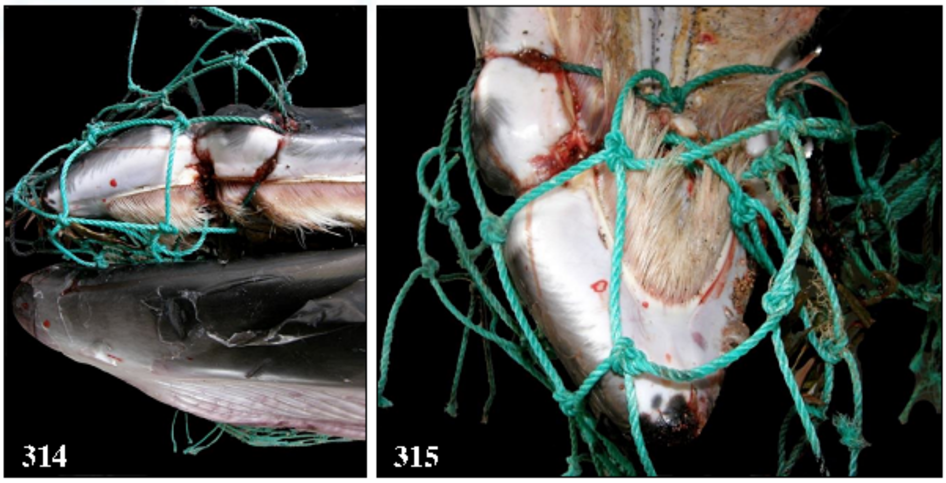


**Figure 1.** Entanglement in net of a young Minke whale (*Balaenoptera acurostrata*) stranded in the Canary Islands. A piece of net was wrapped around the upper and lower jaw and the dorsal and pectoral fins (Extracted from Diaz-Delgado 2015).

A high prevalence of entanglement has been reported in the United Kingdom. In 5 to 19% of the Minke whales observed each year were found entangled in Cornwall, between 2002 and 2015 (Table 1). Observations of entanglement of other cetacean species were also documented (Grampus griseus, Phocoena phocoena, Tursiops truncatus, Megaptera novaeangliae, Physeter macrocephalus) with prevalence values up to 8% for data sets over 10 specimens. In Halichoerus grypus, Allen et al., (2012) reported entanglement rates from 3.6 % to 5% between 2004 and 2008 (14 cases per year) (Table 1). Entanglement resulted in body constriction or/and wounds, and were mainly due to fisheries materials (for 14 out of 15 cases where debris were identifiable).

**Table 1:** Prevalence and material/items cited as responsible for entanglement of biota in the literature (INDICIT project area). Cnd.= condition; (AS) at sea, (OL) on land, (DEB) marine litter of user origin, such as plastic package, baler twine (rafia etc) or other (stake nets etc…), (FG) fishing gear : (L) lines (long lines & monofilament line pieces, hooks…), (N/T) pieces of nets or trawl nets, (R) piece of single rope (mooring, pot etc), (UNK) material/item not specified, (L) lesion, (D) death.

| Species | Country/ Ocean basin-region | Years | Cnd. | N | N entangled | Circumstances | Item/ Material | Effect | Reference |
|---|---|---|---|---|---|---|---|---|---|
| CETACEAN | | | | | | | | | |
| *Globicephala macrorhynchus, Balaenoptera acurostrata, Stenella coeruleoalba* | Spain | 2006-2012 | | 320 | 4 (1.25%) | NA | FG | NA | Diaz-Delgado, 2015 |
| COMMON DOLPHIN | | | | | | | | | |
| *Delphinus Delphis* | Scotland | 1995-1999 | | 37 | 1 (2.7%) | OL | FG (N/T) | NA | SAC Veterinary Science Division, 2000 |
| | United Kingdom | 2011 | Dead | 89 | 7 (7.9%) | OL | FG | NA | Deaville et al, 2011 |
| | Greece (North Aegean Sea) | 1998-2013 | Dead | 17 | 1 (5.9%) | OL | UNK | NA | Milani et al 2017 |
| RISSO'S DOLPHIN | | | | | | | | | |
| *Grampus griseus* | Scotland | 1995-1999 | | 34 | 1 (2.9%) | OL | UNK | NA | SAC Veterinary Science Division, 2000 |
| HARBOUR PORPOISE | | | | | | | | | |
| *Phocoena* | United Kingdom | 2011 | | 322 | 9 (2.8%) | OL | FG | NA | Deaville et al, 2011 |
| | United Kingdom | 2014 | | 336 | 1 (0.3%) | OL | FG | NA | Deaville et al, 2014 |
| | Germany | 1990-2014 | Dead | 4006 | 5 (0.1%) | OL | FG (N/T, L), | NA | Unger et al, 2017 |
| | Greece (North Aegean Sea) | 1998-2013 | Dead | 7 | 2 (28.5%) | OL | UNK | NA | Milani et al, 2017 |
| MINKE WHALE | Scotland | 1995-1999 | | 50 | 4 (0.08%) | OL | FG (R) | D | SAC Veterinary Science Division, 2000 |
| *Baleanoptera acutorostrata* | United Kingdom | 2002 | | 18 | 2 (11.1%) | OL | UNK | NA | Sabin et al, 2003 |
| | United Kingdom | 2003 | | 18 | 1 (5.55%) | OL | FG (N/T) | L | Sabin et al, 2004 |
| | United Kingdom | 2005 | | 14 | 1 (7.1%) | OL | UNK | NA | Sabin et al, 2006 |
| | United Kingdom | 2008 | | 19 | 1 (5.3%) | OL | UNK | D | Deaville et al, 2008 |
| | United Kingdom | 2010 | | 16 | 1 (6.2%) | OL | FG | NA | Deaville et al, 2010 |
| | United Kingdom | 2011 | | 14 | 1 (7.1%) | OL | UNK | NA | Deaville et al, 2011 |
| | United Kingdom | 2013 | | 16 | 3 (18.7%) | OL | FG | NA | Deaville et al, 2013 |
| | United Kingdom | 2015 | | 18 | 3 (16.7%) | OL | FG | NA | Deaville et al, 2015 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BOTTLENOSE DOLPHIN | | | | | | | | | |
| *Tursiops truncatus* | United Kingdom | 2007 | | 3 | 1 (33.3%) | OL | FG | NA | Deaville et al, 2007 |
| STRIPPED DOLPHIN | Greece (North Aegean Sea) | 1998-2013 | Dead | 15 | 1 (6.7%) | OL | UNK | NA | Milani et al, 2017 |
| *Stenella coeruleoalba* | Greece | 2010-2016 | | 120/yr | 36/yr (30%) | OL | FG (N/T) | NA | Frantzis, com pers 2017 |
| | Greece | NA | Alive | NA | 1 | OL | DEB | NA | Frantzis, com pers 2017 |
| FIN WHALE | United Kingdom | 2007 | | 3 | 1 (33.3%) | OL | UNK | NA | Deaville et al, 2007 |
| *Baleanoptera physalus* | | | | | | | | | |
| HUMPBACK WHALE | United Kingdom | 2014 | | 1 | 1 (100%) | OL | UNK | NA | Deaville et al, 2014 |
| *Megaptera novaeangliae* | United Kingdom | 2015 | Alive | 1 | 1 (100%) | OL | FG (R) | D | Deaville et al, 2015 |
| SPERM WHALE | United Kingdom | 2011 | | 9 | 1 (11.1%) | OL | UNK | NA | Deaville et al, 2011 |
| *Physeter macrocephalus* | Greece | 2000-2017 | Alive, Dead | NA | 2 | OL | FG (L, N/T) | L, D | Frantzis, com pers 2017 |
| | France (Mediterranean) | NA | Alive | NA | 1 | OL | FG (N) | NA | GECEM, com pers 2017 |
| | France (Mediterranean) | NA | Alive | NA | 3 | AS | FG (N) | D | GECEM, com pers 2017 |
| GREY SEAL | Germany | 1990-2014 | Dead | 255 | 3 (1.2%) | OL | FG (L, N/T) | L, D | Unger et al, 2017 |
| *Halichoerus grypus* | UK (Cornwall) | 2004-2008 | | NA | 58 (2004: 5%; 2008 : 3.6%) | NA | FG (L, N/T, R) | NA | Allen et al 2012 |
| HARBOUR SEAL | Germany | 1990-2014 | Dead | 2326 | 6 (0.25%) | OL | FG (L, N/T), DEB | L, D | Unger et al, 2017 |
| *Phoca vitulina* | | | | | | | | | |
| SEAL | The Netherlands | NA | | 350/yr | 20/yr (6.5%) | AS, ON | FG (L, N/T), DEB | NA | De Vries et Bakker Paiva, com pers 2017 |
| *Halichoerus grypus; Phoca vitullina* | | | | | | | | | |

In Germany, Unger et al (2017) reported 14 cases of entanglement (5 harbour porpoises, 6 harbour seals, 3 grey seals) out of 1622 examined carcasses, between 1990 and 2014 (one case each 2 years), along the North Sea and Baltic Sea (Figure 2). The materials responsible for these entanglements were also generally fishing related debris entangling individuals around the neck, flippers or body, and causing deep lesions, suppurative ulcerative dermatitis, severe suffering and death (Figure 3). One harbour seal got the neck entangled by a rubber band (Figure 3).

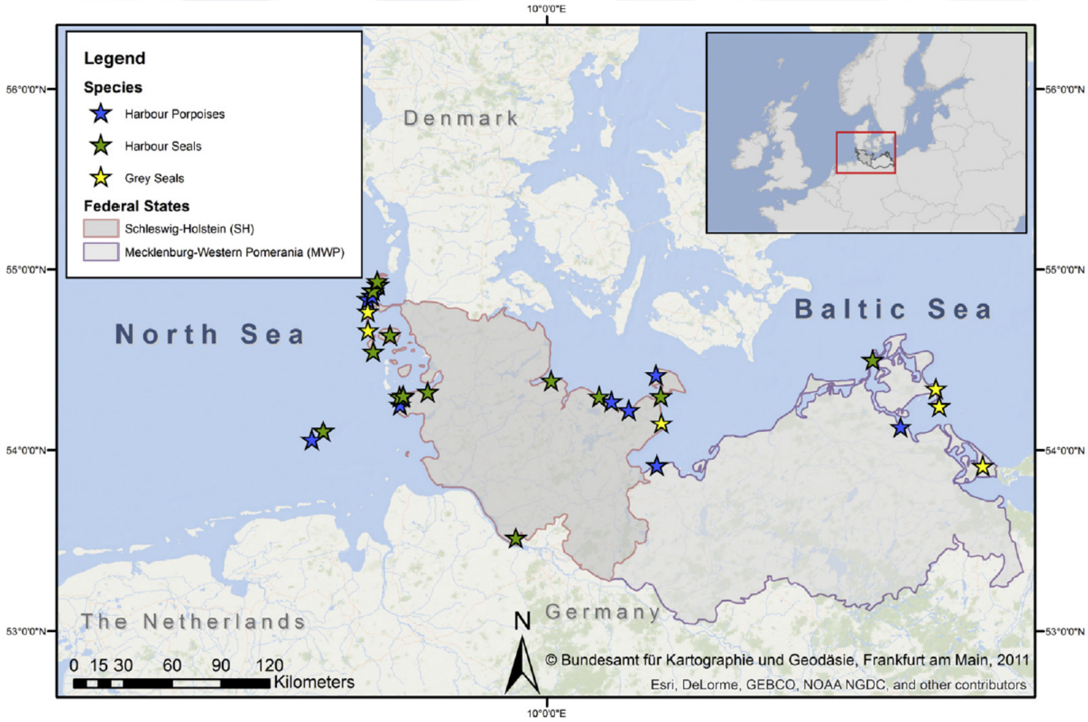


**Figure 2.** Stranding locations of harbour porpoise, harbour seals and grey seals carcasses with marine debris findings in Germany (1990-2014) (After Unger et al., 2017).

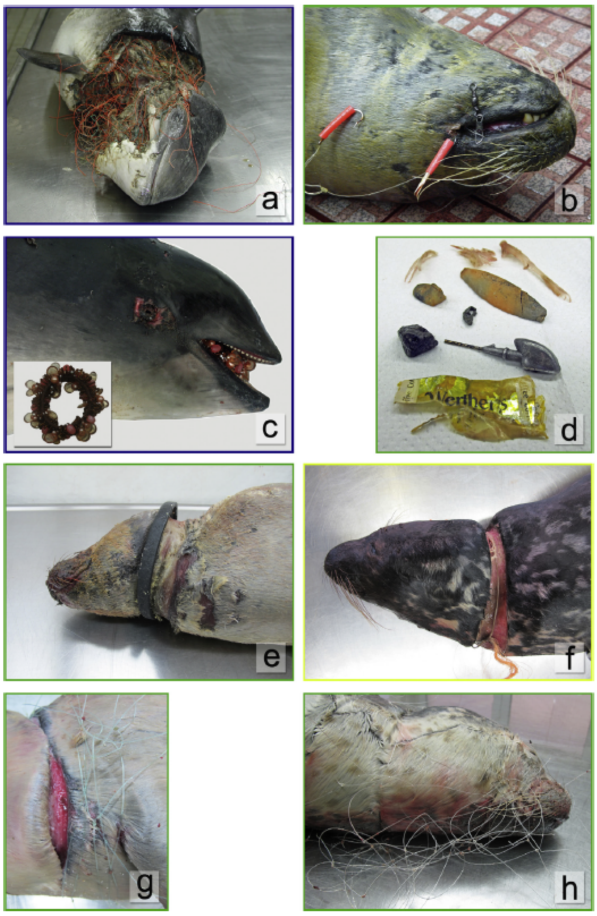


**Figure 3.** Materials responsible for the entanglement and consecutive lesions in marine mammals found stranded in Germany (1990-2014) (Photograph e: rubber band around the neck of a harbour seal. Other photographs: fishing material entangling or ingested by harbour porpoises (a,c), harbour seals (b,d,g,h), and grey seals (f)).

Seals are also impacted by debris, e.g. in the Netherlands. A study carried on the Dutch coast between 1985 and 2010 found that entanglement was more prevalent in grey seals than in harbour seals. Mean numbers of 1.5 grey seals and 0.6 harbour seal were observed entangled per year (Werner et al 2016), generally by pieces of ghost trawl nets and gill nets, juveniles being the most frequently impacted.

Thus, in the literature, the numbers of entangled specimens and prevalence vary a lot, according to species and years. However, the total number of observations is sometimes very low and the material responsible for entanglement mainly originates from fishing gears, or is not described. For example, in northern Aegean sea, Milani et al (2017) observed that the prevalence of entangled *Phocoena phocoena* was 28.5%. However, the total number of observations was low (N=7) and the material responsible for entanglement was not described (Table 1).

Our survey allowed us to collect additional information within the project area on sensitive species, which consisted in 2 seal species - *Halichoerus grypus* and *Phoca vitulina* (France and Netherlands), a spermwhale *Physeter macrocephalus* (Greece, France) and 2 dolphin species - *Stenella coeruleoalba* and *Tursiops truncatus* (Tunisia & Greece) (Table 1). Dolphins and spermwhales were found stranded dead, with, in one case, a dolphin being rescued and released. The number of observations per year was low, except for *Stenella* *coeruleoalba* in Greece, where a mean number of 30% out of 120 specimens are found entangled per year (Frantzis, comm. pers.), all in nets.

French, German and Dutch naturalists monitor the colonies of *Halichoerus grypus* and *Phoca vitulina*, with stranding networks and rescue centres operating all year long. In the Netherlands, from the 350 seals rescued per year by the Seal centre Pieterburen (the main Dutch rescue centre, supported by 100 volunteers), 20 individuals (6.5%) are found entangled yearly (Bakker Paiva & de Vries, comm. pers.). In France, few animals are found entangled each year (Monnet & Van Canneyt, comm. pers.). According to Bakker Paiva & de Vries (comm. pers.), the number of cases observed in the Netherlands, UK, Germany and France is increasing over years.

Depending on the cases, entangled animals are observed alive by NGOs' naturalists during their monitoring operations on the beaches used by seals colonies, or found dead on the coast. In some cases, animals are transferred to dedicated rescue centres for medical care. Most frequently, the material responsible for the entanglement is fishing nets, lines or ropes (Figures 3 & 4). Fishermen, and the fisheries observers who embark for monitoring catch and bycatch, also alert occasionally the NGOs and rescue centres when seals are caught accidentally alive. When close relationships with fishermen are permanent, these alerts may allow the naturalists to conclude that stranded animals entangled in pieces of gears are certainly due to abandoned or lost gear debris (Bakker Paiva & de Vries, comm. pers.) rather than to active fishing.


**Figure 4.** Seals found entangled in the northern coasts of France between 2006 and 2011. (Courtesy: Picardie Nature).

Both juveniles and adults are impacted by entanglement. In the case of juveniles, several individuals have been observed growing while remained entangled, during months or years, suffering from all complications related to the constriction of tissues by strapping debris. Strangling became also more intense with time and mobility.

### Spatiotemporal variability

Through our survey and data collection, few data were collected regarding the spatiotemporal variability in marine mammals. Activity reports and publications from the United Kingdom stranding networks and scientific institutions, provide some information in their investigation area. In *B. acurostrata*, globally the prevalence of entanglement increased from 2002 to 2015 in Cornwall (Table 1). According to Bakker Paiva & de Vries (comm. pers.), the number of entangled seals observed in the Netherlands, UK, Germany and France is increasing year after year.

### Factors of sensitivity

Age and specific mammal behaviour could be the main factors influencing the occurrence of entanglement. In two questionnaires, the exploratory and playful behaviour of juveniles and even adults, were referred as the reasons why individuals might have been entangled. Some types of debris items could also be more attractive than others, for unknown reasons. However, since this probably depends on the specificities of each species and even on circumstances, and due to the high number of existing marine mammal species, a deeper study is needed to confirm this hypothesis.

For example, the influence of age was proposed by Siebert et al. (1994), who observed that the common porpoises trapped in nets were mainly subadult/ juveniles, while Arbelo et al. (2013) observed that this age class only represented 50% of cases of entanglement. In the Canary islands area, Diaz-Delgado (2015) observed that 6/11 (54.5%) of entangled individuals were adult animals, and 2/11 (18.2%) juveniles or subadults. However, for a better interpretation of the results, information about the demographic structure of populations would be needed.

### Constraints and feasibility of monitoring

Entangled marine mammals are observed on land by stranding networks or at sea by different types of observers (Table 1).

A number of cetacean species have been observed in several countries of the project area (Table 1). Stranding networks exist in a number of countries, at least in the occidental part of the project area. The number of stranded specimens may be very high annually; in France for example, several hundreds of marine mammals are found each year on the French coasts (Van Canneyt et al, 2016; Dars et al 2017), but like in United Kingdom, they can consist in few observations for each of the different species observed. In France, the data collection procedure does not include specifically entanglement as a cause of morbidity/ mortality, nor the different kinds of materials responsible for the entanglement, both of them would be necessary to perform statistical analysis. The definition of entanglement itself often refers to interactions with fishing activities, which are the main cause of mortality and stranding, rather than to debris. Furthermore, stranded specimens are often decomposed, and the proportion of in depth examinations is very low. As an example, the 2016 stranding network annual report for marine mammals in France (Dars et al 2017), reveals that 30% of 1251 external examinations showed signs of accidental capture, and for 45 in depth examinations, the causes of mortality were divided in: natural cause, pathology, bycatch and other anthropogenic causes (which includes entanglement and other causes).

Observation at sea makes it difficult to identify the material responsible for the entanglement. Identifying this material is easier on land, on dead stranded animals or on live ones, stranded or not. This is the case in seals, since they use not only marine habitat, but also terrestrial habitat, and maybe rescued in rehabilitation centres. Other marine mammals are rarely rescued, because of their size and/or the necessity to benefit special infrastructures.

In France, 40 to 50 seals are rescued each year in 6 rescue or first aid centres, while in the Netherlands, this number goes up to 350 (De Vries et Bakker Paiva, comm. pers.). The distribution of these species is restricted to the occidental northern part of our project area. However, since an increasing number of entanglements are reported each year, seals could represent a good indicator species for measuring the impact of marine debris through entanglement, specifically in this area.

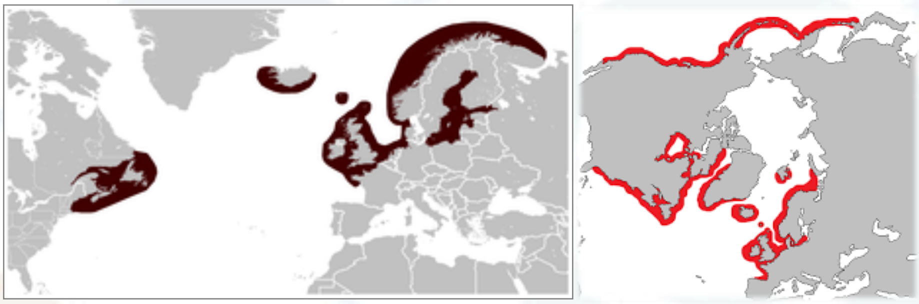

**Figure 5.** Distribution of Halichoerus grypus (left, in brown; after wikimedia) and of *Phoca vitulina* (right side, in red, after https://www.marinemammalscience.org/facts/phoca-vitulina).

In conclusion, while monitoring devices already exist in several parts of the project area, using marine mammals as indicator species faces several constraints and possible sources of bias. The difficulty to distinguish true entanglement in marine debris from capture during active fishing operations is a major constraint and source of bias, which prevented for years the TSG ML to consider an entanglement indicator within the MSFD (Galgani, comm. pers). According to Bakker Paiva & de Vries (comm. pers.), the fishing material found on entangled seals in the Netherlands consist in discarded gears. Accidental captures are clearly identified since fishermen call the rescue centre when a capture is occurring. Furthermore, in seals, some biologists consider that the prevalence of entanglement may be underestimated, since weakened animals tend to sink to the seabed rather than being washed ashore (Hazekamp et al., 2010). This might also be the case in other marine mammal species. Marine debris themselves may degrade more or less quickly, and signs of entanglement may have disappeared and thus not be detected by observers (Werner et al 2016). Another source of bias is the fact that most marine mammal species are migratory species, and/ or may move at a high speed, which makes it difficult for interpreting monitoring results. Even so, monitoring entanglement in marine mammals may be significant in several ways. For example, given the wide range of seals (Figure 5) and the increasing prevalence of entanglement (see also Werner et al 2016) in the HELCOM and OSPAR areas, in particular where large amounts of debris have been reported (Galgani et al 2000), a long term monitoring is of great interest for managers and conservationists. The data are also of high significance for assessing the impact of marine litter pollution in hotspots areas, where the risk to encounter debris is high, and for evaluating the consequences of entanglement in marine debris on specimens/populations of seal species.

## *Sea turtles*

Entanglement in marine floating debris (including ghost fishing gear) is known to cause lacerations, increased drag - which reduces the ability to forage effectively or escape threats - and may lead to drowning or death by starvation (Nelms et al., 2016). Important effects have been described even in the reproductive output of turtle rookeries, as described by Sanchez-Sierra (2017) in the loggerhead colony of Cape Verde, where rear flipper lesions in nesting females could incapacitate them to dig their nest, making these females not productive for the population.

## Sensitive taxa, occurrence and circumstances

All sea turtle species are sensitive to entanglement. However, our survey identified three sensitive species within the project area: the loggerhead *Caretta caretta*, the green turtle *Chelonia mydas*, and the leatherback *Dermochelys coriacea* (Table 2). Duncan et al. (2017) reported, from the consultation of 106 experts' opinions in 43 countries, up to 95.5% of entanglement in 0 to 4100 strandings per year sea turtles. They evaluate that entanglement cause an annual mortality of around 1000 individuals in the prospected areas, which is likely to be underestimated since an unknown proportion of entangled turtles strands or is eaten by predators (Hart et al 2006).

Data have been collected through our survey for *C. caretta* from seven countries (France, Greece, Italy, Portugal, Spain, Turkey and Tunisia), one country for *C. mydas* (Greece), and two countries (France and Spain) for *D. coriacea* (Table 2). This data consists in qualitative and quantitative information included in the questionnaire responses and in the datasets (France, Greece, Spain and Turkey). Complementary information was extracted from the literature and activity reports (Table 2).

The common sensitive species on which data of entanglement is available in the major part of the project area are the *C. caretta*, and in a lesser extent *D. coriacea* (Table 2). However, since *Lepidochelys kempii* is frequent in the study area, particularly in the OSPAR area, but also in the Mediterranean, this species can also be affected (see for example Carreras et al, 2014).

According to certain survey answers (Greece, Italy, Portugal), more data could be available in the future, given an agreement between data producers and users, or new national decisions including sea turtles as species to be monitor within the MSFD monitoring program (Greece). Videos might also represent a source of data, since several cases of entanglement were found on internet and social networks, in particular in Macaronesia. For Spain, Greece, Tunisia, Turkey, Portugal and France, data was collected during stranding network activities, which were alerted through an emergency phone number provided to fishermen (professional or sport), recreational boats in case of incidental captures or at sea observations, and coastal users in case of stranding.

For Portugal, data also came from oceanographic or fishing campaigns and in the case of Italy, data came from an aerial survey dedicated to marine mammals and sea turtle census.

Available data consisted in numbers of entangled individuals, and in main types of items responsible for the entanglement (fishing gear, debris or plastics). In a lesser extent, data included the material composing debris items, and the effects of entanglement on turtles.

**Table 2.** Results of the literature analysis and surveys carried out on the feasibility of an entanglement indicator in sea turtles (present project)

(AS) at-sea, (OL) on land, (AE) Aerial, (DEB) marine litter of user origin, such as plastic package, baler twine (rafia etc) or other (stake nets etc…), (FG) fishing gear : *(L) lines (long lines & monofilament line pieces, hooks…), (N/T) pieces of nets or trawl nets, (R) piece of single rope (mooring, pot etc)* (L) lesion, (CD) carapace distortion, (A) Amputation, (D) death, (yr) year.
*References in italics indicate that datasets were available.*

| Species | Country/ Oceanic basin | Years | Condition | N | N entangled (%) | Circumstances | Item/ Material | Effect | References |
|---|---|---|---|---|---|---|---|---|---|
| LOGGERHEAD TURTLE | | | | | | | | | |
| *Caretta caretta* | France (Mediterranean) | 2010-2016 | | 103 | 9 (8.7%) | AS, ON | FG, DEB | NA | *RTMMF com pers 2017* |
| | France Atlantic | 1997 | | 8 | 1 (12.5%) | | FG (L) | | Duguy, Morinière & Meunier, 1998 |
| | Turkey | NA | Dead | 38 | 12 (31.6 %) | OL | DEB | A | *Kaska et al 2017, present study* |
| | Tunisia (Strait of Sicily) | 2013 | Alive | NA | 1 | AS | FG (L) | L, CD | Chaieb, present study |
| | Portugal (Madeira) | 2002-2017 | Dead | NA | 1/yr | AS | FG (L, N/T), DEB | D | Alves, com pers 2017 |
| | Portugal | 2007-2017 | Alive, Dead | NA | ~10/yr* | OL | FG (N/T) | L, A, D | Santos, present study |
| | Portugal | 1978-2013 | Dead | 386 | 96 (24.9%) | OL | FG (L, N/T) | | Nicolau et al, 2016 |
| | Portugal (Azores) | 2004-2008 | Alive | NA | 3 | OL | FG (L), DEB | A, D | Barreiros and Raykov, 2014 |
| | Italy (Sardinia) | 2008-2017 | Alive, Dead | 176 | 27 (15.3%) | AS, OL | FG (L, N/T), DEB | | Camedda, present study |
| | Italy (Tyrrhenian sea) | NA | | NA | 1 | AE | DEB, FG (N/T) | NA | Lauriano, com pers 2017 |
| | Italy (Sicily) | 1994 | Alive | NA | 1 | AS | DEB, FG (R) | | Bentivegna et al, 1995 |
| | Spain (Mediterranean) | 1994-2017 | Alive, Dead | 1415 | 38 (2.7%) | OL, AS | DEB, FG (L, N, R) | L | *UVEG, present study* |
| | Spain (Gran Canaria) | 1998 - 2014 | Alive, Dead | 1860 | 945 (50.8%) | OL | DEB, FG (L, N/T, R) | | Oros et al., 2016 |
| | Spain (Canary Islands) | 2003 - 2009 | | 1538 | 892 (58.0%) | | DEB, FG (L, N/T, R) | | Fariñas-Bermejo et al., 2017 |
| | Spain (Canary Islands) | 1998-2003 | Alive | 1983 | 906 (45.69%) | OL | FG (N/T, R), DEB | | Calabuig & Liria Loza, 2007 |
| | Greece (Rhodes Island) | 1984-2011 | Alive, Dead | 152 | 17 (11%) | OL | FG (L) | | Corsini-Foka et al, 2013 |
| | Greece | 2012-2017 | Alive, Dead | 79 | 7 (8.8%) | OL | FG (L) | L, A, D | *Corsini-Foka, com pers 2017* |
| LEATHERBACK TURTLE | | | | | | | | | |
| *Dermochelys coriacea* | Spain (Mediterranean) | 1994-2017 | Alive | 10 | 1 (0.1%) | OL | FG | NA | *UVEG, present study* |
| | Spain (Canary Islands) | 1988-2016 | | 13 | 2 (15.4%) | | DEB, FG (L,N,R) | | Liria-Loza A. per comm |
| | Portugal | 1978-2013 | Dead | 275 | 49 (17.8%) | OL | FG (L, N/T, R) | | Nicolau et al, 2016 |
| | United Kingdom | 2014 | | 9 | 2 (22.2%) | | FG (R) | | Deaville et al, 2014 |
| | France (Atlantic) | 1988-2016 | | 405 | 27 (6.7 %) | OL | FG (L, R), DEB | NA | *RTMAE CESTM, com pers 2017* |
| | France (Atlantic) | 1988-2016 | Dead, Alive | 1265 | 33 (2.6 %) | AS | FG (L) | NA | *RTMAE CESTM, com pers 2017* |
| GREEN TURTLE | | | | | | | | | |
| *Chelonia mydas* | Greece | 2012-2017 | Dead | 14 | 3 (21.4%) | OL | FG (L), DEB | L, D | *Corsini-Foka, com pers 2017* |
| | Greece (Rhodes Island) | 1984-2011 | Alive, Dead | 42 | 6 (14%) | OL | FG | | Corsini-Foka et al, 2013 |
| | Spain (Canary Islands) | 1998-2016 | | 24 | 3 (12.5%) | | DEB, FG (L, N, R) | | Liria-Loza A. per comm |
| | Turkey | 2001 | Dead, Alive | 23 | 10 (43.5%) | OL | FG (N/T) | | Kaska et al, 2004 |

In Macaronesia, currently, data is being collected following a pilot protocol, which includes the INDICIT consortium within the sea turtle litter ingestion data sheet. However, already available data was found in the literature. In the Macaronesian waters, cases of entanglement of sea turtles by marine debris has been described since the 90's. Dellinger (2003) described the entanglement in ghost fishing gears as one of the mortality causes of juvenile loggerheads in the Madeira Islands waters (Portugal). In the Canary Islands, 45.7% of the loggerheads found stranded from 1998 to 2003 (N = 906 individuals) were found entangled in nets, plastic, ropes and other floating debris (Calabuig & Liria-Loza, 2007). One of the most important debris causing turtle entanglement in the Canarian waters, were the plastic mesh bags and shade cloth used in agriculture (raffia sacks). Almost all entangled turtles stranded were found alive and most of them successfully recovered (87%), but the high impact caused by the debris on turtles in the region is demonstrated (Calabuig & Liria-Loza, 2007). In 2012, Calabuig (2012) indicated that 50% of the turtles stranded in the Canary Islands were entangled in marine debris, while Fariñas-Bermejo et al. (2016) showed that marine debris affected the 58.7% of turtles of loggerhead stranded from 2003 to 2009 in all the Canarias Archipelago.  Entanglement due to marine debris was the most important stranding cause, increasing from 42.98% in 2003 to 66.36% in 2009. A wider analysis conducted by Oros et al. (2016) on the morbidity and mortality of stranded turtles in the Canary Islands from 1998 to 2014, indicated that the most frequent cause of morbidity was the entanglement in fishing gear and/or plastics (50.8%, N=945), while only 11.5% of the turtles affected by entanglement were found dead. Although entanglement has been reported as a common cause of morbidity and mortality in sea turtles (see Nelms et al., 2016 for a review), the prevalence of entanglement in the Canary Islands is higher than that reported in other surveys. In addition, entanglement on floating materials like plastic is an increasing problem (Casale et al., 2010), but it is remarkable that the use of synthetic raffia in the Canary Islands is very common.

In mainland eastern Spain (Valencia community), out of the 38 loggerhead turtles found entangled between 1995 and 2017 (2.7% of the total registered individuals, ie one individual each two years), 30% were found entangled in debris (plastic packaging, raffia, balloons etc.). In 70% of the entanglement events, the materials responsible were ghost fishing gears (Table 2). Only one entangled leatherback was observed.

In France Mediterranean, nine cases of entangled loggerhead turtles were observed out of 103 turtles (less than 0.1% or less than one individual per year) stranded between 2010 and 2016. In France Atlantic, 2.6% (N=33) of leatherbacks observed at sea, and 6.7% (N=27) of leatherbacks observed stranded were found entangled between 1988 and 2016 (one individual per year), while in the loggerhead in 1997, one case (12%) were observed entangled. Since accidental capture and entanglement by debris were not discriminated, we could not calculate the incidence of true debris.

In Portugal, two responses to our questionnaire indicate that depending on the location, the number of entanglement observations varied from one to ten loggerhead turtles per year, three other responses do not quantify the prevalence of entanglement. The calculation of the prevalence was then not possible. Barreiros and Raykov (2014) recorded 3 entangled loggerhead individuals in longlines and debris in the Azores, during the period 2004-2008. On the mainland of Portugal, Nicolau et al (2016) analysed data collected by stranding networks during the period 1978-2013, and reported 96 cases (24.9%) of entanglement in loggerhead, and 49 cases (17.8%) in the leatherback, all due to fishing gear material (Table 2).

In Greece (Rhodes Island), between 1984 and 2011, respectively 11% and 14% of stranded or rescued loggerheads and green turtles were entangled in fishing gear material (Corsini Foka et al, 2013). Between 2012 and July 2017, Corsini Foka (comm. pers.) observed that respectively 9% and 21% of the stranded or rescued loggerhead and green turtles were entangled (Table 2 and Figure 6). In the area of Zakynthos island, both inside and outside the

National park, between 2014 and 2016, 20 sea turtles (*Caretta caretta*) were found entangled in fishing gear (nets, hooks, lines and longlines) (Dimitriadis, pers.com.).


**Figure 6.** Pictures of 10 sea turtles stranded in Rhodes Island and adjacent islands (Greece), between 2012 to July 2017, showing entanglement (evident or testified by persons) or wounds evidently caused by entanglement (Photos: Archive of the Hydrobiological Station of Rhodes-Hellenic Centre for Marine Research. Legends of the pictures indicate the number of the individual [X], the location of finding in Greece, and the species code (CC: *Caretta caretta*, CM: *Chelonia mydas*)).

In Turkey, the prevalence of entanglement was high (31.6%).

In Tunisia, only one case of entanglement was cited throughout our survey, and a few others documented with pictures.

In Italy, one case of entanglement was reported in a questionnaire answer, consisting in a loggerhead observed in the Tyrrhenian Sea during an aerial survey. The CNR-database showed 15.3% among on 170 loggerhead turtles observed being entangled in Sardinia between 2008 and 2017. Bentivegna et al (1995) reported also one case of entanglement with debris in Sicily (Table 2).

Despite the fact that we did not get any reply from the United Kingdom to our survey, we found information in Deaville et al (2014), who reported two entangled leatherback turtles in 2014.

In almost all cases, pieces of fishing gears were responsible for entanglement, while debris of user origin were cited in a lesser extent, except in Spain and Italy. Given the fact that no standard protocol/typology discriminating the different materials responsible for entanglement and circumstances was available at the time of this data collection, it was not possible to assess the proportion of each kind of entanglement circumstances. In particular, it was not possible to differentiate entanglement during active fishing, entanglement in pieces of gears brought with turtle after bycatch and liberation, entanglement in true debris from fishery origin and entanglement in other debris (plastic and others). In Turkey, France Atlantic and Spain, respectively 58.3%, 8.3% and 30% of the entanglements were due to materials of an origin other than fishery, and 10% in Italy (Sardinia), where several debris were constituted of materials from both origins. In the frame of further analyses, besides a standard protocol and phenology, pictures could permit to identify the materials responsible for the entanglement and the types of observed impact (Figure 6).

When documented, the effects observed on entangled animals were death, different kinds of external lesions, carapace distortion and amputations. Depending on the intensity of affections, individuals found alive were rescue successfully or died during care operations (Figure 6).

### Spatiotemporal variability

Data collected during our survey showed a spatial variability in the prevalence of entanglement especially for the loggerhead (from 2.6% in Spain to 31.6% in Turkey). However, the stranding network activity reports, published from observations in the Canary Islands, revealed the highest prevalence up to nearly 59% (Fariñas-Bermejo et al. 2016) in the project area.

For the leatherback turtles, the prevalence varied from 0.1% (Spain Mediterranean) to 22.2% (UK). However, this species is less abundant in the Mediterranean compared to the Atlantic side of France (Table 2). Available data are to date insufficient or were sent too late to evaluate precisely any spatiotemporal variability of a possible sea turtle entanglement indicator at the whole project scale.

In the Canarias waters, Fariñas-Bermejo et al. (2016) observed larger numbers of entangled turtles stranded in the western islands, which could be related to the greater influence of the Canary current coming from the north of the archipelago. Oros et al. (2016) found that entanglement observed in stranded turtles in the Canary Islands from

1998 to 2014 occurred frequently during all seasons, but was significantly more prevalent in summer and fall.

Data collected in the Canary Islands showed a temporal variation, the prevalence of loggerhead entanglement increased from 45.7% during the period 1998 to 2003 (Calabuig & Liria-Loza, 2007), to 58.7 % of entangled turtles during the period 2003 to 2009 (Fariñas-Bermejo et al., 2016).

In Greece, the number of entangled green turtles increased in the Rhodes region between the 2 periods of observation (14% between 1984 and 2011; 21% between 2012 and July 2017), whereas around Zakynthos island, the number of entangled loggerhead turtles was almost constant during 2014-2016. In the area of the Aegean archipelago, it is difficult to estimate the variability as there are many remote islands and coasts.

In Italy, the occurrence of entanglement in the loggerhead varied from 9.2% to 26.5% between respectively 2008 and 2012 (121 observations) and 2013 to 2017 (49 observations)

For other regions, while almost all questionnaire responses recorded an increase in the number of observations over time, there is a need of precise data for further studies in order to assess the temporal variability.

### Factors of sensitivity

Data was insufficient for evaluating sensitivity factors. Age, which may be related to a certain degree to variability in behaviour or perception, could influence attraction, as found by Duncan et al. (2017) who assess that sea turtle adults (especially in *Dermochelis coriacea and Lepidocelys olivacea*) would be more impacted. However, in response to our surveys, while some data mentioned the size of turtles (Spain, France, Turkey, Greece, Italy), in loggerhead turtle for example, the mean size of stages from which we could deduct the age different depending on genetic populations to which individuals belong (according skeletochronological and growth studies, see for example Casale et al, 2011; Poviano et al., 2011; Lenz et al, 2016), is. In Italy, the data reported by CNR (Camedda, unpublished data) showed 1 among 4 loggerheads smaller than 40 cm being entangled compared to 12.2% (14 among 115) of the larger individuals (between 40 and 77 cm). In Greece (Rhodes region), the data communicated by Corsini Foka (unpublished data; comm. pers.) reported immature and mature animals clearly distinguishable by size: small individuals (20 to 21 cm CCL n-t[2]) were entangled/embedded in bags while large individuals (59 to 76.5 cm CCL) were entangled in fishery materials. More information could allow more powerful tests in the future.

### Constraints and feasibility of monitoring

Few information is available on the constraints for monitoring entanglement in sea turtles.

The large distribution of loggerhead turtle makes this species suitable for monitoring in the Mediterranean and the Macaronesia, the monitoring of leatherbacks being more pertinent in the Atlantic regions, and of green turtles specifically in the Eastern Mediterranean. Despite the lack of knowledge regarding the variability in the sensitivity factors according to species, the general propensity to be entangled is probably equivalent in all species, thus all *Cheloniidae* and *Dermochelyidae* species could be

[2] Carapace curved length notch to tip

considered for monitoring, rather than one species in particular. At this stage, no biological constraints were identified which could be source of bias. However, more studies are needed.

In terms of methods, the abundance of specimens seems to vary according to countries, however, more data exist and would be available upon contractual procedure with those data owners (for example, in Greece, the ARCHELON rescue centre, the National Park in Zakynthos, the School of Veterinary Medicine at the University of Thessaloniki, etc.), and/or after translation of grey activity reports into English language. The intensity of the observation effort might also be increased and provide more data, if a long term monitoring was structured and launched by the members States of EU and RSCs. No common approach exists for collecting data. Data banking is particular for each owner and no validated method exists. However, the INDICIT partners have included several entanglement parameters within their standard data sheet, for the collection of information about marine debris ingested by sea turtle and new standardized data will now be produced.

From the seven countries which participated in the survey, data were mainly collected at sea or on land (Table 2) by stranding networks, rescue centres, or biologists (fisheries observers, meteorologists, oceanographers and marine protected areas). One observation of an entangled loggerhead was, however, interestingly collected during an aerial survey (Italy). This latter kind of sampling device does rarely permit (unless good conditions of visibility and special permissions to fly at low altitudes) to identify the species, or the size or sex of individuals.

At this stage of the data collection, the representativeness of data is partial, since in some cases the sampling devices act only locally (e.g. Rhodes, Valencia Community etc.) rather than nationally (e.g. Tunisia, France). The available data already showed the possibility to discriminate state/ areas in the perspective of establishing the state of environment. However, data representativeness is related to the level and scale of observation effort, which may be deployed with large variations each year, depending on weather forecast (presence of observers at sea or on the coast) or available funds. A long term monitoring and means provided for it would then contribute to improve the data availability and their representativeness. Furthermore, since sea turtles are listed on the red list of endangered species by IUCN and in several European directives and conventions (CMS, CITES, Bern & Barcelona conventions etc.), data assessing the impact of entanglement on sea turtle individuals and populations would also be significant for assessing their population health and tendencies. In certain countries like Greece, the increase of fishing gears set and left in the sea with very limited control by authorities, and the increase of insufficiently managed litter produced by residents and tourists were emphasized as of particular concern.

The regular occurrence and prevalence of entanglement in three species of sea turtles (*D. coriacea, C. mydas* and *C.caretta*) in the project area, makes them a suitable taxon for monitoring and indicates that a long term monitoring could be feasible. The stranding networks and rescue centres are probably the best devices for collecting the data on entanglement, since they work together with all kinds of sea users. A wide network of operational sea turtles rescue centres already exist[3] (Ullman, and Stachowitsch, 2015) and collects data. However, funds are crucial for maintaining this observation effort, since some turtles need care for several months before recover. As an example, the Hydrobiological Station of Rhodes (Greece) estimates the cost up to 300 euros per month (Corsini Foka, comm. pers.). Furthermore, in certain areas like Greece, with a lot of rocky

[3] Map sea turtles rescue centers

islands, the logistic constraints for reaching and monitoring the coasts are particularly hard (Corsini Foka, comm. pers.). The main limitation of this possible indicator is the present incapacity to distinguish debris from materials coming from active fishing activities.

## *Birds*

### Sensitive taxa, occurrence and circumstances

While numerous species of birds have been reported to be sensitive to entanglement (see part « analysis of literature »), three species were cited as being sensitive, in publications related to the project area and in our surveys (Table 3): European shag - *Phalacrocorax aristotelis* (France), Scopoli's Shearwater - *Calonectris diomedea* (France, Greece, Tunisia) and northern Gannet - *Morus bassanus* (France, Netherlands).

**Table 3:** Prevalence and material/items cited as responsible for entanglement of biota in the literature for birds (INDICIT project area)

(BS) Boat survey, (IN) In nest, (OL) On land, (DEB) marine litter of user origin, such as plastic package, baler twine (rafia etc) or other (stake nets etc.), (FG) fishing gear: *(L) lines (long lines & monofilament line pieces, hooks…), (N/T) pieces of nets or trawl nets, (R) piece of single rope (mooring, pot etc),* (UNK) material/item not specified, (L) lesion, (D) death, (yr) year.

References in italics indicate that datasets were available.

| Species | Country/ Ocean basin | Years | Type | N | N entangled (%) | Circumstances | Item/ Material | Effect | References |
|---|---|---|---|---|---|---|---|---|---|
| NORTHERN GANNET | | | | | | | | | |
| *Morus bassanus* | Cantabrian Sea | 2007-2010 | NA | 2245 | 8 (0.36%) | BS | FG (R, N/T, L) | NA | Rodriguez et al, 2013 |
| | Mediterranean West | 2007-2010 | NA | 266 | 0 (0%) | BS | FG (R, N/T, L) | NA | Rodriguez et al, 2013 |
| | Mediterranean South | 2007-2010 | NA | 97 | 0 (0%) | BS | FG (R, N/T, L) | NA | Rodriguez et al, 2013 |
| | Gulf of Cádiz | 2007-2010 | NA | 926 | 1 (0.15%) | BS | FG (R, N/T, L) | NA | Rodriguez et al, 2013 |
| | Eastern Canary Island | 2007-2010 | NA | 14 | 0 (0%) | BS | FG (R, N/T, L) | NA | Rodriguez et al, 2013 |
| | Mauritanian waters | 2007-2010 | NA | 124 | 25 (20.16%) | BS | FG (R, N/T, L) | NA | Rodriguez et al, 2013 |
| | United Kingdom | 1996–1997 ; 2005-2010 | NA | NA | 525 | IN | DEB | NA | Votier et al, 2011 |
| | Germany (Helgoland Island) | 1983-1984 | Dead | 28 | 8 (29%) | OL | FG (N/T, R), DEB | NA | Vauk & Schrey, 1987 |
| | Germany (Helgoland Island) | 1983-1984 | Live | 313 | 8 (2.6%) | OL | FG (N/T, R), DEB | NA | Vauk & Schrey, 1987 |
| | Germany (Helgoland Island) | 2014 | NA | 656 | 17 (2.6%) | IN | UNK | NA | Schulz et al in prep |
| | Germany (Helgoland Island) | 2015 | NA | 684 | 24 (3.5%) | IN | UNK | NA | Schulz et al in prep |
| | France (North Sea) | 2015 | NA | 9 | 2 (1.9%) | OL | FG (N/T) | D | Legroux & Pischiutta, 2015 |

| Species | Country/ Ocean basin | Years | Type | N | N entangled (%) | Circumstances | Item/ Material | Effect | References |
|---|---|---|---|---|---|---|---|---|---|
| GUILLEMOT | | | | | | | | | |
| *Uria aalge* | Germany | 2014 | NA | 2880 | 32 (1.1%) | IN | UNK | NA | Schulz et al in prep |
| | Germany | 2015 | NA | 3381 | 34 (1.0%) | IN | UNK | NA | Schulz et al in prep |
| NORTHERN FULMAR | | | | | | | | | |
| *Fulmarus glacialis* | Germany | | NA | 67 | 2 (1.8%) | NA | UNK | NA | Schulz et al in prep |
| | Germany | 2014 | NA | NA | NA (2.6%) | NA | UNK | NA | Schulz et al in prep |
| | Germany | 2015 | NA | NA | NA (3.5%) | NA | UNK | NA | Schulz et al in prep |
| EUROPEAN SHAG | | | | | | | | | |
| *Phalacrocorax aristoteli* | France (Toulinguet) | 2013 | Alive, Dead | NA | 3 | IN | FG (L) | NA | *Cadiou, 2013* |
| | France (Toulinguet) | 2014 | Alive | NA | 1 | IN | FG (L) | NA | *Cadiou, 2014* |
| SHEARWATER | | | | | | | | | |
| *Calonectris diomedea* | Greece (Strofades Islands) | 2007-2017 | NA | 5550 | NA (10-20%/yr) | IN | DEB, FG (L) | NA | *Karris, com pers 2017* |
| | Tunisia (Cap Bon) | 2007-2017 | NA | 10/yr | NA | AS, OL | FG | L | *Benmessaoud, present study* |

In France, bird stranding was monitored by NGOs on the beaches each year, and several colonies of breeding birds were also monitored. Adult shags and northern Gannets often use marine debris such as ropes, pieces of fishing nets and different kinds of other debris as materials for building their nests. From 2012 to 2014, litter was found in 12 to 98% of the shag's nests, in different locations (Table 4, Cadiou 2013 & 2014 and Cadiou & Fortin 2015). Shags - *Phalacrocorax aristotelis* are impacted by the entanglement and variable numbers of adult and immature are entangled by the beak or legs, and often dead (Cadiou, comm. pers.).

Entangled northern Gannets - Morus bassanus are regularly observed dead stranded or in nest, and alive in nest or flying off the cliff (Figure 7), in several breeding sites. The prevalence ranged from relatively low values 0.05 to 0.2% (adults and chicks, all dead in nest) (Provost et al. 2015 & 2016 in Cadiou, pers. comm.) to 22% (Legroux & Pischiutta, 2015) (Table 4). The litter debris responsible for this entanglement are mainly fishing materials.

**Table 4.** Proportion of nests of European Shag Phalacrocorax aristoteli including marine litter and types of debris in several breeding colonies in France.

(DEB) marine litter of user origin, such as plastic package, baler twine (rafia etc) or other (stake nets etc…), (FG) fishing gear: (L) lines (long lines & monofilament line pieces, hooks…), (N/T) pieces of nets or trawl nets, (R) piece of single rope (mooring, pot etc).

| Country/ Ocean basin | Years | % nests with litter | Item/ Material | References |
|---|---|---|---|---|
| France (Toulinguet) | 2013 | NA (89%) | FG | Cadiou, 2013 |
| France (Tas de Pois) | 2012 | NA (12%) | FG | Cadiou, 2013 |
| France (Tas de Pois) | 2013 | NA (36%) | FG | Cadiou, 2013 |
| France (Parc Naturel Marin d'Iroise) | 2014 | NA (81%) | DEB, FG (L) | Cadiou, 2014 |
| France (Archipel de Molène, Ouessant, archipel des Sept-Îles) | 2014 | NA (20%) | DEB, FG (N/T, R) | Cadiou & Fortin, 2015 |
| France (Les Fourches and la presqu'île de Crozon) | 2014 | NA (80 %) | DEB, FG (N/T, R) | Cadiou & Fortin, 2015 |

**Figure 7.** Marine debris entangling the beak and leg of two northern Gannets - *Morus bassanus*, in France (courtesy Guy Flohart- GON).

In Greece, from 10 to 20% of the *Calonectris diomedea* breeding population (5,500 pairs) monitored since 2007 by the Technological Educational Institute (TEI) of the Ionian Islands in the Strofades islands, are subject to entanglement each year (Karris, pers. comm.). Despite this fact, it was not possible to assess the impact on the population, but debris were identified as mainly plastics bottle lids and in a lesser extent, to pieces of fishing lines.

In Tunisia (Cap Bon), Benmessaoud (pers. comm.) reported a mean number of 10 entangled *Calonectris diomedea* individuals per year, observed at sea or stranded, with internal and external lesions. However, it was not clear if this entanglement was due to litter, since the variations in frequency are probably linked to the fisheries activities intensity (trawlers and longlines).

In the Netherlands, very few birds were found entangled by the volunteers working within the stranding network coordinated by the Seal centre Pieterburen (Bakker Paiva & de Vries, pers. comm.).

### Spatiotemporal variability

Few data were obtained through our surveys regarding the spatiotemporal variability in the entanglement of seabirds. In Tunisia (Cap Bon), the number of entangled *Calonectris diomedea* increased in summer. Since, individuals were found entangled mainly in fishing gear materials, this increase suggests that entanglement was related with the increase of fishing effort during this season (Benmessaoud, present study).

In Spain and Mauritania, Rodriguez et al. (2013) found a strong spatial variability of entanglement prevalence in non-breeding northern Gannet - *Morus bassanus*, between six different wintering sites (Table 3 and Figure 8). The flying birds were counted with binoculars from the vessels during the different fisheries observer campaigns and at different seasons. No entangled bird was observed in the Mediterranean and the Canary Islands, while in Mauritania, the incidence was exceptionally high (20.2%).

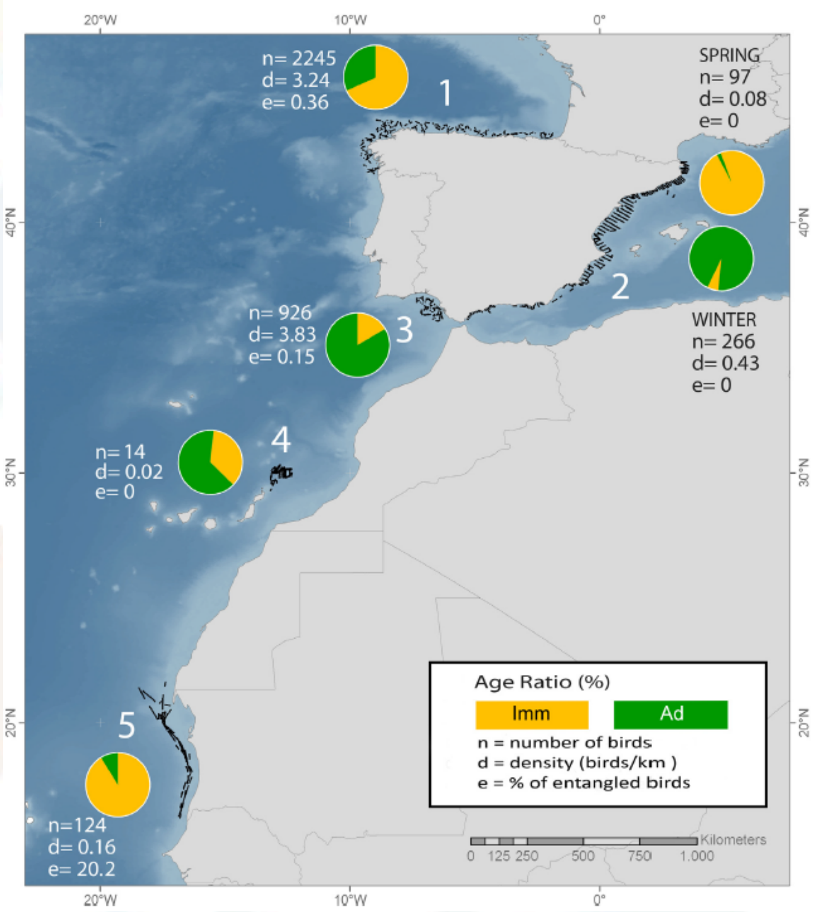


**Figure 8.** Spatiotemporal variability of the entanglement in the northern Gannet - *Morus bassanus*, in wintering areas (after Rodriguez et al 2013) (1 = Cantabrian Sea, 2 = Western Mediterranean, 3 = Gulf of Cádiz, 4 = Eastern Canary Islands, 5 = Mauritanian waters; The black lines indicate the transect locations; The pie charts represent the frequency of occurrence of the age classes (Imm= immature birds of 1–4 calendar year, and Ad = adult birds); For each region, the number of birds counted (n), the estimated average density (d) and the occurrence of entanglements (e, in %) are also shown).

### Factors of sensitivity

Foraging strategy and behavioural characteristics may be one of the factors acting for sensitivity of seabirds.

In the shearwater breeding population of Strofades islands, Karris (pers. comm.) proposed that the entanglement of individuals might be the outcome of: i) high mobility of shearwaters according to their dual foraging strategy during the breeding period (Karris 2014); and ii) intensive fishing effort in the Ionian Sea (Karris et al. 2013).

In northern Gannet - *Morus bassanus*, the « plunge diving behaviour » might explain the high rate of entanglement, which occurs when they dive bomb into a drift plastic object or to catch fish, being concentrated under those objects (see Rodriguez et al. 2013, for references). Furthermore, Rodriguez observed that the most frequent debris responsible for the entanglement in his study area, were the red objects, followed by the greens, white and yellow objects.

The age of the birds may also constitute a sensitivity factor. In *Morus bassanus* for example, Rodriguez et al (2013) found a higher entanglement prevalence in immatures (1st and 2nd calendar years).

### Constraints and feasibility of monitoring

The state of the art and testimonies obtained through our surveys indicate that seabirds may represent a good indicator species for the entanglement, since they give some evidence that monitoring is feasible depending on approaches.

The beach surveys also constitute a valuable sampling device, since a standard protocol (Beach Bird Survey) is already used in several countries for monitoring, for example in the frame of OSPAR (monitoring oiled Guillemots).

The northern Gannet has already been emphasized as a relevant indicator species, and at least two approaches could be applied in this wide species' range. In Germany, Schulz et al. (in Werner 2017, pers. comm.), developed a standard protocol after MSFD TSG ML (2013), in which each colony of northern Gannet - *Morus bassanus* is visited at least three times per year to estimate the minimum entanglement rates. This method allows to assess the quantifiable mortality rates due to different types of plastic litter, and generates a minimal disturbance of breeding birds and source-specific information on mortality risk. In non-breeding (southern wintering) areas, the standard vessel based approach proposed by Rodriguez et al. (2013), could be valuable in the perspective of long term monitoring programs, after testing the method in other areas.

In Mediterranean, the shearwater - *Calonectris diomedea* is susceptible to entanglement, however this species probably does not represent an interesting indicator species, since entanglement is mainly due to the active fishing rather than to the debris (Cadiou, pers. comm.).

## Fish

### Sensitive taxa, occurrence and circumstances

At a global scale, entanglement has been reported in 89 species of fish (Kühn et al 2015), which are often deeply cut by the debris in which they are entangled (Barreiros and Guerreuro, 2014). Entanglement has been reported to reduce mouth opening, which hampers foraging and gill ventilation (Sazima et al, 2002 in Kühn et al, 2015), reduce mobility and several deleterious effects (see Kühn et al 2015 for detailed description and references).

Elasmobranches may be caught in large amounts in ghost fishing gears. In Atlantic, Large et al. (2009) observed high quantities of sharks entangled in ghost fishing gears (*Centrophorous squamosus* - 6.2 ton and *Somniosus microcephalus* - 1 ton) in Ireland and the UK waters (Table 5), while in Cape Verde, López-Jurado et al (2003) reported entanglement in 2 other species (*Ginglymostoma cirratum* - 2 tons and *Pristis pectinata* - 12 tons) (Table 5).

**Table 5:** Prevalence and material/items cited as responsible for entanglement in the literature for elasmobranch (INDICIT project area and Atlantic) (DEB) marine litter of user origin, such as plastic package, baler twine (rafia etc) or other (stake nets etc...), (GFG) fishing gear, (N/T) pieces of nets or trawl nets

| Species | Country/ Ocean basin | Years | N | N entangled (%) | Item/Material | References |
|---|---|---|---|---|---|---|
| BLUE SHARK | | | | | | |
| *Prionace glauca* | Mediterranean sea | 2016 | NA | 1 | DEB | Colmenero et al, 2017 |
| | Atlantic | 2016 | NA | 5 | DEB | Colmenero et al, 2017 |
| LEAFSCALE GULPER SHARK | | | | | | |
| *Centrophorous squamosus* | Atlantic (Ireland, UK) | 2005 | NA | 6.2 ton | GFG (N/T) | Large et al, 2009 |
| GREENLAND SHARK | | | | | | |
| *Somniosus microcephalus* | Atlantic (Ireland, UK) | 2005 | NA | 1 ton | GFG (N/T) | Large et al, 2009 |
| NURSE SHARK | | | | | | |
| *Ginglymostoma cirratum* | Atlantic (Cape Verde Islands) | 2001 | NA | 2 | GFG (N/T) | López-Jurado et al., 2003 |
| TOPE SHARK | | | | | | |
| *Galeorhinus galeus* | Atlantic (Cape Verde Islands) | 2001 | NA | 1 | GFG (N/T) | López-Jurado et al., 2003 |

In Spain, Colmenero et al (2017) recently reported cases of juvenile blue shark - *Prionace glauca* (Table 5), entangled in plastic straps around their gill region, causing several degrees of injury on the dorsal musculature and pectoral fins.

During our survey, entanglement in this species was also mentioned in one response from Portugal. This phenomenon was further substantiated by F. Vandeperre (IMAR, present survey), who reported at least 30 observations from a fisheries observer program of the Portuguese pelagic longline fleet, which corresponds to a prevalence of approximately 1/1000. In almost all cases, blue sharks were imprisoned in a ring-shaped object: plastic rings, plastic straps. Since straps were often those used for the packaging of fish bait, some fishermen therefore started cutting the straps before unfortunately throwing them in the sea. Pictures obtained during our survey show deep wounds due to lines, which have been lacerating and cutting the tissues (Figure 9a). A swordfish - *Xiphias gladius* was also found with a plastic ring around the rostrum (Figure 9b).

a) b)

**Figure 9.** Effects of entanglement in fish:
**a)** Blue shark - *Prionace glauca* ; **b)** Swordfish - *Xiphias gladius* (courtesy of IMAR).

## Spatiotemporal variability

No information was available about the spatiotemporal variability of entanglement in fish in the project area.

## Factors of sensitivity

Given the recurrent pattern of entanglement in blue sharks, F. Vandeperre (IMAR, present survey) proposed that it would represent a specific interaction, probably related to some characteristic behaviour. Cliff et al. (2002, in Kühn et al 2015) suggested that predators such as sharks, are at risk of entanglement when their prey fish using debris as a shelter. According to Bird (1978, in Kühn et al 2015) and Nunes et al. (2017), sharks' investigative behaviour to floating objects may be a leading cause for approximation to these objects, which may result in their entanglement. Thus, behavioural characteristics such as neophilia (i.e. attraction to novel objects), may be a determining factor for higher sensitivity to entanglement.

## Constraints and feasibility of monitoring

Sharks species, such as the blue shark - *Prionace glauca*, are wide ranging (Figure 10), and fishing target or bycaught species susceptible to be monitored on a regular basis by fishery observer programs. However, our survey collected only one record of entangled sharks within the project area, which confirmed the report from Spain published by Colmenero (2017).

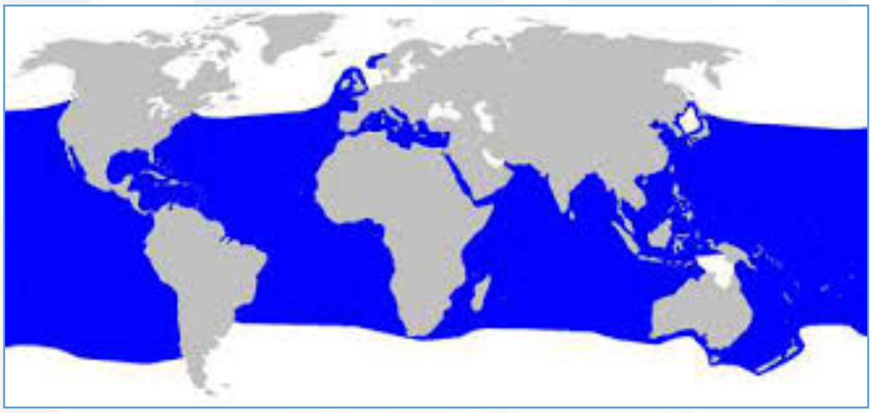

**Figure 10.** Distribution (in blue colour) of the blue shark *Prionace glauca*
(after sharksider.com)

At our project scale, according to Poisson (comm. pers.), expert in halieutics, who lead a working group on elasmobranches' conservation in the frame of FAO GFCM, data about entanglement in sharks is too rare for conducting a constraint analysis or for using sharks as indicator species of entanglement in debris. Moreover, this expert mentioned that in certain areas, sharks may be entangled intentionally by fishermen before releasing them at sea, which would bias the data regarding the prevalence of entanglement in those species.

Despite the questionnaires provided by Portugal showed that data are available from fishery observer campaigns, we still lack information on the existence of data on entanglement in fish in the project area. To address this question, it would be necessary to contact each institution in charge of fisheries monitoring, which represents a long work. Fishery observer campaigns represent probably the most appropriate tool for monitoring entanglement in fish. We searched the internet for the existence of diver networks that could have some monitoring activity or data, but without any result.

Consequently, we cannot conclude about the feasibility of an entanglement indicator based on fish monitoring at this stage.

## *Invertebrates*

### Definition of entanglement in invertebrates

In the case of invertebrates, "entanglement" with marine debris also includes «smothering». Smothering is often observed on the seabed, especially affecting epibenthic invertebrates, such as corals and sponges. Since smothering is often difficult to distinguish from entanglement, and is often reporte as «entanglement», we decided to include both types in the present feasibility study.

### Sensitive species, occurrence, circumstances of observation and factors of sensitivity

Entanglement has been reported for many groups of invertebrates (crabs, lobsters, echinoderms etc.). However, our literature review suggests that epibenthic organisms (such as hydrozoans, corals and sponges) have been more commonly reported. As a result, the following sections are centred on these sessile organisms, presenting interesting characteristics to consider them as potential indicators of entanglement.

#### Sensitive taxa and occurrence

The analysis of the published literature on entanglement in the marine biota revealed that 104 different taxa of epibenthic invertebrates were reported entangled in marine debris (Table 6). Entanglement was reported for corals in shallow tropical reefs (e.g. Chiappone et al. 2005), down to gorgonians inhabiting deep submarine canyons (e.g. Mordecai et al., 2011). Generally, fishing gear (especially monofilament line and gills nets) is mostly responsible for those accounts (90%), while entanglement in plastic bags and other plastic items have also been observed (e.g. Sheehan et al. 2017), but is far less common.

Most literature reporting entanglement in epibenthic invertebrates comes from the deep sea (>200m). Cold-water corals and sponge are known to form large aggregations (that are also termed "Marine Animal Forests") that are widespread throughout the deep sea of the MSFD region (Figure 11), forming three-dimensional structures that are important for a wide variety of organisms. Epibenthic organisms found in the deep ocean have life history characteristics, making them extremely vulnerable to anthropogenic disturbances. Some cold-water corals have growth rates <1mm per year, with life span exceeding 1000 years (Carreiro Silva et al., 2013). Therefore, recovery of cold-water coral assemblages from disturbance occurs extremely slowly (Althaus et al., 2009; Williams et al., 2010). Such organisms are considered to form Vulnerable Marine Ecosystems (VMEs), under certain criteria defined by the FAO guidelines, in support of the United Nations General Assembly (UNGA) resolution 61/105 (FAO, 2009). The FAO has included a set of criteria that should be used, individually or in combination, for the identification process of VMEs, specifically: the uniqueness or rareness, the functional significance of the habitat, the fragility and the structural complexity. Typical taxonomic VME groups include stony corals, black corals, gorgonians, stylasterids, sea-pens and habitat-forming sponges (deep-sea astrophorids, hexactinellids or lithistids). These taxonomic groups are widespread in the deep sea and could be considered as being indicators of entanglement in marine debris.

**Table 6:** Sensitive invertebrate species, prevalence and types of material responsible for the entanglement at the global scale (**DRG** - Derelict fishing gear ; **DRL** - Derelict fishing line ; **DRN** - Derelict fishing nets).

| taxon | species name | years | % entangled | material | country/ area | references |
|---|---|---|---|---|---|---|
| 65 species | | 2002 | | DFG | Puget Sound | Good et al. 2010 |
| *CORALS* | *Acanthogorgia hirsuta* | 2013 | 1,00% | DFG | Sardinian slope | Cau et al. 2017 |
| | *Antipathella subpinnata* | 2011 | | DFL | St.Vincent Canyon, Portugal | Oliveira et al. 2015 |
| | *Antipathella subpinnata* | 2013 | 12,00% | DFG | Sardinian slope | Cau et al. 2017 |
| | *Antipathella subpinnata* | 2010-2011 | | DFL | Tyrrhenian Sea | Angiolillo et al. 2015 |
| | *Antipathes dichotoma* | 2013 | 2,00% | DFG | Sardinian slope | Cau et al. 2017 |
| | *Antipathes dichotoma* | 2010-2012 | | DFL | Tyrrhenian Sea | Bo et al. 2014 |
| | *Astrospartus mediterraneus* | 2011 | | DFL | St.Vincent Canyon, Portugal | Oliveira et al. 2015 |
| | *Callogorgia verticillata* | 2009 | | DFL | French Mediteranean | Fabri et al. 2014 |
| | *Callogorgia verticillata* | 2013 | 9,00% | DFG | Sardinian slope | Cau et al. 2017 |
| | *Callogorgia verticillata* | 2010-2011 | | DFL | Tyrrhenian Sea | Angiolillo et al. 2015 |
| | *colonial zoanthid P. mammillosa* | 2001 | 19,60% | DFL | Florida Keys, USA | Chiappone et al. 2005 |
| | *Corallium rubrum* | 2013 | 18,00% | DFG | Sardinian slope | Cau et al. 2017 |
| | *Dendrophyllia cornigera* | 2013 | 7,00% | DFG | Sardinian slope | Cau et al. 2017 |
| | *Dendrophyllia cornigera* | 2010-2012 | | DFL | Tyrrhenian Sea | Bo et al. 2014 |
| | *Dentomuricea cf. Meteor* | 2010 | | DFL | Azores | Pham et al. 2013 |
| | *Errina dabneyi* | 2009-2011 | | DFL | Azores | Rodríguez and Pham. 2017 |
| | *Eunicanella verrucosa* | 2015 | | DFG | SW England | Sheehan et al. 2017 |
| | *Eunicella cavolini* | 2013 | 23,00% | DFG | Sardinian slope | Cau et al. 2017 |
| | *gorgonians (Octocorallia),* | 2001 | 55% | DFL | Florida Keys, USA | Chiappone et al. 2005 |
| | *gorgonians (Octocorallia),* | 1989-2011 | | Plastic bag | Monterey Canyon | Schlining et al. 2013 |
| | *Leiopathes glaberrima* | 2013 | 7,00% | DFG | Sardinian slope | Cau et al. 2017 |
| | *Leiopathes glaberrima* | 2010-2012 | | DFL | Tyrrhenian Sea | Bo et al. 2014 |
| | *Lophelia pertusa* | 2009 | | DFL | French Mediteranean | Fabri et al. 2014 |
| | *Lophelia pertusa* | 2011 | | DFN | Cap de Creus Canyon, Spain | Tubau et al. 2015 |
| | *Madrepora oculata* | 2009 | | DFL | French Mediterranean | Fabri et al. 2014 |
| | *Madrepora oculata* | 2013 | 7,00% | DFG | Sardinian slope | Cau et al. 2017 |
| | *milleporid hydrocorals* | 2001 | 17% | DFL | Florida Keys, USA | Chiappone et al. 2005 |
| | *Oculina* | 2004-2005 | | DFL | SE USA | Bauer et al. 2008 |
| | *Ophiuroidea* | 2011 | | DFL | St.Vincent Canyon, Portugal | Oliveira et al. 2015 |
| | *Paramuricea clavata* | 2013 | 7,00% | DFG | Sardinian slope | Cau et al. 2017 |
| | *Paramuricea clavata* | 2010-2011 | | DFN | Tyrrhenian Sea | Angiolillo et al. 2015 |
| | *Paramuricea clavata* | 2010-2012 | | DFL | Tyrrhenian Sea | Bo et al. 2014 |
| | *Paramuricea macrospina* | 2010-2011 | | DFL | Tyrrhenian Sea | Angiolillo et al. 2015 |
| | *Parantipathes larix* | 2013 | 6,00% | DFG | Sardinian slope | Cau et al. 2017 |
| | *Pocillopora meandrina* | 1998 | 65,00% | DFL | Hawaii | Yoshikawa and Asoh, 2004 |
| | *Pocillopora meandrina* | 2002 | | DFL | Hawaii | Asoh et al. 2004 |
| | *Savalia savaglia* | 2010-2011 | | DFL | Tyrrhenian Sea | Angiolillo et al. 2015 |
| | *scleractinian corals (Scleractinia)* | 2001 | 30,90% | DFL | Florida Keys, USA | Chiappone et al. 2005 |
| | *Viminella flagellum* | 2010 | | DFL | Azores | Pham et al. 2013 |
| | *Viminella flagellum* | 2013 | 1,00% | DFG | Sardinian slope | Cau et al. 2017 |
| | *Viminella flagellum* | 2010-2011 | | DFL | Tyrrhenian Sea | Angiolillo et al. 2015 |
| | | 2011-2013 | | DFL | Indian Ocean | Woodall et al. 2015 |
| *CRABS* | *Chionoecetes opilio* | 1988 | | DFN | Oregon, USA | June, 1990 |
| *ECHINODERMS* | *Echinodermata* | 2011 | | DFL | St.Vincent Canyon, Portugal | Oliveira et al. 2015 |
| | *Gracilechinus alexandri* | 2011 | | DFL | Cap de Creus Canyon, Spain | Tubau et al. 2015 |
| *SEA ANEMONES* | *Amphianthus sp.* | 1999 | | Plastic | Hausgarten, Arctic | Bergmann and Klages. 2012 |
| | *Bathyphellia margaritacea* | 1999 | | Plastic | Hausgarten, Arctic | Bergmann and Klages. 2012 |
| *SEA URCHINS* | *Cidariscidaris* | 2007 | | DFL | Nazare Canyon, Portugal | Mordecai et al. 2011 |
| *SPONGES* | *Cladorhiza gelida* | 1999 | | Plastic | Hausgarten, Arctic | Bergmann and Klages. 2012 |
| | *Geodia cydonium* | 2014-2015 | | DFN | Meditranean | Melli et al. 2017 |
| | *Pseudotrachya hystrix* | 2009-2011 | | DFL | Azores | Rodríguez and Pham. 2017 |
| | | 2011-2013 | | DFL | Indian Ocean | Woodall et al. 2015 |
| | *Demospongae* | 2001 | 13% | DFL | Florida Keys, USA | Chiappone et al. 2005 |

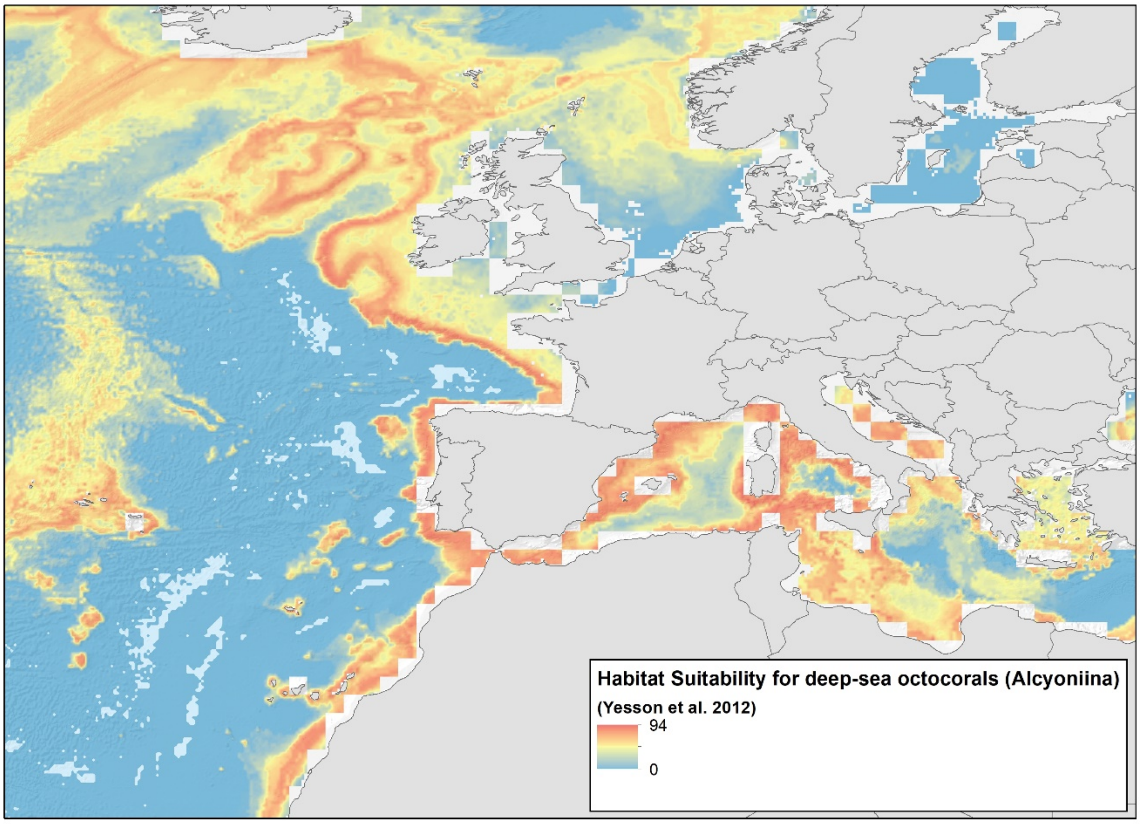


**Figure 11.** Habitat suitability (species distribution models) for deep-sea corals (*Alcyoniina*) within the MSFD regions
(After Yesson et al. 2012).

Spatiotemporal variability

There is a direct link between the occurrence of entanglement in epibenthic invertebrates and the spatial distribution of fishing effort. Typically, high levels of entanglement are reported for fishing grounds where the use of longlines and gills nets is more widespread (e.g. Bo et al. 2014; Pham et al. 2013; Rodriguez and Pham 2017; Oliveira et al. 2015). Less entanglement is found on areas where bottom trawling is the dominating fishing gear. This is primarily because, such areas are ploughed by the gear, leaving little epibenthic organisms left behind (Watling and Norse, 1998). Furthermore, bottom trawls are less easily lost when compared to longlines or nets. Nevertheless, Figure 12 shows that entanglement of epibenthic organisms has been reported throughout the MSFD region.

There is very little data for assessing any change in the level of entanglement of epibenthic invertebrates over time. Most information on entanglement of epibenthic invertebrates is opportunistically obtained from video footage that were collected for other purposes. Future efforts should focus in compiling existing video footage across the area of interest for establishing baselines for entanglement in epibenthic invertebrates.

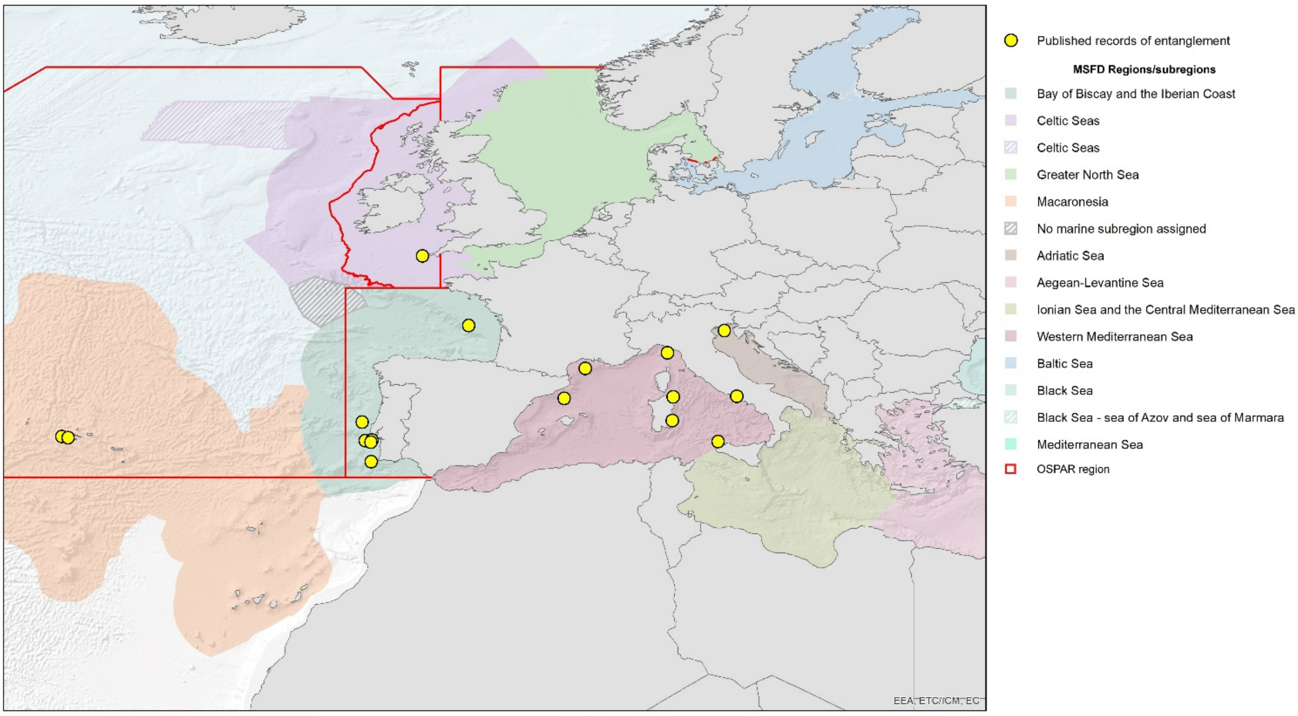


**Figure 12.** Published records of entanglement within MSFD regions.

## Factors of sensitivity

The most common organisms found entangled are corals and sponges, probably due to their complex morphologies, but also because they form dense aggregations. As a result, larger and more complex species, such as gorgonians, black corals, scleractinians and habitat forming sponges are more susceptible of being entangled in debris. Accordingly, these morphologically complex taxa are more often reported as being entangled than other smaller morphotypes. On a small seamount off the Azores, entanglement was assessed for two cold-water coral species of contrasting morphologies that are found in equal abundance (Pham et al., 2013). Entanglement in the most complex species was far more frequent than in the other simpler whip-like coral.

# Constraints and feasibility of monitoring

## Distribution of species and availability of data

Epibenthic invertebrates, and in particular corals and sponges, are abundant and wide spread in the MSFD region (see Figure 11; Yesson et al. 2012; Davies and Guinotte, 2011; Howell et al. 2016; Salomidi et al. 2009) and OSPAR area. Report of entanglement in marine debris started to emerge due to the wider use of underwater imaging technologies to explore the seafloor. Nowadays, campaigns for exploring the seabed are common in the framework of national explorations (for example *Medseascan* in France or EMEPC in Portugal), scientific endeavours or commercial exploitation of seabed resources (e.g. seabed mining or oil exploitation). These campaigns, which operate in coastal areas or in the deep sea, collect hours of videos, which are generally available from oceanographic institutes upon request or directly from websites (see for example *cartographie.aires-marines.fr*). Consequently, a high amount of data is already available for obtaining a powerful assessment of entanglement in marine debris.

Biological constraints

Epibenthic invertebrates may represent good bioindicators for entanglement, depending on the complexity of their morphologies. No biological constraints were identified for corals, whereas for sponges, according to Consoli (pers. comm.) their ability to grow and entirely cover the marine debris over time represents a biological constraint which makes it difficult to use them as indicator species, unless they are used under specific conditions (see below).

Methodological constraints

Despite their quite high cost, underwater imaging technologies such as Remotely Operated Vehicles (ROV) and towed cameras are now widely used (Scotti et al, 2017; Iokeimidis et al 2015). Methods used for monitoring/exploring the seabed are tailored according to each dive's objective, but generally follows a similar outline, which results in underwater footage that is georeferenced. The images are then annotated, providing a wide variety of information (Durden et al. 2016) (Figure 13).

For monitoring purposes, a specific sampling scheme has to be defined (length of transects, distance above the seafloor for adequate image resolution etc.). In particular, the locations should be chosen according to strict criteria, based on the level of the available knowledge for the area. For example, a good understanding of fishing activities and oceanographic conditions should be taken into account for a correct use of the indicators. Knowledge of fishing effort is crucial for site selection, since variability in entanglement could directly reflect changes in fishers' behaviour. If monitoring is being done in an area that may become a less productive fishing ground, decrease in the occurrence of entanglement would simply reflect changes in fishing effort.
Once collected, protocols for image annotation and analysis needs to be defined. An important constraint, which has no simple solution, lies in determining how the occurrence of entanglement is linked to the number of litter items. For instance, a high occurrence of entangled corals in an area might not be caused by separate litter items, but by a single longline of several kilometres long.

Another important issue to solve, for a correct use of such indicator, is the interactions between litter items. An area with a high abundance of lost longline will increase the likelihood of further gear to be lost (Cho et al. 2011). Such bias will be important to take into account when evaluating the indicator.

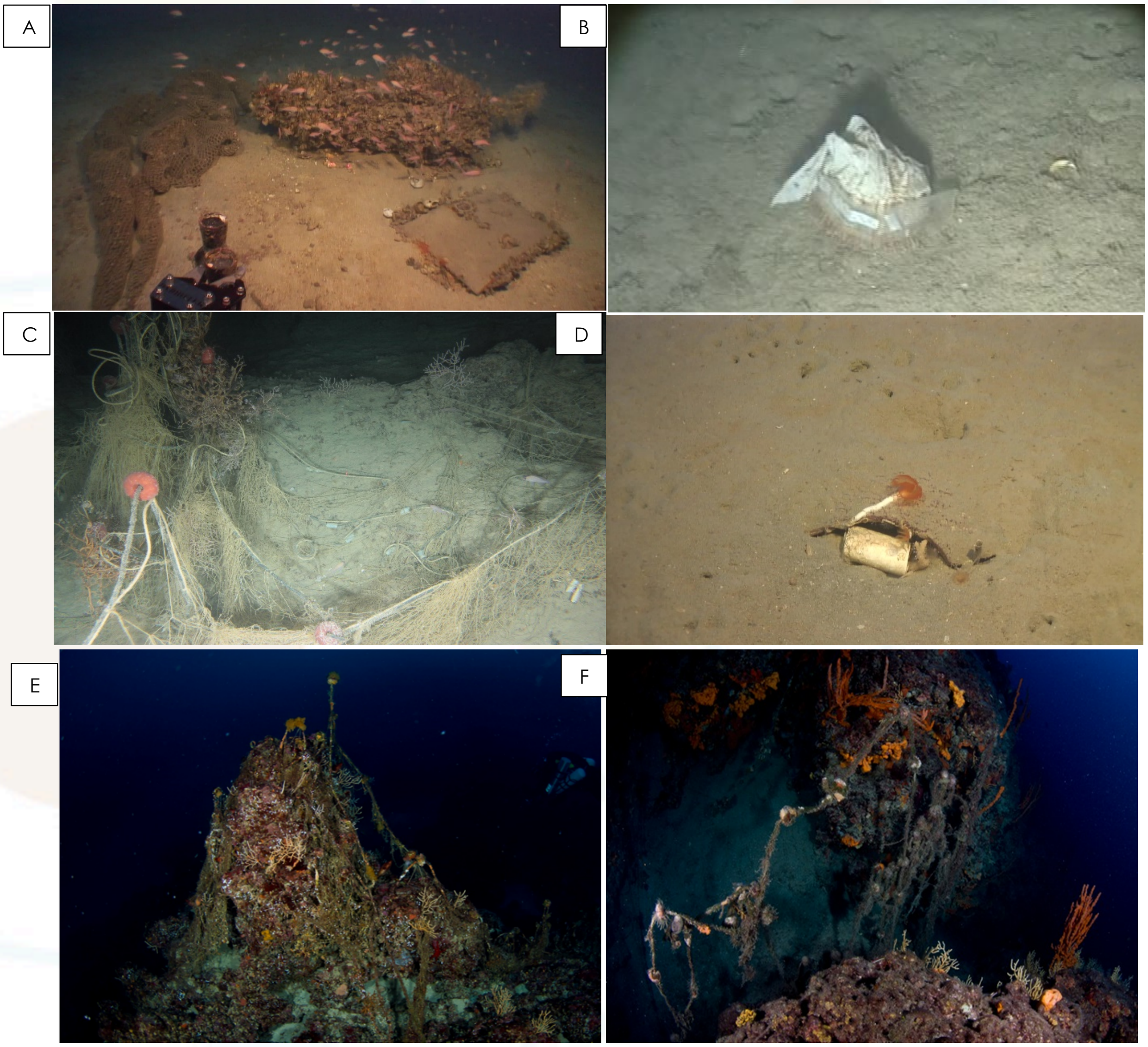


**Figure 13.** Imaging entangled invertebrates on the sea floor with a ROV *((A) fishing net and metal beverage can (left) in Greece (courtesy C. Iokeimidis/ SEABED campaign; (B) : urchin covered by plastic sheets in Marseille, France (courtesy F. Galgani/CYATOX campaign); (C) : corals entangled by fishing material, Italy (courtesy S. Canese/ISPRA); (D)* **:** *beverage metal can covered by a ceramic tile having a polychaeta attached on it (courtesy C. Iokeimidis/ SEABED campaign)); (E), and(F): Ghost nets on Mediterranean coralligenous communities, Korinthiakos gulf, Greece (Photo credit Yiannis Issaris@HCMR).*

## Environmental constraints

Cold-water corals and sponges are mostly dominating the deep ocean, restricting our monitoring to a certain type of litter items, mostly related to fishing gears.

## Logistic constraints

The methods used for studying/monitoring epibenthic species request vessels and specific equipments (crane, ROV, cameras, etc.); in this respect, the main constraint of this method might be its cost, which can be high depending on the vessel used. However, oceanographic means are present in quite a number of EU and RSCs Members States (e.g. IFREMER in France, HCMR in Greece, ISPRA and national fleet in Italy, IEO in Spain, EMPC in Portugal etc.). The oceanographic means operated in Mediterranean Sea and other RSC areas could be mutualized and represent useful data collection devices for a long term monitoring, upon a multiannual basis (see conclusion). It is important to note that there is a risk associated with using ROVs in areas with high abundance of lost fishing gear, due to the potential entanglement of the ROV itself (Pham, pers. Obs.).

### Conservation and regulatory constraints

Several species of epibenthic invertebrates are listed as endangered/vulnerable, through various international directives and agreements. Considering that the proposed method for monitoring the indicator will only rely on imaging techniques, there is no regulatory constraints in using these species as indicator species. Moreover, data related to pressures on these taxa should be of particular interest for managers/administrations, for assessing the conservation state and trends of populations/ habitats, as well as the efficiency of conservation measures for species.

## Conclusion

In the Mediterranean, RAC SPA (2017) already suggested using benthic invertebrates as entanglement indicator, since it offered the possibility of monitoring this impact at a wide range of depths. This report confirms the potential of epibenthic invertebrates, in particular deep-water corals, not only in the Barcelona convention area, but also in the EU, OSPAR and HELCOM areas. This group of organisms is widespread, and sufficiently abundant for relevant monitoring programs. According to Consoli (pers. comm.), monitoring activities could, for example, be operated each 6 years by ROVs, on selected sampling areas on a basis of 20 days at sea and 4 months of work for analysis of images/data. Monitoring of entangled corals would have to be performed in areas with rocky substrate, since on soft (sand) bottoms, specimens may have suffered from active trawling, which cannot permit to attribute the impact to litter only. In addition, the morphological complexity of different coral species should be taken into account when defining indicators, since entanglement largely depends upon this factor. The main constraint of this method might be its cost, which can be high depending on the vessel used. However, the oceanographic means operated in Mediterranean Sea and other RSC areas could be mutualized and represent useful data collection devices for long term monitoring. The present feasibility study suggests that « marine debris entangling corals » could be proposed as a candidate indicator of impact of marine debris on marine biota, and that further work should be undertaken in order to collect and share data which would permit tests, and develop this indicator (criteria, methods/ common approach, thresholds, common banking procedure etc.). Another proposal would be, to consider more globally an indicator like « marine debris entangling (including smothering) epibenthic invertebrates ». In the first case, the metric could be the number of individuals entangled or damaged (typology to be defined), while in the second case, the metric could consist in the number of individuals entangled/smothered by debris, or the surface of invertebrate communities covered by debris.

# IV – CONCLUSION

## 1. Data availability and existing resources for monitoring

Our feasibility study permitted to partly identify existing networks of stranded/injured/rescued animals in nine countries of the project area. Despite the fact that the INDICIT partners contacted a lot of stakeholders within their respective countries, they obtained a limited percentage of responses to their solicitations. However, these first results will be valuable for completing the list of database availability per country/organization/project on marine litter compartments, currently under preparation by MSFD TSG ML (Galgani, 2017 comm. pers.). In this table, which purpose was to list data holders, databases types, methodologies, data amounts (entry/ surveys), area covered, time covered, metadata, the compartment "entanglement" was only documented for Germany (for sea bird colonies) and Spain ("INDICIT" cited in the perspective of further information). Our results in listing all the stakeholders who collect data per country, local sector and taxon of interest are thus significant, although incomplete. More time is requested in order to list all data holders, generate confidence and agreements for sharing information. Efforts should thus continue during and after the project, in order to complete the list, and share it widely between stakeholders and managers, which will be useful for further investigations, monitoring building, coordination of studies and harmonization of methodologies.

According to countries, the networks we identified are uni or multi-taxon targeted, acting for conservation or scientific purposes, investigating at national or local scale, managed by volunteer-NGOs, authorities or scientists. Part of those networks are very much dependent on sea users for alerts (fishermen/sailors, fisheries observers and other sea users).

Our results in the identification of the adequate resources necessary for the assessment of an entanglement indicator showed that skills, human and logistical resources, exist at some level. However, the majority of the existing data are collected by volunteers (NGOs, rescue centres) and fund raising (public and private), the other part by professional scientific oceanography/fishery monitoring networks, or by national entities responsible for the protection of the environment (biodiversity and marine protected areas).

Unless for fish, on which very few data seem available at our project scale, data are available at different levels, according to the distribution of the other megafauna species and observation effort; databases are managed by all the data producers. For invertebrates, given the development of imaging technology on seabed, much more data than those published in scientific publications is certainly available within oceanographic institutions/projects (Galgani, 2017 com. pers.).

In summary, structures producing data in the project area are heterogeneous in their target and governance, and do not benefit from stable human/financial resources, which may ensure a constant data flow on entanglement for each taxon. The need for financial resources was highlighted in the majority of the responses to our survey. Some of the respondents proposed the involvement of local societies and authorities, and to develop citizen science platforms and initiatives. However, the majority of respondents declared that if the funds and standard protocols/ typology (types of impact and litter responsible for entanglement, with clues for distinguishing bycatch material) were available, they would be ready to investigate more deeply the feasibility of an entanglement indicator in marine mammals, birds or turtles. In this perspective, the number of data would then be much larger than actually is available. Concerning an epibenthic invertebrate indicator of entanglement/smothering indicator, listing oceanographic institutions/projects and extracting data has to be planned in the framework of a dedicated survey.

## 2. Knowledge and methodology

Several constraints have been identified for an entanglement indicator, and factors influencing the analysis of trends were examined (Table 7), which prevented the MSFD TG ML to consider entanglement as an indicator of impact of marine debris for several years (Galgani 2017, pers. com.).

**Table 7:** Factors influencing the analysis of trends of entanglement of fauna at sea (after RAC/SPA 2017, modified from UNEP/MAP, 2015)

| Detection | Sampling and detection bias |
|---|---|
| The entanglement happens due to isolated events distributed over a wide distribution area | Practically no direct and systematic sampling has been done and there are few long-term studies |
| The litter responsible for entanglement is not always identifiable at sea because it is not very, or only partially, visible | Inadequate sampling methods, to be improved |
| Dead animals are hard to see because they float on the surface and are sometimes caught up in the litter | Strandings represent an unknown part of the total number of entanglements |
| Animals that are entangled disappear after death by sinking or predation | Counting stranded animals does not take into account surviving animals and those taken in small litter |
| | Entangled animals spend longer feeding* out at sea than near the shore |
| | Some entanglements reflect interaction with active fishing gear rather than lost nets |
| | Many observations are not declared or published or are but in an anecdotal way |
| | Few data available before 1980 |

In Megafauna taxa (marine mammals, birds, turtles), the main constraint found in our study was the difficulty to distinguish an entanglement caused by "true" debris from that due to fishing gears. The only fishing material debris which we found distinguishable were balls of ropes of different materials, cross sections and colours. Consequently, a pilot protocol, which the INDICIT consortium is currently testing, could include three categories for describing the items responsible for the entanglement: fishing gears pieces, balls or ropes and debris of user source. However, this categorization would not permit to discriminate monofilament "true" fishing debris. According to Sacchi (pers. com), expert in halieutics, "Fantared" european project, carried out by partners specialized in halieutics, showed that it was quiet impossible to distinguish entanglement by drifting fishing material from that of a capture by fishing gears. A risk approach could be more pertinent, like it was studied for debris ingestion (Darmon et al, 2016), an entanglement risk index could be evaluated using quantities of fishing material abandoned or thrown at sea calculated through extrapolation of Fantared results updated with current fishing activities data and debris abundance data provided by oceanographic campaigns working on the water column or on the bottom in canyon areas, where debris are numerous.

## 3. Perspective and recommendations

The present deliverable was edited in November 2017, or month 10 across INDICIT project duration, and updated until March 2018 (month 14).

The results of the present state of the art and feasibility study was further discussed at the third INDICIT meeting in Montpellier, February 6-7, 2018, and on the following day with the External Advisory Board, which provided valuable comments and advice considered in this report.

The present document is a first feasibility study of an entanglement indicator. Despite the fact that we collected valuable data, information and contacts, not all available information was collected, due to the limited availability of data producers and experts. More information will certainly be gathered until the end of the project duration. In the meantime, the "ingestion and entanglement data sheet for sea turtles" will allow the collection of new data, which will be useful for further studies and tests.

Given the level of knowledge currently identified, several recommendations merge from the present study, which could be considered for further steps of the development of an entanglement indicator (Table 8). Like it was proposed by RAC/SPA (2017) for the Mediterranean, our feasibility study shows that monitoring entanglement could consider several zoological groups (cetaceans, birds, reptiles, fishes, and invertebrates) organised by compartments (beaches via stranding networks, surface during oceanographic campaigns, and seabed, thanks to underwater means of observation).

**Table 8.** Knowledge requested and recommendations for next steps of developing an indicator of entanglement of biota by marine litter (modified after RAC/SAP 2017 and present work)

| Constraints | Type of knowledge | Action requested | Recommendation |
|---|---|---|---|
| biological | number of species to monitor | choose small number of target species or whole taxon | draw a proposal |
| | biology and life cycle | test data sets | continue data collection |
| | probability of encountering litter | mapping risk areas | study |
| | prevalence /rate of entanglement | Priority at risk populations | draw a proposal |
| | description of induced pathologies | list criteria for diagnosis | veterinary workshop |
| environmental | confirm significance/ representativity as a pollution indicator | test data sets | study |
| methodological | standard protocols (ROV-invertebrates; examination-megafauna) | improve/ develop protocols | disseminate/ test protocol proposal |
| | criteria distinguishing entanglement/strangling due to litter from active fishing gear. | draw typology of material responsible for entanglement | workshop with halieutic experts |
| | identify possible factors interfering with results | acquire knowledge on movements, speed of decomposition | study |
| | organise data collection taking into account seasonal variations (litter -fishing activity, tourist season; species - migration) | acquire / compile knowledge | study |
| | quality assurance and banking arrangements and procedures | survey | pursue survey with data producers |
| logistic | cost of the monitoring | survey | pursue survey with data producers |
| | accessibility of samples and data | survey | draw a recapitulative table per country |
| | prior existence of permanent (no constraints related to seasonal variations) or seasonal data collection arrangements | survey | draw a recapitulative table per country |

As a first step, it is proposed to continue/ promote data collection during and after the INDICIT project, in order to potentiate the first efforts developed through our survey. Pilot and existing protocols could be tested on sea turtles, seals and sea birds with data producers interested in performing pilot monitoring. In particular, the INDICIT protocol proposes to collect data on entanglement on sea turtles as optional parameters. Concerning an epibenthic invertebrate indicator of entanglement, we recommend 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.  In pursuing efforts initiated during our survey, more knowledge will be acquired regarding the needs for rationalisation of existing data collection arrangements and procedures.

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## QUESTIONNAIRE USED FOR THE FEASIBILITY STUDY OF INDICATOR 2
## (Entanglement of biota by marine debris)

1. Do you spend much time at sea or around the sea? (yes or no)

If yes, professional or recreational?

2. Have you ever observed any marine animals entangled in litter? (yes or no)

If yes, in which species/taxa? (e.g. marine mammals, sea birds, sea turtles, fishes, other to be specified)

3. Do you have data/ information about this topic? (yes or no)

If yes, would you accept to share the data with INDICIT? (yes or no)

4. If yes,
   4.1. would you prefer us to contact you by phone for a discussion (yes or no)?
   4.2. in the other case, please fill hereafter:

What kind of information is available?

- ☐ report in ……………………………………………….(language);
- ☐ data table;
- ☐ publication (please send us the pdf file or cite the reference);
- ☐ otherwise, please describe your observations hereafter, giving as much information as possible:

| |
|---|
| *Species:* |
| *Number of individuals observed entangled per year:* |
| *Size of individuals: *(For turtles use this references: small = hand size; medium = forearm size; big = arm size; very big = more than arm)* |
| *Location (if possible coordinates in decimal degrees or precise location):* |
| *Circumstances of observation (at sea, stranded, other):* |
| *Type of debris responsible for entanglement (if possible be specific about the main type of debris; i.e. lost fishing line, ghost nets, plastic bags etc.):* |
| *Impact on the individual (wound, lesion and location on the body, death etc., if possible classify from the most frequent to the less frequent type of impact)* |
| *Were the individuals rescued?:* |
| *Which percentage survived?:* |
| *How much did it cost to rescue one individual (rough evaluation):* |
| *Since when do you observe cases of entanglement in your region?:* |
| *Do you observe a tendency (increase or decrease)?:* |

5. Why and how did, in your opinion, the individuals got entangled?
   *If possible, describe the circumstances and/or factors which, according to your observations, could favor the entanglement (many debris at sea, attractiveness of some debris, low visibility/perceptivity, high mobility, behavior linked to the stage of development/size etc.)*

6. Do you think that entanglement is easy to monitor in your region (frequency, existing observation networks and logistics, etc.)?

7. Do you think that the real number of entanglements is higher than the level you observed?

8. In your opinion, how could the monitoring be improved?

9. Do you have pictores of entanglement and if so, would you accept to share them with INDICIT? (yes or no)

# PILOT STUDY FOR THE IMPLEMENTATION OF INDICATOR **“MICRO-PLASTIC INGESTION BY FISH AND SEA TURTLES”** IN THE AREAS OF THE MARINE STRATEGY FRAMEWORK DIRECTIVE AND THE REGIONAL SEA CONVENTIONS **OSPAR, HELCOM AND BARCELONA**

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INDICIT
INDICATOR • IMPACT • TURTLE

# INDEX

# I – SUMMARY

Microplastics are small pieces of plastic litter < 5mm in diameter, they include production pellets/powders and engineered plastic microbeads and plastic items that are degraded and consequently fragmented into microplastic particles. The impacts of microplastic debris in the marine environment is not completely known, the investigation of impact of microplastic in this last decade is increasing and understanding the effects of microplastics on marine organisms is only at the beginning. Exists different pathways by which marine organisms interacts with microplastics, ingestion is the most likely interaction with microplastics for many organisms particularly when feeding mechanisms do not allow to discriminate between particles. Monitoring the ingestion of litter is a complex task, indentifying interactions between marine litter and marine organism depends on the quantity and quality of data collection.

Marine Strategy Framework Directive, Ospar, Helcom, Barcelona Conventions, need to develop indicators to determine impacts of litter on marine life (biota). The sea bird *Fulmarus glacialis* was proposed as possible indicator for the Northern European waters and the Loggerhead turtle *Caretta caretta* for the Southern European waters. For marine turtles, most part of the authors analysed marine debris ingestion greater than 5mm in diameter, but in the recent years others started to analyse until 1mm, following the Fulmar procedures. Most part of them did not differenciated macro (>5mm) and micro (<5mm) debris ingestion assuming that a part of the micro-debris derived from macro-debris fragmentation inside the digestive tract. Actually, a big concern to undertand the real impact of marine debris on marine biota is to identify if micro-debris ingested by megafauna species are derived from macro-debris fragmentation or by trophic transfer or both. Moreover, the number of publications on litter ingestion by fish is quickly increasing and many species are documented not only in laboratory experiments but also on field. The ingestion of microplastics by fishes presents a strong potential for developing monitoring purposes on ingestion of litter by marine organism. This report analyses the feasibility of "microplastric ingested by turtles" and "microplastic ingested by fish" as possible indicators of debris impact. The choice of a good indicator species is discussed taking into account the habitat and trophic information of the species, the sensitivity to litter, the wide distribution and commercial importance (for fish). A review of the literature on micro-debris ingested by turtles and fishes has been conducted, information on the type of study, sampling procedure, geographical location, species examined and their functional group, laboratory analyses, plastic frequency of occurrence, size of plastics and type, was extracted from each article. Each paper was critically examined and data collated to allow for a comparative analysis and general overview. A proposal of selection criteria and candidate fish target species to be used as indicators for monitoring microplastic ingestion are suggested.

# II – CONTEXT

## 1. INDICIT objective

The INDICIT proposal focuses on indicator 10.2.1 (10DC3 in the revised com Decision) associated to Descriptor 10 ("Marine litter") of the Marine Strategy Framework Directive (MSFD): "*Trends in the amount and composition of litter ingested by marine animals.*" For this indicator, the Commission Decision (2010/477/EU) expresses the need for further development based on the experience of some sub-regions and on emerging knowledge about other impacts besides the ingestion of litter by marine organisms. Therefore, the primary task for the implementation of an appropriate monitoring programme is to develop standardized tools to be used for investigating trends in the amount and composition of litter ingested by marine animals that cover all the MSFD marine regions.

Given their propensity to ingest litter, their wide distribution and the large range of habitats used during their life, sea turtles, in particular the loggerhead species - *Caretta caretta*, was proposed as a possible indicator. If the standard monitoring of the Good Environmental Status (GES) through the indicator

"Debris ingested by sea turtles" has a scientific and technical basis to be implemented; for other debris impacts, in particular micro-plastic debris items ingestion by other biota, more information is necessary. Indeed, this indicator should measure the ingestion of debris items sized from 1 mm, but do not focalize on the ingestion of micro-litter which, as defined by the MSFD's guidance on monitoring of marine litter in European Seas, corresponds to the particules inferior to 5 mm (JRC, 2013), which is probably of high concern, given their probable high concentration in the marine environment through e.g., fragmentation. Therefore, a most exhaustive analysis of micro-litter occurrence in loggerhead turtle should be conducted, as well as the study of other species (e.g., fish), which also has some potential to measure litter impact through ingestion. Scientific and technical background is needed to evaluate the feasibility of the implementation of such indicator as a monitoring tool. The aim of this report is to analyse all studies based on the published and grey literature, as well as on the collection and analysis of available data sets covering the MSFD, the Barcelona, OSPAR, HELCOM and Black sea Conventions areas regarding marine debris ingestion by turtle and by fish, in order to verify the possibility to use the "Micro-plastic litter ingested by turtle" and "Micro-plastic liter ingested by fish" as new indicators of debris impact.

## 2. The relevance of microplastics as an environmental contaminant

Whilst macroplastic debris has been the focus of environmental concern for some time, it is only since the turn of the century that tiny plastic fragments, fibers and granules, collectively termed ''microplastics'', have been considered as a pollutant in their own right (Cole et al., 2011). Microplastics are small pieces of plastic litter <5mm in size (Barnes et al., 2009, Gago et al., 2016). They can be broadly categorised as having entered the environment either as particles that are already <5mm, generally described as primary microplastics, or as having formed as a consequence of the fragmentation of the larger items in the environment, then described as secondary microplastics (Veiga et al., 2016, Gago et al., 2016).

Primary microplastics include production pellets/powders and engineered plastic microbeads, used in cosmetic formulations, cleaning products and for industrial abrasives. In contrast, secondary microplastics come from larger plastic items that are degraded and consequently fragmented, mostly due to weathering degradation, into microplastic particles (GESAMP, 2016). Both microplastic types (primary and secondary) exist in marine ecosystems at high concentrations. It has been estimated that about 245 tonnes of microplastics are produced each year which end up in water, where they are likely to become ingested and incorporated into the bodies and tissues of marine organisms (Auta et al., 2017). The widespread presence and the degradation and fragmentation of plastic are one of the key factors causing microplastics to be ubiquitous in the marine environment. While there is extensive literature on the loss of mechanical integrity of plastics with weathering on both land and beach as well as in the ocean environments (GESAMP 2016), micro-plastics impacts on marine fauna is less known. Microplastics enter the marine environment via different pathways. The microplastics beads present in cosmetics such as scrubs, toothpastes or air-blasting media, and in clothing, can enter the aquatic environment through industrial or domestic drainage systems. Similarly, synthetic fibers from clothing produce microplastic sheds that are washed into water or wastewater treatment plants as effluents (Auta et al., 2017). Microplastics are introduced also in rivers via wind and storm sewers (Zalasiewicz, et al., 2016).

Investigations on the presence of microplastics in the marine environment started in the 2000's. Recently, research has shown that microplastics have ubiquitously permeated the aquatic ecosystem, up to the Polar Regions and the mid-ocean islands (Lusher et al., 2015). Auta et al. (2017) evaluate the distribution of microplastics in the marine environment to represent 89% in North East Atlantic ocean, in North-western Atlantic 60%, in Jade Bay (Southern North Sea) 70 %, in Portuguese coast 53% and in the Mediterranean Sea 74%. However, the distribution of microplastics would tend to be 'patchy'. At different scales, from global (accumulation zones), to regional and very local (e.g. Langmuir cells) scales, the amount of plastic can vary by orders of magnitude. Models can aid in identifying hot-spots, especially while their ability to accurately simulate plastic behaviour and pathways is improving. As hot-spots are areas where the density of microplastics are highest, models might easily simulate these areas than their lower density counterparts (GESAMP, 2016). Numerical modelling has been applied to track back or hind cast from where plastics in the ocean may have come, and these same approaches are used for micro-plastic. Hind casting is particularly useful for source identification, especially where accumulation regions have been identified. Ocean circulation models are further used to identify where oceanic accumulation zones are most likely to occur. Coupling such tools and approaches with species distribution maps and other ecological information, it is possible to combine disparate data types to predict or identify hotspots of risk for taxa to encounter microplastic (Hardesty et al., 2017). Based on recent model results, at least 5.25 trillion plastic particles weighing 268,940 tons are currently floating at sea (Eriksen et al., 2014). As the abundance of microplastics increases, its bioavailability to marine organisms also increases. The colour, density, shape, size, aggregation and abundance of these tiny plastic particles affect their potential bioavailability to marine organisms. Biological interactions of microplastics with marine biota are the key to understanding the movement, impact and fate of microplastics in the marine environment (Auta et al., 2017).

Different pathways by which marine organisms interact with microplastics exist. Ingestion is the most likely interaction with microplastics for many organisms, particularly when feeding, discrimination between particles being not possible (Avio et al., 2017). Microplastics ingestion and the risk of transfer into the food web has been shown on several species, such us pinnipeds (McMahon et al., 1999; Eriksson and Burton, 2003), seabirds (Franeker et al., 2011), marine mammals (Fossi et al., 2012, 2014) and basking shark (Fossi et al., 2012, 2014). However many pressing questions regarding their impacts remain unsolved (Lanzarote Declaration, 2016). The interaction and the effect of microplastics in marine turtle and fish are detailed in the following paragraphs. Although laboratory studies have demonstrated that litter can impact fishes, the evidences in nature are only limited. As has been the case with other forms of contamination leading to pollution, it is important to consider responses across several levels of biological organization to evaluate, interpret and/or predict reliably the net effect of contaminants on wildlife (Rochman et al., 2016).

In general, the damage reported in large vertebrates following plastic ingestion, induces physical injuries to the different portions of the digestive tract, i.e. oesophagus, stomach, and intestine (Beck & Barros 1991; Baird & Hooker 2000; Mauger et al., 2002; Pierce et al., 2004; Jacobsen et al., 2010; Poppi et al., 2012; Stahelin et al., 2012). The damage may vary from perforations, inflammations and ulcerations, that are not necessarily lethal but do affect the functionality of the digestive system and the health of the individual. Hard microplastics usually present irregular shape with sharp edges that can penetrate intestinal lining and cause mechanical injuries and ulceration. Among the most obvious consequences of microplastics is the physical blockage of the digestive organs and interference with feeding. Accumulated plastic in stomachs may slow down overall digestion when normal food parts simply cannot pass to specific parts of the stomach or the gut. Anywhere in the digestive tract, plastics may cause partial blockage or constipation reducing the amount of food that can pass, and causing weakening and emaciation of the individual. In the digestive tract, even without blockage, volume occupied by plastic waste reduces the space available for food intake. Impacts may operate by physical-mechanical effects, chemical toxicity or combined effects. The hydrophobic properties and large surface area to volume ratio of microplastics (fragments smaller than 5 mm in size), can lead to the accumulation of contaminants, such as heavy metals and polychlorinated biphenyls (PCBs) from the marine environment. These chemicals, and those incorporated during production (such as plasticizers) can leach into biological tissue upon ingestion, potentially causing cryptic sub-lethal effects that have rarely been investigated (Koelmans, 2015). Indirectly ingested microplastics may pass their contaminants adsorbed through the cell membranes into the predator body tissues and organs, where they can accumulate and lead to chronic effects (Wright et al., 2013). The implications of trophic transfer, of both the microplastics and their associated toxins, are as yet unknown (Cole et al., 2013; Wright et al., 2013; Reisser et al., 2014) and worthy of investigation.

In recent years, a considerable number of experimental studies have been conducted on the ingestion and potential impacts from microplastic ingestion. These experiments have been done in particular on lower food web levels, including in fishes (Mattsson et al., 2014; Luis et al., 2015; Cedervall et al., 2012; Peda et al., 2016). Fish fed with microplastics in laboratory conditions, showed structural histopathological alterations in the distal intestine, such as widening of lamina propria, detachment of mucosal epithelium from *lamina propria*, shortening and swelling of villi, vacuolation of enterocytes, increase of goblet cells and hyperplasia of goblet cells, and loss of regular structure of serosa (Peda et al. 2016). In vitro exposure of fish neutrophils to nano-plastics is causing significant increase in the degranulation of primary granules and in the release of neutrophil extracellular traps (Greven et al. 2016), outlining the stress response of chief phagocytic cells. Such responses indicate the potential of nanoplastics to interfere with fish disease resistance, although such a hypothesis was not tested until now. This is, however, confirmed for non-plastic nanoparticles (Jovanovic et al. 2015). Ingestion of nano-plastics (<25 nm) is changing fish metabolism by altering the ratio of triglycerides to cholesterol in the blood serum, as well as the distribution of cholesterol between muscle and liver (Cedervall et al. 2012). Metabolic changes in micro- and nano-plastics exposed fish also include up regulation of fatty acids and down regulation of amino acids (Lu et al. 2016).

Concerning marine turtles, indirect ingestion of microplastics may occur when eating prey items, such as cnidarians, molluscs and crustaceans, which have been shown to ingest and assimilate microplastic particles in their tissues (Parker et al., 2005; Casale et al., 2008, Cole et al., 2013; Wright et al., 2013). This indirect ingestion may lead to sub-lethal effects that are difficult to identify, quantify and attribute to plastic ingestion as opposed to other water quality issues (Baulch and Perry, 2014; Vegter et al., 2014; Gall and Thompson, 2015).

## 3. Indicators description

The Commission Decision (COM DEC) 2010, identified indicators to characterize marine litter, including micro-particles (particules inferior to 5 mm), in the different marine environmental compartments. One category of them targets the impacts of litter on marine life (biota), emphasising that this indicator needs to be further developed.

The Technical Group on Marine Litter, established by EC in 2010, developed a Guidance on monitoring of marine litter (JRC, 2013) furnishing general approaches and strategies for marine litter monitoring. This guidance recommended the development of a general protocol for the measure of trends and regional differences in ingested litter in fish. However, it was not considered already applicable due to limited knowledge. The implementation of the new COM DEC defined the criteria 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*", and specifications on the units for the measuring: "*D10C3: amount of litter/micro-litter in grams (g) and number of items per individual for each species in relation to size (weight or length, as appropriate) of the individual sampled*".

In this view, the ingestion of microplastics by fish presents a strong potential for developing monitoring purposes on ingestion of litter by marine organisms. OSPAR, HELCOM and Barcelona Conventions have also included an analogous indicator in their monitoring programme and are currently testing the use of fish for this scope, underlying that a rigorous monitoring protocol needs to be developed.

During the last years, a growing body of publications on ingestion by fish and many species documents plastic ingestion, not only in laboratory experiments but also in the field (Fig. 1). Different studies have demonstrated the ingestion of microplastics in several commercial fish species, coming from pelagic and benthic tropic levels and at different geographical areas (Tab. 2 and 4). The heterogeneity of applied methods and results of these different studies need a deep analysis before any further considerations.

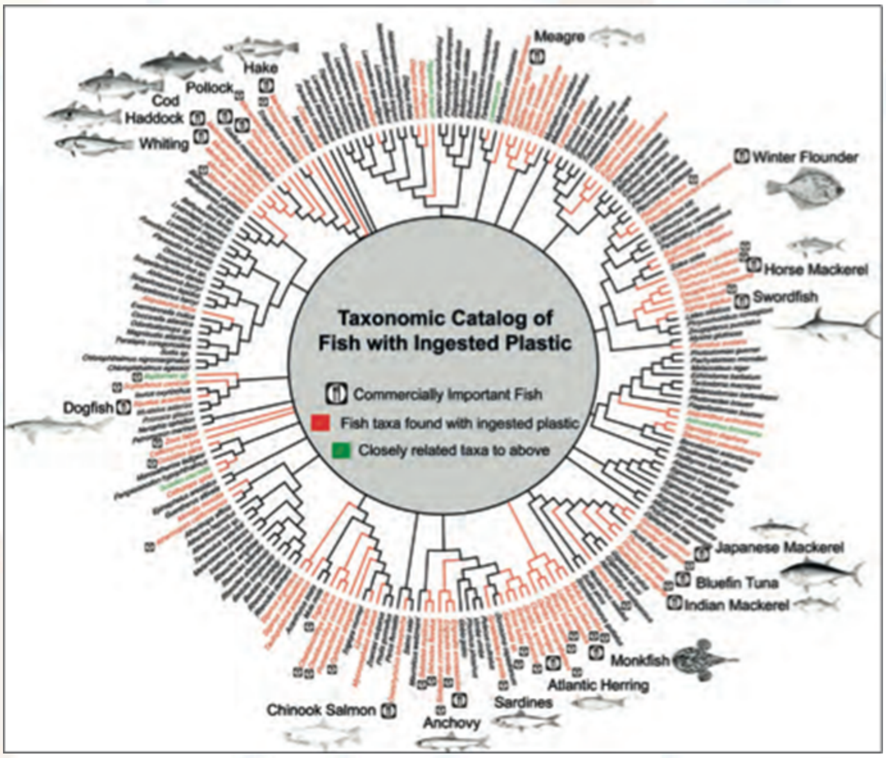


**Fig. 1.** Fish species found to contain microplastics (in red) (GESAMP, 2016).

Direct consumption of plastic fragments has been documented in all marine turtle species (e.g. Carr, 1987; Bjorndal et al., 1994; Hoarau et al., 2014). In the recent years, the ingestion of microplastics in particular, has also been described, but most of the studies concerning the impact of marine debris on sea turtles concern only macro-debris ingestion. Currently, and based on the MSFD Directive, researchers have adapted their methodologies to analyse ingested debris up to 1 mm size for marine turtles (Casale et al., 2008, 2016; Lazar & Gracan, 2011; Cameda et al., 2014; Nicolau et al., 2016). Nevertheless, the majority of studies did not separate micro-debris (< 5mm) from macro-debris, one of their hypotheses being that small pieces of debris may derive from the fragmentation of the macro-debris within the digestive tract. However, it is also possible that microplastic ingestion is unrelated with macro-debris fragmentation (Pham et al., 2017), underlying the necessity to consider a specific collection of ingested microdebris by sea turtles, and differentiate them from the macrodebris. Actually, a big concern to undertand the real impact of marine debris on marine biota is to identify if micro-debris ingested by megafauna species are derived from macro-debris fragmentation, by trophic transfer or both.

### 1. Methods

A search of the available original peer-reviewed literature was performed using databases (updated in March 2017) such as ProQuest Aquatic Science Collection, Science Direct, Scopus and Google Scholar.

For fish, in each database we used a combination of selected keywords: “marine” and “fish” and “ingestion” or “uptake” associated to “plastic” or litter” or “debris” or “microplastic” or “microdebris”. Bibliographic search was also based on two scientific social media, ResearchGate and Mendeley. From each article, we mined information on the type of study (on field and laboratory), laboratory processing of samples, geographical location, species examined and their functional group, sampling technique, plastic frequency of occurrence, type and size of plastics for items inferior to 5 mm.

For marine turtles, we used the combination of selected keywords: “marine”, “turtle”, “ingestion”, “microplastic”, “microdebris”, “microlitter”, “debris” and “uptake”. For each article, we focused on methodologies to identify if micro (specifically of size 1-5 mm as defined by JRC (2013)) and macro plastic ingestion was analyzed separately.

### 2. Results

#### a) Output of the search

Related with fish, 35 articles were found (last update in March 2017). First papers investigating plastic uptake in fish were from 1970’s (Carpenter et al., 1972; Kartar et al., 1973; Kartar et al., 1976). However, only in the last decade such studies became more common in this currently dynamic field of research, with a rapid increase in published papers (Fig. 2) and in the number of investigated species (Tab. 1 and 3).

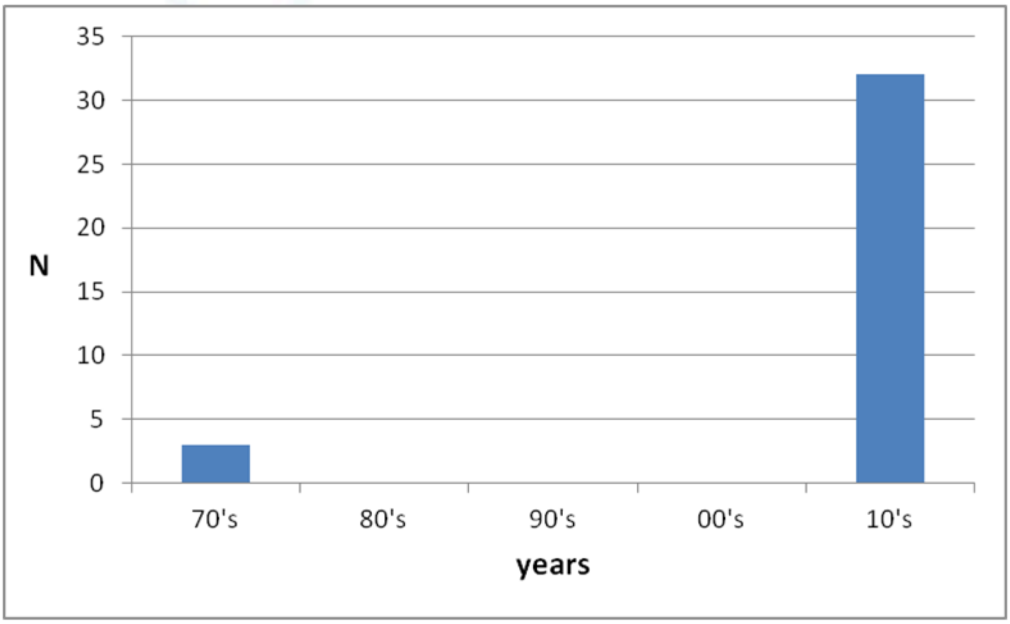


**Fig. 2.** Number of published peer-reviewed papers over time for plastic ingestion by fish (as updated in March 2017).

In marine turtles, an important constraint is that most part of the studies focused only on macro debris (greater than 5 mm or more) ingestion (see the literature review specific to Indicator "Litter ingested by sea turtles" in the INDICIT dedicated report). In the recent years, researchers have adapted their methodologies to include debris until 1 mm size (8 publications described below, Table 4), but conducted in the same way. Manual extraction by visual observation of gut content extracted from the digestive tract is the most commonly used technique (Tomas et al., 2002; Santos et al., 2015; Fukuoka et al., 2016; Nicolau et al., 2016), including both macro and microdebris. Nevertheless, this technique is not adequate for microplastic collection, due to the difficulties to detect smaller debris (< 5mm), which could remain camouflaged into the organic content. Only two studies included smaller and independent sieves to analyse debris according to size, such as Lusher et al. (2015), which used 0.118, 0.5 and 1 mm mesh sieves to analyse debris ingestion on beaked whale (Mesoplodon mirus). Pham et al. (2017) also separated the ingested macro (> 5mm) and microdebris (< 5mm) in loggerhead turtles captured in the North Atlantic gyre (Azores, Portugal).

### b) List of species and biological features

## *Fish*

In order to understand the perspective for the implementation of the indicator on marine micro-debris ingested by fish, two tables filled with information from the literature analysis were completed. Twenty-five papers linked to field studies were selected and compared. Table 2 contains information on 159 analysed fish species, their habitat, the geographical area and the sampling techniques used. The species studied by more than one author are reported in Table 3. Nineteen species were studied by more than two authors, six by more than three authors and two by more than four authors. Each species was included in their habitat type according to the classification of Fishbase (Fig. 3). The number of studies included in the category "habitat demersal" is 34% (referred to the number of species sampled by the authors), "bentho-pelagic" 19%, "pelagic-neritic" 15%, "reef associated" 12%, "bathy-pelagic" 9%, "bathy-demersal" 4% and "pelagic-oceanic" 7% The habitat types is an useful criteria for the choice of the indicator target species as the habitat information can give information on marine litter accumulation areas and trophic information about the species (that can influence marine litter ingestion). Sampling collection techniques are reported, but the knowledge on this issue is only at a preliminary stage. Studies on plastic uptake are often carried out in the framework of samples collected to investigate the feeding ecology of one or more species, or samples coming from routine surveys for monitoring fish stocks. The kind of sampling technique may influence exposure to microplastics. For example, a bottom-trawling net may re-suspend sediments with microplastics and potentially have more wear and tear compared to a mid-water trawl fishing net. Davison & Asch (2011) investigated the incidence of plastic ingestion during sampling, by comparing four types of nets. Results showed that the percentage of fish that had ingested plastics did not significantly differ amongst nets. Comparing these results with an analogous study by Boerger et al. (2010), the authors considered that the higher percentage of fish ingesting plastics found in that study (35%) could be attributed to the influence of feeding within the net, since manta trawl was deployed for a longer time, up to 5.5 hrs.

**Table 1.** List of fish species investigated for macro and microplastic ingestion and geographical distribution of studies.

| Fish species | Habitat | Geographical area | Sampling technique | Author |
|---|---|---|---|---|
| *Centrophorus granulosus* | Bathy-demersal | Eastern Mediterranean Sea (Ionian sea) | Deep water Long-lines | Anastasopoulou et al., 2013 |
| *Pteroplatytrygon violacea* | pelagic-oceanic | | | |
| *Etmopterus spinax* | Bathy-demersal | | | |
| *Galeus melastomus* | Demersal | | | |
| *Raja clavata* | Demersal | | | |
| *Raja oxyrinchus* | Bathydemersal | | | |
| *Scyliorhynus canicula* | Demersal | | | |
| *Squalus acanthias* | Benthopelagic | | | |
| *Squalus blainville* | Demersal | | | |
| *Brama brama* | pelagic-neritic | | | |
| *Conger conger* | Demersal | | | |
| *Epigonus telescopus* | Bathydemersal | | | |
| *Helicolenus dactylopterus* | Bathydemersal | | | |
| *Lepidopus caudatus* | Benthopelagic | | | |
| *Merluccius merluccius* | Demersal | | | |
| *Micromesistius poutassou* | Bathypelagic | | | |
| *Molva macrophthalma* | Demersal | | | |
| *Mora moro* | Bathypelagic | | | |
| *Nettastoma melanurum* | Bathypelagic | | | |
| *Pagellus bogaraveo* | Benthopelagic | | | |
| *Phycis blennoides* | Benthopelagic | | | |
| *Polyprion americanus* | Demersal | | | |
| *Scedophilus ovalis* | Benthopelagic | | | |
| *Scorpaena elongata* | Demersal | | | |
| *Sudis hyalina* | Bathypelagic | | | |
| *Xiphias gladius* | pelagic-oceanic | | | |
| | | | | |
| *Argyrosomus regius* | benthopelagic | Eastern Mediterranean Sea (Turkish territorial waters) | Trawl net | Guven et al., 2017 |
| *Caranx crysos* | reef | | | |
| *Dentex dentex* | benthopelagic | | | |
| *Dentex gibbosus* | benthopelagic | | | |
| *Diplodus annularis* | benthopelagic | | | |
| *Lagocephalus spadiceus* | demersal | | | |
| *Lithognathus mormyrus* | demersal | | | |
| *Liza aurata* | pelagic-neritic | | | |
| *Mullus barbatus* | demersal | | | |
| *Mullus surmuletus* | demersal | | | |
| *Nemipterus randalli* | demersal | | | |
| *Pagellus acarne* | benthopelagic | | | |
| *Pagellus erythrinus* | benthopelagic | | | |
| *Pagrus pagrus* | benthopelagic | | | |
| *Pelates quadrilineatus* | reef | | | |
| *Pomadasys incisus* | demersal | | | |
| *Sardina pilchardus* | pelagic-neritic | | | |
| *Saurida undosquamis* | reef | | | |
| *Sciaena umbra* | demersal | | | |
| *Scomber japonicus* | pelagic-neritic | | | |
| *Serranus cabrilla* | demersal | | | |

| Fish species | Habitat | Geographical area | Sampling technique | Author |
|---|---|---|---|---|
| *Siganus luridus* | reef | | | |
| *Sparus aurata* | demersal | | | |
| *Trachurus mediterraneus* | pelagic-oceanic | | | |
| *Trigla lucerna* | demersal | | | |
| *Umbrina cirrosa* | demersal | | | |
| *Upeneus moluccensis* | reef | | | |
| *Upeneus pori* | demersal | | | |
| | | | | |
| *Sardina pilchardus* | pelagic-neritic | Adriatic sea | Commercial demersal trawling | Avio et al., 2015 |
| *Squalus acanthias* | benthopelagic | | | |
| *Merluccius merluccius* | demersal | | | |
| *Mullus barbatus* | demersal | | | |
| *Chelidonichthys lucernus* | demersal | | | |
| | | | | |
| *Trachinotus ovatus* | pelagic-neritic | Central Mediterranean Sea (strait of Messina) | Trolling lines and artificial bait | Battaglia et al., 2016 |
| | | | | |
| *Xyphias gladius* | pelagic-oceanic | Central Mediterranean Sea | Harpoon, hook and lines, and drifting longline | Romeo et al., 2015 |
| *Thunnus thynnus* | pelagic-oceanic | | | |
| *Thunnus alalunga* | pelagic-oceanic | | | |
| | | | | |
| *Mullus barbatus* | demersal | Eastern Ionian Sea (Corfu Island) | Trawl surveys | Torre et al,,2016 |
| *Citharus linguatula* | demersal | | | |
| *Pagellus erithrinus* | demersal | | | |
| *Sardina pilchardus* | pelagic-neritic | | | |
| | | | | |
| *Boops boops* | demersal | Western Mediterranean Sea (Balearic island ) | Bottom trawl nets and purse seine | Nadal et al., 2016 |
| *Gadus morhua* | demersal | North sea | Trawling and fish traps (fyke nets) | Brate et al., 2016 |
| | | | | |
| *Scyliorhinus canicula* | demersal | NE Atlantic | Bottom trawl | Bellas et al., 2016 |
| *Merluccius merluccius* | demersal | | | |
| *Mullus barbatus* | demersal | Mediterranean sea | | |
| | | | | |
| *Gadus morhua* | benthopelagic | North sea | Grande Ouverture Verticale (GOV) trawl | Foekema et al., 2013 |
| *Merlangius merlangus* | benthopelagic | | | |
| *Melanogrammus aeglefinus* | demersal | | | |
| *Clupea harengus* | benthopelagic | | | |
| *Trachurus trachurus* | pelagic-neritic | | | |
| *Eutrigla gurnardus* | Demersal | | | |
| *Scomber scombrus* | pelagic-neritic | | | |
| | | | | |
| *Limanda limanda* | demersal | North Sea; Baltic Sea | Trawling (bottom and | Rummel et al., 2016 |
| *Platichthys flesus* | demersal | | | |

| Fish species | Habitat | Geographical area | Sampling technique | Author |
|---|---|---|---|---|
| *Gadus morhua* | demersal | | pelagic) | |
| *Clupea harengus* | benthopelagic | | | |
| *Scomber scombrus* | pelagic-neritic | | | |
| | | | | |
| *Benthosema glaciale* | pelagic-oceanic | NE Atlantic | Midwater trawl | Lusher et al., 2016 |
| *Myctophum punctatum* | bathypelagic | | | |
| *Notoscopelus kroyeri* | pelagic-oceanic | | | |
| *Lampanyctus crocodilus* | bathypelagic | | | |
| *Maurolicus muelleri* | bathypelagic | | | |
| *Stomias boa boa* | bathypelagic | | | |
| *Nemichthys scolopaceus* | bathypelagic | | | |
| *Arctozenus risso* | bathypelagic | | | |
| *Xenodermichthys copei* | bathypelagic | | | |
| *Argyropelecus spp* | bathypelagic | | | |
| | | | | |
| *Alosa fallax* | pelagic–neritic | NE Atlantic Coastal waters near Lisbon and Tagus river | Trawling and markets | Neves et al., 2015 |
| *Argyrosomus regius* | Benthopelagic | | | |
| *Boops boops* | demersal | | | |
| *Brama brama* | bathypelagic | | | |
| *Dentex macrophthalmus* | benthopelagic | | | |
| *Helicolenus dactylopterus* | bathydemersal | | | |
| *Lepidorhombus boscii* | demersal | | | |
| *Lepidorhombus whiffiagonis* | demersal | | | |
| *Lophius piscatorius* | Bathydemersal | | | |
| *Merluccius merluccius* | bathydemersal | | | |
| *Mullus surmuletus* | Demersal | | | |
| *Pagellus acarne* | Benthopelagic | | | |
| *Polyprion americanus* | Demersal | | | |
| *Raja asterias* | Demersal | | | |
| *Sardina pilchardus* | pelagic–neritic | | | |
| *Scomber japonicus* | pelagic–neritic | | | |
| *Scomber scombrus* | pelagic–neritic | | | |
| *Scyliorhinus canicula* | demersal | | | |
| *Scyliorhinus canicula* | Demersal | | | |
| *Solea solea* | Demersal | | | |
| *Torpedo torpedo* | Demersal | | | |
| *Trachurus picturatus* | Benthopelagic | | | |
| *Trachurus trachurus* | pelagic–neritic | | | |
| *Trichiurus lepturus* | Benthopelagic | | | |
| *Trigla lyra* | Bathydemersal | | | |
| *Trisopterus luscus* | Benthopelagic | | | |
| *Zeus faber* | Benthopelagic | | | |
| | | | | |
| *Merlangius merlangius* | benthopelagic | NE Atlantic English channel | Trawling | Lusher et al., 2013 |
| *Micromesistius poutassou* | bathypelagic | | | |
| *Trachurus trachurus* | pelagic-neritic | | | |
| *Trisopterus minutus* | benthopelagic | | | |
| *Zeus faber* | benthopelagic | | | |
| *Aspitrigla cuculus* | demersal | | | |
| *Callionymus lyra* | Demersal | | | |

| Fish species | Habitat | Geographical area | Sampling technique | Author |
|---|---|---|---|---|
| *Cepola macrophthalma* | Demersal | | | |
| *Buglossisium luteum* | Demersal | | | |
| *Microchirus variegates* | Demersal | | | |
| | | | | |
| *Trichiurus lepturus* | benthopelagic | SW Atlantic (southeastern Brazil) | Gill-nets | Di Beneditto et al., 2014 |
| *Gadus morhua* | benthopelagic | NW Atlantic | | Liboiron et al., 2016 |
| | | | | |
| *Acanthurus coeruleus* | reef | SW Atlantic (North-Eastern Brazil coast, Salvador Bahia State) | Landing points of artisanal fishery | Miranda et al., 2016 |
| *Caranx crysos* | reef | | | |
| *Dasyatis americana* | reef | | | |
| *Lagocephalus laevigatus* | pelagic-neritic | | | |
| *Lutjanus analis* | Reef | | | |
| *Lutjanus jocu* | Reef | | | |
| *Mycteroperca sp.* | Reef | | | |
| *Paralichthys brasiliensis* | Demersal | | | |
| *Rhizoprionodon lalandii* | pelagic-demersal | | | |
| *Scomberomorus cavalla* | Benthopelagic | | | |
| *Sphyraena guachancho* | pelagic-neritic | | | |
| | | | | |
| *Alepisaurus ferox* | bathypelagic | North Pacific Ocean | Longline fishing grounds | Jantz et al., 2013 |
| | | | | |
| *Katsuwonus pelamis* | pelagic-oceanic | SW Pacific (Indonesia) | Fish market | Rochman et al., 2015 |
| *Rastrelliger kanagurta* | pelagic-neritic | | | |
| *Decapterus macrosoma* | reef | | | |
| *Spratelloides gracilis* | pelagic-neritic | | | |
| *Siganus argenteus* | reef | | | |
| *Siganus fuscescens* | reef | | | |
| *Siganus canaliculatus* | reef | | | |
| *Lutjanus gibbus* | reef | | | |
| *Selar boops* | reef | | | |
| *Atherinopsis californiensis* | pelagic-neritic | NE Pacific (USA) | | |
| *Engraulis mordax* | pelagic-neritic | | | |
| *Scomber japonicus* | pelagic-neritic | | | |
| *Morone saxatilis* | demersal | | | |
| *Oncorhynchus tshawytscha* | benthopelagic | | | |
| *Thunnus alalunga* | pelagic-oceanic | | | |
| *Citharichthys sordidus* | demersal | | | |
| *Ophiodon elongatus* | demersal | | | |
| *Sebastes caurinus* | demersal | | | |
| *Sebastes miniatus* | reef | | | |
| *Sebastes mystinus* | reef | | | |
| *Sebastes flavidus* | reef | | | |
| | | | | |

| Fish species | Habitat | Geographical area | Sampling technique | Author |
|---|---|---|---|---|
| *Engraulis japonicus* | pelagic-neritic | NE Pacific Tokyo bay (urban coastal area) | Sabiki rigs from a pier | Tanaka & Takada, 2016 |
| | | | | |
| *Myctophum lychnobium* | bathypelagic | West Pacific (Mariana Island) | Dip-nets | Van Noord et al., 2013 |
| *Symbolophorus evermanni* | bathypelagic | | | |
| *Centrobranchus andreae* | pelagic-oceanic | | | |
| *Girella laevifrons* | | Southeast Pacific (central coast Chile) | Fishing nets | Mizraji et al., 2017 |
| *Scarthychthys viridis* | pelagic-neritic | | | |
| *Graus nigra* | demersal | | | |
| *Helcogramoides chilensis* | demersal | | | |
| *Auchenionchus microcirrhis* | Demersal | | | |
| | | | | |
| *Mugil cephalus* | benthopelagic | SW Indian (Harbour waters) | Castnet | Naidoo et al., 2016 |
| | | | | |
| *Cathorops spixi* | demersal | Estuary north east Brazil | Otter trawl | Possatto et al., 2011 |
| *Cathorops agassizii* | benthopelagic | | | |
| *Sciades herzbergii* | demersal | | | |
| | | | | |
| *Trachurus declivis* | benthopelagic | Southern Ocean Australia | Fish market | Cannon et al., 2016 |
| *Hyperlophus vittatus* | pelagic-neritic | | | |
| *Conger verreauxi* | Reef | | | |
| *Engraulis australis* | pelagic-neritic | | | |
| *Paragalaxias dissimilis* | Demersal | | | |
| *Thyrsites atun* | Benthopelagic | | | |
| *Hyporhamphus melanochir* | pelagic-neritic | | | |
| *Notolabrus tetricus* | Reef | | | |
| *Aldrichetta forsteri* | Demersal | | | |
| *Mugil cephalus* | Benthopelagic | | | |
| *Gymnoscopelus nicholsi* | Bathypelagic | | | |
| *Dissostichus mawsoni* | pelagic-oceanic | | | |
| *Haletta semifasciata* | Demersal | | | |
| *Platycephalus bassensis* | Demersal | | | |
| *Platycephalus laevigatus* | Demersal | | | |
| *Katsuwonus pelamis* | pelagic-oceanic | | | |
| *Scomber australasicus* | pelagic-neritic | | | |
| *Scorpaena jacksoniensis* | Reef | | | |
| *Sillaginodes punctatus* | Demersal | | | |
| *Sillago flindersi* | Demersal | | | |

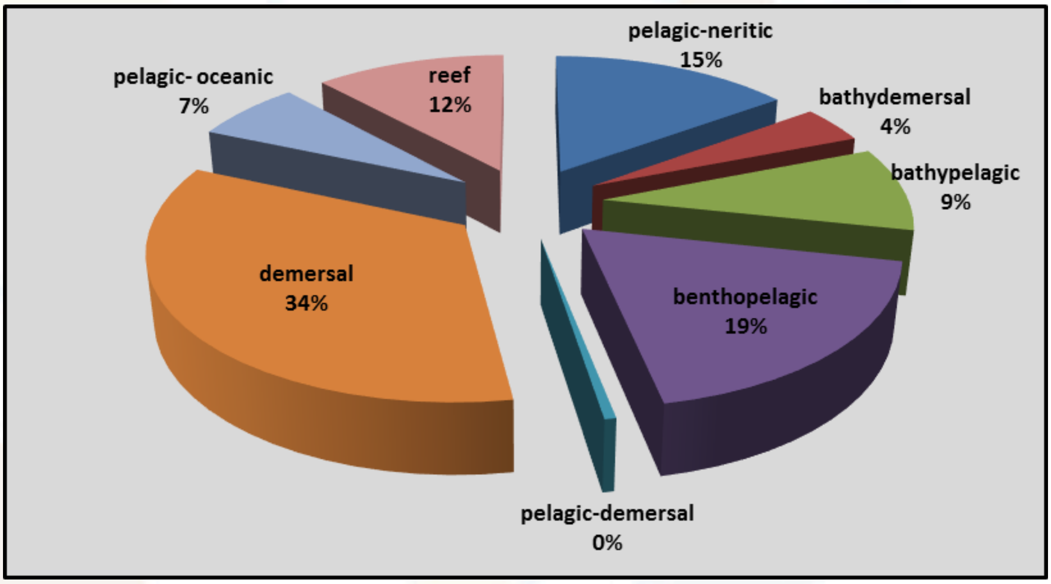


**Fig. 3.** Percentage of studied species subdivided according to their habitat (www.fishbase.org/).

**Table 2.** Species analysed in more than one scientific paper.

| Species analysed by at least 2 papers | Species analysed by at least 3 papers | Species analysed by at least 4 papers |
|---|---|---|
| ***Argyrosomus regius*** | *Scomber japonicus* | *Gadus morhua* |
| ***Boops boops*** | *Scomber scombrus* | *Merluccius merluccius* |
| ***Brama brama*** | *Scyliorhinus canicula* | *Mullus barbatus* |
| ***Caranx crysos*** | *Trachurus trachurus* | *Sardina pilchardus* |
| ***Clupea harengus*** | | |
| ***Helicolenus dactylopterus*** | | |
| ***Katsuwonus pelamis*** | | |
| ***Merlangius merlangius*** | | |
| ***Micromesistius poutassou*** | | |
| ***Mugil cephalus*** | | |
| ***Mullus surmuletus*** | | |
| ***Pagellus erithrinus*** | | |
| ***Polyprion americanus*** | | |
| ***Scyliorhinus canicula*** | | |
| ***Squalus acanthias*** | | |
| ***Thunnus alalunga*** | | |
| ***Trichiurus lepturus*** | | |
| ***Xiphias gladius*** | | |
| ***Zeus faber*** | | |
| **19** | **4** | **4** |

Among the 159 species analysed, 11 (*Argyrosomus regius, Boops boops, Brama brama, Caranx crysos, Helicolenus dactylopterus, Merluccius merluccius, Mullus surmuletus, Polyprion americanus, Sardina pilchardu, Scomber japonicus*) are common, both in the North East Atlantic and the Mediterranean sea, two of the main regions according to the MSFD and consequently to the OSPAR and Barcelona Conventions (Fig. 4).

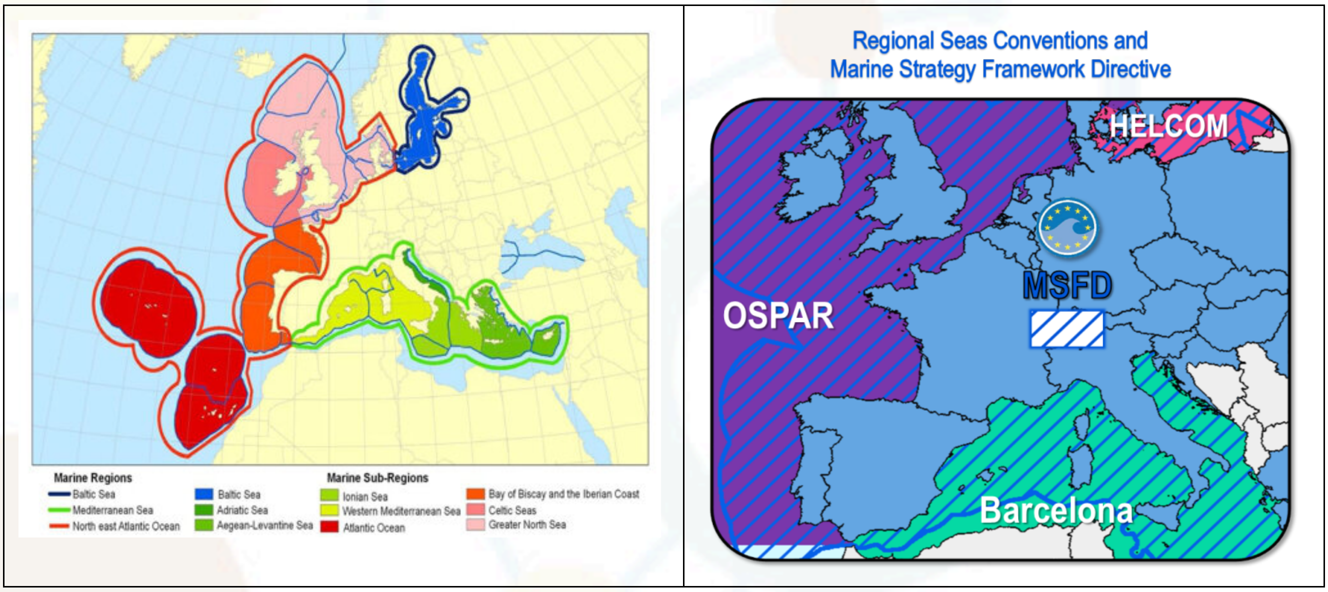


**Fig. 4.** Regions and sub-regions according to the MSFD and the Regional Sea Conventions.

## *Marine turtles*

Direct consumption of plastic fragments is well documented and is observed in all marine turtle species (Carr, 1987; Bjorndal et al., 1994; Hoarau et al., 2014 between others). The differences in debris ingestion among species may also be attributed to differences in species biology and how their digestive systems cope with the debris once ingested. Adult and subadult loggerhead sea turtles have a larger-diameter digestive tract than green turtles of a similar age class. Therefore, they may more readily pass ingested materials (Bugoni et al. 2001). Species could perhaps have different enzymes or microflora that act differently on ingested debris (Bjorndal 1997). Also, carnivorous species (like loggerhead and kemp's ridley turtles) could be less susceptible to debris ingestion than herbivores (green), gelatinous organism feeders (leatherback) and omnivores (hawksbill), or they could be less likely to retain the ingested debris. The selectivity of the feeding behaviour, the occupation of highly polluted areas and the ability to discriminate litter from potential preys could also be the cause, e.g. gelatinous organisms being possibly confounding with soft plastics, (Schuyler et al., 2014). Therefore, feeding ecology and diet, as well as habitat use in relation to areas of high plastic density, determine the likelihood and consequences of plastic ingestion (Bond et al., 2014). These differ among turtle life stages, regional populations and species, meaning that there are likely to be inter- and intra-species variation in the densities and types of plastic encountered and potentially consumed (Schuyler et al., 2014).

Concerning microplastics, a potential issue for carnivorous marine turtle species, such as kemp's ridley, olive ridley (Lepidochelys olivacea), loggerhead, leatherback and flatback (Natator depresus) turtles, is the indirect ingestion of microplastics through consumption of contaminated invertebrate preys, such as cnidarians, molluscs and crustaceans (Parker et al., 2005; Casale et al., 2008).

### c) Reported plastic ingestion

#### *Fish*

Examples of studies demonstrating the presence and amount of microplastics in a range of wild-caught fishes are summarized below. The Table 4 resumes the evidences occurrence, number and size of plastic ingested by the 159 species studied by different authors. The variability in frequency of occurrence of microplastic ingestion is very high among geographical locations. In the Mediterranean Sea, the occurrence of microplastic ingestion (percentage of individuals with ingested microplastic) was: 1.9 % (Anastasopoulou et al., 2013), 58 % (Guven et al., 2017), 28 % (Avio et al., 2015), 24.3 % (Battaglia et al., 2016), 18.2 % (Romeo et al., 2015), 68 % (Nadal et al., 2016), 18.8 % (Bellas et al., 2016). In the North East Atlantic it was reported as: 32 % (Bellas et al., 2016), 3 % (Brate et al., 2016), 2.6 % (Foekema et al., 2013), 5.5 % (Rummel et al., 2016), 11 % (Lusher et al., 2016) 19.8 % (Neves et al., 2015), 36.5 % (Lusher et al., 2013). The reason for this variability is not strictly linked to the quantity of microlitter in the environment, but it could also depend on the different methodologies to assess microplastic ingestion. Even if the data may not be directly comparable, it is really useful to understand the global patterns of ingestion incidence. Choosing different microplastic size classes, by using different mesh of sieves, can lead to a different representation of the incidence of marine microplastic ingestion in fish. For example, Lusher et al. (2013), counted particles with diameter above 130 µm and the percentage of fish with microplastic was 36.5 %, while Rummel et al. (2016) counted particles above 500 µm and the percentage of fish with microplastic ingested was 5.5 %. Guven et al. (2017) used a mesh size of 26 µm and the percentage of fish with microplastic was 58 %. The percentage of fish with ingested litter may increase both with the sampling size (e.g. 1337 individuals in Guven et al., 2017) and the decreasing microplastic diameter. Therefore, the higher percentage of microplastic ingestion occurence inby fish inhabiting the Mediterranean Sea may not be connected to the higher quantity of microplastic in the environment, but can be due to the heterogeneity in methodologies of the above studies. The average number of debris items per fish evidenced by the different authors is not so high, the minimum value being 0.03 ± 0.18 (Rummel et al., 2016), and the maximum value 3.75 (Nadal et al., 2016) and 3.8 (Naidoo et al., 2016). Foekema et al. (2013) showed that more than 80 % of the fish with plastic contained only a single particle. These results may also suggest that microplastics do not accumulate in the digestive tract of these fish for very long periods. The prevalent type of plastic identified by the different studies is fibers: 70 % (Guven et al., 2017), 71 % (Bellas et al., 2016), 65.8 % (Neves et al., 2015), 93 % (Lusher et al., 2016), and 51.2 % (Naidoo et al., 2016). The high percentage of fibers can be related to sampling and analysis contamination (Foekema et al., 2013), or anthropogenic waste. Rochman et al. (2015), comparing fish from Indonesia and USA, showed that the majority (80 %) of anthropogenic debris found in the fish from the USA was composed of fibers from textiles, whereas not a single type of fiber was detected in fish from Indonesia. The reason could be the differences in waste management strategies on land between countries. Indonesia, in the region where fishes were collected, 30 % of solid waste generated is not processed and an increasing amount of waste is directly discarded along the coast, rivers and into drainage channels, thus, it is common for plastic items to end up in the ocean where they degrade into fragments over time. In California, waste management systems are more advanced and thus, it is less common for plastic items to be discarded in the ocean. Fibers from washing machines can remain in sewage effluent, and may be delivered to aquatic habitats in large concentrations via wastewater outfalls.

Nadal et al. (2016) showed that higher amount of microplastic fibers are found in the digestive tract of the assessed omnivorous fish, because it is associated with its wider diet source. The category "fragments" is abundant: 57.5 % (Avio et al., 2015), 34.2 % (Neves et al., 2015), 86 % (Tanaka et al., 2016), 34.6 % (Naidoo et al., 2016), and 23 % (Possatto et al., 2011). Nylon fragments from cables used in fishery activities (subsistence, artisanal and commercial) play a major role in this contamination (Possatto et al., 2011). Pelagic species ingested more fragments, whereas benthic species ingested more fibers, which may be related to the presence of high quantities of fibers on the seabed. Pelagic fish are usually visual predators, and are more likely to confound particles and prey items (Neves et al., 2015).

**Table 3.** Evidence of plastic ingestion according to studies geographical distribution.

| Number of studied specimens | Geographical area | Sampling size and percentage of individuals with ingested plastic | Number of debris items per fish (range or average) | Size of particle range | Prevalent type of plastics | Authors |
|---|---|---|---|---|---|---|
| 26 | Eastern Mediterranean sea (Ionian sea) | 1504 individuals 1.9 % | Range 1-6 average 1.37±0.2 | 5–60 mm | Fragments: hard plastic (56%); plastic bags (22%); fishing gears (19%) | Anastasopoulou et al., 2013 |
| 28 different species 14 families | Eastern Mediterranean sea (Turkish territorial waters) | 1337 individuals 58% | Range 1 – 35 average 2.36 | 0.09 - 12.07 mm | Fibers (70%), hard plastic (20.8%), nylon (2.7%), rubber (0.8%), miscellaneous plastic (5.5%) | Guven et al., 2017 |
| 5 | Adriatic sea | 125 individuals 28% | Range 1 - 1.78 | Range 0.1-5 mm | Fragments (57%), line (23%), film (11%), pellets (9%), Polyethilene (65%), PET (19%), polystyrene (4%), PVC (4%), Nylon 6/T (4%), Polypropylene (4%) | Avio et al., 2015 |
| 1 | Central Mediterranean Sea (strait of Messina) | 115 individuals 24.3% | Range 1-2 | Range 1.52-5.27mm | not assessed | Battaglia et al., 2016 |
| 3 | Central Mediterranean Sea | 121 individuals 18.2% | Average 1.31 | Range 0.63-164.5 mm | not assessed | Romeo et al., 2015 |
| 4 | Eastern Ionian Sea (Corfu Island) | 400 individuals | | | Airborne contamination using the methodology based on hermetic enclosure devices, isolating the working areas during the various processing steps, airborne contamination reduced by 95.3%. | Torre et al.,2016 |
| 1 | Western Mediterranean sea (Balearic island ) | 337 individuals 68% | Average 3.75 Range 2.47- 4.89 | | Filament type (more indicative of sewage origin) and synthetic garments related to washing of textiles | Nadal et al., 2016 |
| 2 | NE Atlantic | 84 individuals 32% | Average 1.20 ± 0.4 | Range 0.38 -3.1 mm | Fibers (71%), followed by spheres (only in red mullets, 24%), films (3.2%) and fragments (1.6%) | Bellas et al., 2016 |
| 1 | Mediterranean sea | 128 individuals 18.8% | Average 1.75 ± 1.14 | Range 0.38 -3.1 mm | | |
| 1 | North sea | 302 individuals 3% | Range 0-2 | Range 3.2 - 41.7mm | polyester, polypropylene, polyvinyl chloride, | Brate et al., 2016 |

| Number of studied specimens | Geographical area | Sampling size and percentage of individuals with ingested sea plastic | Number of debris items per fish (range or average) | Size of particle range | Prevalent type of plastics | Authors |
|---|---|---|---|---|---|---|
| | | | | Average 14.1mm | polystyrene, polyetylene, polytetrafluoroethylene (Teflon), nylon 6.6, styrene acrylonitrile resin and poly(n-butyl methacrylate) | |
| 7 | North sea | 1203 individuals 2.6% | Range 0-4 | average 0.8mm | polyethylene (PE), polypropylene (PP), polyethyleentereftalaat (PET), styreneacrylate (SA), | Foekema et al., 2013 |
| 5 | North Sea; Baltic Sea | 290 individuals 5.5% | *demersal* Average 0.03± 0.18 *pelagic* Average 0.19±0.61 | Range 0.2 - 5mm | Fragment, film, spherule, fiber. 40% polyethilene, polyamide (22%) and propylene (13%) | Rummel et al., 2016 |
| 10 | NE Atlantic | 761 individuals 11% | Average 1.2±0.54 | Range 0.5-11.7mm Average 1.9 mm | Fibers (93%) Fragments (7%) | Lusher et al., 2016 |
| 26 | NE Atlantic (Coastal waters near Lisbon and Tagus river estuary) | 263 individuals 19.8% | Average 1.40 ± 0.66 | Range 0.217 - 4.81mm Average 2.11 ± 1.67 mm | Fibers (65.8%), fragments/particles (34.2%) / polypropylene, polyethylene, alkyd resin, rayon, polyester, nylon and acrylic | Neves et al., 2015 |
| 10 | NE Atlantic English channel | 504 individuals 36.5% | Eange 1-15 Average 1.9 ±0.1 | not assessed | Shape: fibers (68.3%), fragments (16.1%) and beads (11.5%). Materials: rayon, polyamide, polyester, polyethylene, acrylic | Lusher et al., 2013 |
| 1 | SW Atlantic (southeastern Brazil) | 149 individuals 0.7% | Range 0-1 | not assessed | Cigarette | Di Beneditto et al., 2014 |
| 1 | NW Atlantic | 205 individuals 2.4% | Range 0 – 2 | not assessed | Film/sheet plastic threads, fragments | Liboiron et al., 2016 |
| 11 | SW Atlantic (NE Brazil-coast Salvador Bahia) | 32 individuals 22% | Range 1-6 | Range 1-5 mm | Pellets | Miranda et al., 2016 |
| 1 | North Pacific Ocean | 192 individuals 24.5% | Range 1-56 | Range 3.1–723.9 mm | 6 categories: net, rope,fragment, strap,plastic bag,miscellanous | Jantz et al., 2013 |
| 11 | SW Pacific (Indonesia | 140 individuals 28% in | Range 0-21 Average | Average 3.5 ± 1.1mm | Hard fragments, fishing line, film, foam | Rochman et al., 2015 |

| Number of studied specimens | Geographical area | Sampling size and percentage of individuals with ingested plastic | Number of debris items per fish (range or average) | Size of particle range | Prevalent type of plastics | Authors |
|---|---|---|---|---|---|---|
| | | Indonesian; 25% in USA | 1.4 ± 3.7 | | | |
| 12 | NE Pacific (USA)) | | Range 0-10 Average 0.1 ± 0.3 | Average 6.3 ± 6.7mm | Fibers, foam, film, monofilament, fragments | |
| 1 | NE Pacific Tokyo bay (urban coastal area) | 64 individuals 77% | Average 2.3 | Range 0.15 - 1 mm | Fragments 86%-beads 7.3%-filaments 5.3%-foams 1.3%; polyethylene 52%, polypropylene 43.3% | Tanaka & Takada, 2016 |
| 3 | West Pacific (Mariana Island) | 36 individuals: *M. lychnobium* (40%), *C. andreae* (40%), *S. evermanni* (7%) | not assessed | not assessed | not assessed | Van Noord et al., 2013 |
| 5 | Southeast Pacific (central coast Chile) | Omnivore *Girella laevifrons* (N = 16), Herbivore *Scarthychthys viridis* (N = 19), and Carnivores Graus nigra (N = 8) *Helcogramoides chilensis* (N = 3), *Auchenionchus microcirrhis* (N=16) | not assessed | not assessed | not assessed | Mizraji et al., 2017 |
| 1 | SW Indian (Harbour waters) | 70 individuals 73% | Average 3.8 | Range 0.2 -15 mm | Fibers (51.2%), fragments (34.6%), polystyrene (5.0%) films, monofilament line, twine | Naidoo et al., 2016 |
| 3 | Estuary north east Brazil | 182 individuals *Cathorops spixii* 18%, *C. agassizii* 33%, *Sciades herzbergii* 18% | Range 1-10 | not assessed | Nylon fragments 23%; hard plastics also found | Possatto et al., 2011 |
| 21 | Southern Ocean Australia | 342 individuals 0.30% | Range 0-2 | not assessed | Fragments | Cannon et al., 2016 |

Only Pham et al. (2017) analysed separately micro and macro debris ingestion in marine turtles. The study analysed 24 juvenile loggerheads captured accidentally by long line fisheries in the North Atlantic gyre (around Azores Archipelago, Portugal). Microplastics from 1 to 5 mm where found in 58% of the turtles, mostly localized in the intestines (84%), and classified as follows: 87% were plastic fragments, 8% sheets fragments and 5% industrial pellets. This study also concluded that some of the micro plastics found could derive from macrodebris fragmentation into the digestive tract, but an important part of them were probably ingested in another way. Many other studies analysed debris ingestion until 1 mm, including both macro and microplastics, but the results are always shown together without differentiation, preventing any analysis of the specific impact of micro-debris on sea turtles (Table 4).

**Table 4.** List of studies related to debris ingestion in marine turtles, which include microplastics on their sampling methods (Cc = *Caretta caretta*; Cm = *Chelonia mydas*; MED = Mediterranean Sea; ATL = Atlantic Ocean).

| SPECIE | STAGE | AREA /REGION | DEAD/ALIVE | PERIOD | DEBRIS COLLECTED | REFERENCE |
|---|---|---|---|---|---|---|
| Cc (N=54) | Juveniles | MED (Eastern Adriatic Sea) | Dead (Necropsies) | 2001 - 2004 | >1mm (analysed together with macro debris) | Lazar & Gracan, 2011 |
| Cc (N=95) | Juveniles | MED (Lampedusa) | Dead (necropsies), Alive (feces) | 2001-2005 | > 0,4mm (analysed together with macro debris) | Casale et al., 2008 |
| Cc (N= 121) | Juveniles | MED (Sardinia waters) | Dead (necropsies), Alive (feces) | 2008 - 2012 | >1mm (analysed together with macro debris) | Camedda et al., 2014 |
| Cc (N=567) | Juveniles | MED (Lampedusa and Sicily) | Dead (necropsies), Alive (feces) | 2005, 2008, 2009, 2011, 2012, 2014, 2015 | > 0,4mm (feces) (analysed together with macro debris) | Casale et al., 2016 |
| Cc (N=18) Cm (N=27) | | MED (northern Cyprus) | Dead (Necropsies) | 2011 - 2015 | >50micros (analysed together with macro debris) | Duncan et al., 2016 |
| Cc (N=150 ) | Juveniles | MED (Sardinia, Tuscany, Lazio and Campania) | Dead (Necropsies) | 2011 - 2014 | >1mm (analysed together with macro debris) | Matidi et al., 2017 |
| Cc (N=95) | Juveniles | ATL (Portugal mainland) | Dead (Necropsies) | 2010-2013 | >1mm (only analysed macro debris >5mm) | Nicolau et al., 2016 |
| Cc (N=24) | Juveniles | ATL (Azores, Portugal) | Dead (Necropsies) | 1996-2016 | Analysed macro (>5mm) and micro (1-5mm) debris separately | Pham et al., 2017 |

# IV - SUMMARY OF THE KNOWLEDGE AND PERSPECTIVES FOR THE IMPLEMENTATION OF THE INDICATOR

## 1. Methodology used and potential sources of error

### a) Methodologies for sampling and processing microplastics

#### *i.* Field sampling of individuals

Although the relatively low number of studies carried out to observe the microplastics ingestion in environmentally exposed fish, a wide range of habitats in which fish have been found to ingest micro-plastics have been reported in literature, including offshore, coastal or estuarine waters. Litter ingestion between fish species showed a high variability in respect with the geographical location, functional groups, size and feeding behaviour. Consequently, also capture methods vary among studies: Benthic species are caught by otter trawls (Lusher et al., 2013) or deep water long-lines (Anastasopoulou et al., 2013), while mesopelagic and epipelagic fish species are captured by manta trawl (Boerger et al., 2010), long-lines (Choi and Drazen, 2013), midwater trawls (Davison and Asch, 2010) and also dip-nets (Davison and Asch, 2010, Van Noord et al., 2013). A random collection of recently captured specimens at the landing points of artisanal fisheries, were also adopted in studies of plastic ingestion (Miranda and de Carvalho-Souza, 2015). Not surprisingly, such a wide range of collection techniques is reported. First of all, this may be because knowledge on this issue is only at a preliminary stage, and investigations are not still focused on key species or functional groups for which scientific community has found a consensus. Obviously, the available budget can limit the choices of researchers, regarding the sampling method to be adopted, so that studies on plastic ingestion are often carried out in the framework of samples collected to investigate the feeding ecology of one or more species, or of samples coming from routine surveys for monitoring fish stocks.

In sea turtles, few collection techniques are reported. Dead turtles could be directly and entirely frozen until laboratory analysis or only the digestive tract after the direct necropsy of the individual is frozen. In laboratory, manual extraction by visual observation of gut content collected from the digestive tract is the most used technique in debris studies (Tomas et al., 2002, Santos et al., 2015; Fukuoka et al., 2016; Nicolau et al., 2016). These studies generally collect debris greater than 5mm and/or 1mm, including both, macro and micro-items. Nevertheless, microplastics (< 5 mm) could remain hidden or camouflaged into the organic content.

#### *ii.* Isolation of the ingested micro-plastics

The high quantity of biotic material and anthropogenic particles contained in fish and/or turtle stomach can hide the presence of microplastic, interfering with their identification and raising the issue of the isolation of the ingested microplastics. The methodological difficulties related to this part of the analyses can partly explain the relatively low numbers of studies specifically addressed to microplastics ingestion in wild populations. However, the number of studies on this issue has increased highly in the last years, in parallel to the strong rise of concern on the topic of plastic contamination in the environment as a whole.

After sampling in laboratory, or directly during field sampling, the entire gastrointestinal tracts (GIT) is dissected from the tip of the esophagus to the vent, and prepared for sorting or entire GIT conservation, respectively. The isolation of micro-plastics content can be made through a range of protocols that can be resumed in two major techniques: direct visual inspection or digestion of biotic material.

Visual inspection was one of the most commonly used methods for the identification of microplastics. The fresh stomach contents can be prepared for processing through a density separation by buoyancy tests, in hyper-saturated saline solution, or a previous desiccation in oven (Avio et al., 2015).

Regarding the digestion of biotic material, most protocols use potassium hydroxide (KOH 10%) (Foekema et al., 2013; Rochman et al., 2015; Lusher et al., 2016; Tanaka & Takada, 2016). Karami et al., (2017) showed that, among all the digesting treatments, incubating fish tissues in 10% KOH at 40 °C was the best balance of efficiently eliminating the biological materials while being inert to most of the tested plastic polymers. Other protocols use NaOH in digesting fish tissues at room temperature (RT, 25 °C), 40, 50, or 60 °C. Potassium hydroxide solution fully eliminated the biological matrices at all temperatures but it is corrosive against plastic polymers across all the temperatures (Karami et al., 2017). The same problems happen using HNO3 treatments that fully digested the fish tissues, but also fully reduced the recovery rate of most or all of the polymers (Karami et al., 2017; Lusher et al., 2017).

Oxidizing agents such as hydrogen peroxide (H2O2), are also tested for the digestion of the biological material. Avio et al., (2015) reported that using 15% H2O2 solution there was only a minimal modification of polymers spectra, confirming that microplastics were efficiently extracted without any damage to the polymers. The H2O2 treatment at 50 °C efficiently digested the biological materials, although it altered the colour of polyethyleneterephthalate (PET) fragments and formed some foamy white coloured particles in the digestate.

Likewise fish laboratory analysis, most part of the studies in marine turtles used manual extraction by visual observation of the digestive gut. In some cases, researchers conducted chemical or enzymatic digestion of the digestive tract, where each section (esophagous, stomach and intestine) is cut into small pieces that are digested by chemicals (KOH 10% or H2O2) (Lusher et al., 2016; Ostiategui-Francia et al., 2016) or enzymatic solutions (Duncan et al., 2016; Caron et al., 2016). In this way, all organic tissues are degraded and only inorganic materials remain in the sample. On live turtles, analysis are carry out through faeces analysis, which present lower quantities of biotic material. In this case, the organic material could be directly digested by chemical products (KOH or H2O2).

### *ii*. Identification of micro-plastics

Following digestion or other isolation protocols, the application of a technique for the identification of pre-sorted particles is crucial, in order to generate reliable data. Studies often did not include any verification of the nature of the particles. In this way, a possible bias is introduced when all particles are classified as synthetic polymers by applying only the visual method, because organic particles can be erroneously isolated and counted, such as grain sediments or fragments of mussel shells, due to a partial digestion, which can occur during the isolation procedure. Nonetheless, this aspect can be particularly relevant for studies that did not include any identification of polymer particles by spectroscopic analysis. A useful technique in case of doubt, when particles do not break when pressed with forceps, consists in applying a hot metal tip on the particle (Bellas et al., 2016).

Studies that have adopted a polymer anidentification protocol, used micro-spectroscopy, such as the Fourier transform infrared (FT-IR) (Foekema et al, 2013; Lusher et al, 2013; Neves et al, 2015; Brate et al, 2016; Tanaka and Takada, 2016), or the Raman spectroscopy (Collard et al, 2015).

The use of very specific analytical method, such as spectroscopy, requires expensive instrumentation, but is needed for an accurate identification of the polymer particles. Raman spectroscopy is comparable to the FT-IR method. However, the smaller diameter of the laser beam in Raman spectroscopy relative to FT-IR, allows the identification of microplastics as small as a few μm in size. The non-contact analysis of Raman spectroscopy offers the benefit that the microplastic samples remain intact for possible further analysis (Shim et al., 2013).

## b) Minimising contamination

According to GESAMP (2016), the methodological difficulties for isolating particles from biota partly explain why only a few studies specifically addressed the occurrence of microplastics in marine organisms. The extraction and quantification of microplastics from organisms is especially challenging, because the plastic pieces may be masked within biological material and tissues. Different protocols have been proposed on the extraction of microplastics from marine invertebrates after a pre-digestion of organic matter, using solvent properties and pH (basic or acid) for sample treatment.

Visual examination is the most common method used to assess size and quantities of microplastics in biota samples, although the contamination and possible sampling bias should carefully be reduced or avoided.

- *Avoidance of the contamination in laboratory*

Considering contamination in laboratory, it has been shown that textile micro-fibers are the main items collected during the microlitter detection, both in samples and in laboratory contamination (Foekema et al., 2013; Dris et al., 2015). This maybe because micro-fibers are ubiquitous in the marine ecosystems (Phuong et al., 2016), in the atmospheric fallout (Dris et al., 2015), as in the everyday human working and living environment and they can be easily transported by the air (Torre et al., 2016). Fibers can originate from the examiner's clothing and/or already present in the laboratory room. Airborne microfibers could also be conveyed by workers' movements into the laboratory area, considering that it is unlikely that a completely clean search room can be obtained (Roux et al., 2001). This demonstrates serious bias that may falsify the gut content analysis, resulting in overestimation of the real microfibers ingestion rate.

The MSFD Technical Group on Marine Litter with the "Guidance on Monitoring of Marine Litter in European Seas" released by the Joint Research Centre, suggest that the procedural contamination should be less than 10% of the average values determined from the samples themselves (MSFD TG ML, 2013).

Studies on microlitter ingested by marine biota have adopted two different methodologies in order to overcome background contamination by microfibers:

1)Reduction and mitigation of the contamination, when microfibers are included in the analysis;

2)Exclusion of microfibers from the final data, to avoid risks of artefacts.

To reduce background contamination many tricks are applied by researchers and the analyses being generally performed in a low foot traffic zone. Naidoo et al. (2016) specify that all Petri dishes had been rinsed, dried with compressed air and checked under a microscope for any plastic contaminants. Forceps used for examining digestive tract contents were checked first for plastic contamination under a dissecting microscope. In addition, a cotton laboratory coat was worn to ensure that synthetic fibers from clothing did not contaminate samples. In order to prevent any accidental external contamination by microplastics, Avio et al. (2015) always used pre-filtered (0.45 mm mesh) deionized water and hypersaline solution, and in addiction they rigorously cleaned with filtered deionized water and air dried all the materials used for dissection and during the different steps of extraction and analysis. Moreover, all sample processing was performed in a clean airflow cabinet, to exclude external contamination from fibers.

According to Lusher et al. (2017), researchers should process samples in a laminar flow hood (e.g. cell or algal culture unit) or alternatively a fume-hood. Glassware, benches and equipment should be rinsed with deionized water, ethanol or acetone, prior to use and environmental filters (e.g. glass fiber filters) can be placed near equipment to quantify external contamination.

Different researchers suggest the use of blank samples during all the stages of the procedure, from the dissection of the animal until the detection under microscope (Davidson & Dudas, 2016; Nuelle et al., 2014; Dris et al., 2015).

Recently, Torre et al. (2016), were able to demonstrate that applying microscope cover methodology, as well as using a glove box for sample handling and filtration, airborne microfibers (synthetic or natural) contamination is drastically reduced. In this study, a plastic cover isolating the stereomicroscopic observation area was used, and the filtration was performed under a pyramid glove box, where incorporated gloves permitted sample handling with no interference from the external environment. The number of microfibers decreased 95.3% when a microscope cover was used during the visual observation and 86.4% when the glove box was used during the gut contents handling and filtration.

The second methodology has also been found in several works (Davison & Asch, 2011; Fries et al., 2013; Foekema et al., 2013; Besseling et al., 2015). However, excluding microfibers may bias the quantification and interpretation of the effects of marine microlitter pollution. Moreover, clean lab protocols could be considered expensive or difficult to be applied (Woodall et al., 2015).

- *Avoidance of the contamination in the field*

While laboratory contamination could be reduced, microplastic contamination during field sampling is really hard to reduce, but different bias should be avoided.

For fishes, which are always caught using polymer nets, trap and rope, minimization of contamination could result in cleaning all the equipment before sampling and reduce as much as possible the exposure of the animals to the fishing gear. Then sampling period should be kept as short as practically realistic. The use of trawling net with long hauls, should be avoided: scraping the net on the bottom create and re-suspend microplastic particles. Moreover, macroplastics are continuously captured and fragmented during the haul. The result will be a cloud of fishes and plastic in the end cod of the trawling net.

For marine turtles, stranded animals always present important sources of contamination, such us coastal or sand microplastics, fibers or plastics fragments derived from the material causing entanglement on the turtle or deposited in the place, or microplastic can adhere during their transport. For this reason, it is very important to conduct exhaustive lavages of the digestive tract in the laboratory before opening and extracting the gut content.

On live turtles, where water tank will be filtered to collect faeces samples, the avoidance of microplastic contamination can be really difficult. Firstly, filtering systems are required in the water circuit before entering into the sampling tanks, in order to avoid a contamination by water. Secondly, the turtle itself must be well washed before placing into the tank to avoid contamination derived from the stranding place or the transport. Finally, microplastics could be transported by air and deposited in the water surface of the tanks. Turtle tanks isolation being really difficult to achieve, so the collection of “control samples” in empty tanks could be an alternative to identify the quantities and the qualities of plastics due to air contamination in the field sampling area.

- *Mode of capture (fish)*

Gut evacuation times for animals are varied, and some animals might excrete microplastic during the capture and manipulation on board and in the net, prior to laboratory analysis (Welden and Cowie, 2016). In such cases, the time between sample collection and the preservation of the animal must be as short as possible. Care must also be taken to minimize handling stress or physical damage. Fish have been observed regurgitating their stomach content. The main cause of regurgitation in fish is thought to be related to the expansion of gas in the swim bladder: this causes the compression of the stomach and might, in extreme cases, result in total stomach inversion (Bowman, 1986). The compression of a catch in the cod end might induce regurgitation in fish (Bowen et al., 1996). The likelihood of regurgitation increases with depth of capture, and gadoids are more prone to regurgitation than flat fish. Piscivorous predators are prone to regurgitation, due to their large distensible oesophagus and stomach (Daan and Neth, 1973; Bowman, 1986). Regurgitation may bias the stomach content estimation, affecting consumption estimates and the presence of plastic debris. At the same time, microplastic has a low potential for bio-magnification in fish, because the trophic level of the species has no influence on the ingested quantity of microplastics (Guven et al. 2017).

Laboratory studies have identified that nano- and micro- plastics can adhere to external appendages of marine organisms (Cole et al., 2013), this for fish could results as adherence occurred during sampling, on board or storage, and is unrepresentative of microplastic ingestion. Organisms that spend long time in nets are subject to additional stress that increases the likelihood of regurgitation or stomach inversion and artificially increases contact time between microplastics and biota; this could facilitate microplastic ingestion and adherence to external appendages (Lusher et al., 2017). According to this study, the collection from commercial fish markets is not ideal, as the researcher will have less control, if any, on the method of capture and the handling conditions of transport. Where applicable, researchers should work closely with fishermen to ensure animals are sampled appropriately and adequate information on the capture procedure is collected.

### c) Possible impacts of microplastics

Marine litter has been widely demonstrated to impact animals, with direct lethal or sub-lethal effects. Litter ingestion would alter the biological and ecological performance of individuals, compromising an individual's ability to capture food, digest food, sense hunger, escape from predators, and reproduce, as well as decreasing body condition and compromising locomotion, including migration (CBD, 2012). Animals may ingest many types of litter and synthetic materials are the most commonly reported. Studies that prove ingested debris as the direct and sole cause of death are rare (Sievert & Sileo 1993; Colabuono et al., 2009), maybe because documentation of direct mortality in nature is extremely difficult. Interactions between biota and microplastics are high, and there is growing evidence that microplastics can incite significant health effects in exposed organisms. Microplastics ingestion is of particular concern, since they are widely distributed and of small sizes, hence a wide range of organisms may ingest them. The smaller the particle, the greater is their availability to small animals (Werner et al., 2016). The UNEP yearbook 2011 identified marine microplastics to be one of the main global emerging environmental issues. The fragmentation of plastic litter can be caused by abiotic factors or through animal digestion processes (Kühn et al., 2015).

Fish may intentionally ingest microplastics by mistaking particles for a natural prey (e.g., plankton) or unintentionally, if the microplastics were already present inside or adhered to the prey. Despite a few surveys indicating that predatory fish are at a higher risk of ingestion of marine litter (including microplastics de facto) (Anastasopoulou et al. 2013; Choy and Drazen 2013; Deudero and Alomar 2015), it appears that there is no correlation between the number of ingested particles and either fish length, fish mass, or species placement within the food web (Guven et al. 2017). Only the type of habitat (pelagic fish vs benthic fish) seems to influence the quantity of ingested microplastics. Pelagic fish do ingest more micro- plastics compared to benthic fish species, regardless of whether they are predatory species or not.

In marine turtles, feeding ecology and diet, as well as habitat use in relation to areas of high plastic density, determine the likelihood and consequences of plastic ingestion (Bond et al., 2014). This is likely to differ among turtle life stages, regional populations and species, meaning that there are likely to be inter- and intra-species variations in the densities and types of plastic encountered and potentially consumed (Schuyler et al., 2014). Differences in debris ingestion by species and their impact may also be attributed to differences in the biology of the animals and how their digestive systems cope with debris once ingested.

In other hand, it has been suggested that plastics might act as a vector facilitating the transport of chemicals to organisms upon ingestion in two different ways. Microplastic might provide a pathway facilitating the transport of harmful chemicals to organisms. Experimental studies have shown that phthalates and BPA affect reproduction in all studied species and generally inducing genetic aberrations (Oehlmann et al., 2009). Some plastics contain additives potentially harmful plasticisers, antimicrobials and flame-retardants, which are incorporated during manufacture and could be released in the organisms after ingestion (Rochman & Browne, 2013; Oehlmann et al., 2009). In addition, plastics are known to sorb persistent organic pollutants from water concentrating them on the item surface. In this way, pollutants on plastic items can become orders of magnitude greater than in the surrounding water facilitating chemical transfer to biota (Mato et al., 2001; Teuten et al., 2007).

Microplastic exposure has been associated with a suite of negative health effects, including increased immune response, decreased food consumption, weight loss, decreased growth rate, decreased fecundity, energy depletion and negative impacts on subsequent generations (Von Moos et al., 2012; Besseling et al., 2011; Wrigth et al., 2013; Huerta et al., 2016; Sussarello et al., 2016; L onnstedt and Eklov, 2016).

Microplastics have also been shown to readily accumulate waterborne persistent organic pollutants, including pesticides, solvents and pharmaceuticals, which may pose further health effects such as endocrine disruption (Wardrop et al., 2016). Laboratory studies indicate that microplastics can cause various negative effects in fish: physical damage, change in lipid metabolism, change in behaviour, as well as cytotoxicity. Rochman et al., (2013; 2014) showed evidence of sorbed chemicals transfer from plastic to fish, including indications for health effects.

Ingestion of microplastics is not necessarily harmful per se. Over millions of years, fish frequently ingested other indigestible material (e.g., sand particles) or partially digestible material (e.g., fish scales, wood, shells of macroinvertebrates, etc.) and have thus evolved to deal with unwanted gastrointestinal non-food material (Javanovic, 2017).

Retention time within the organism is an important consideration: some animals have been shown to retain plastics for several weeks (Browne et al., 2008), while animals that regurgitate indigestible stomach contents on a daily basis or species quickly passing such items through the intestines, possibly being less susceptible to chemical transfer because of the lower exposure time. Gut evacuation times for animals are varied (0 to 52 hours for fish, Lusher et al., 2017). Therefore, some animals might regurgitate microplastics prior to analysis (Bromley, 1994). In general, it is related to the expansion of gas in the swim bladder: this causes the compression of the stomach and might, in extreme cases, result in total stomach inversion (Bowman, 1986). For these reasons, it is possible to affirm that chemical contamination by plastic occurs also in nature, but the extent of this phenomenon will depend on different factors, such as the ingestion rate, the degree of plastic retention in the digestive tract, the types of plastic and the chemical contaminants.

According to Javanovic review (2017), the occurrence of microplastics in the gastrointestinal tract of fish is ephemeral and with low accumulation, although translocation to the liver may occur.

## 2. Perspectives for the implementation of the indicator

Monitoring the ingestion of litter is a complex task, identifying interactions between marine litter and fauna depends largely on data collection. The choice of a good target or indicator species is a major element when developing a monitoring strategy. Fossi et al., (2017) defined six criteria (background information, habitat information, trophic information and feeding behaviour, spatial distribution, commercial importance and conservation status, documented ingestion of marine litter) for the choice of sentinel species of different taxa (inverterbrates, telosts, elasmobranchs, sea turtles, seabirds).

### a) "Microplastic debris ingested by fish"

Taking into account the data analysed into this report, the main criteria we have chosen for the selection of target species on fish are: **habitat and trophic information, sensitivity to litter, wide distribution and commercial importance**.

The ingestion of microplastics by various fish species has been well documented since the beginning of the current decade (Boerger et al. 2010; Davison and Asch 2011) and the literature on the subject has been steadily increasing.

Lantern fish (*Myctophidae*) in the Pacific Oceans are recognized as species that commonly ingest plastics (Boerger et al. 2010; Davison and Asch, 2011; Van Noord, 2012). Literature results showed that 9.2% of Myctophids in the North Pacific gyre area had plastic in the stomach. In the European region, among 10 fish species collected by Lusher et al. (2013), showed 36.5% individuals as containing plastic, with the inclusion of very small fibers. Foekema et al. (2013) found overall a lower 2.6% of individuals with plastic fragments in the stomach among seven common species in the North Sea, but they did not include fibers in the study. Romeo et al. (2015) recently reported that about 18% of large pelagic fishes in the Mediterranean (tuna, albacore, swordfish) had plastic litter in their stomachs. The occurrence of plastic particles in the digestive tracts of 64 Japanese anchovy (*Engraulis japonicus*) sampled in Tokyo Bay has been reported. Plastic was detected in 49 out of 64 fish (77%), with 2.3 pieces on average and up to 15 pieces per individual (Tanaka and Takada, 2016). Nevertheless, the ingestion of litter by fishes is less well described than for birds or marine turtles, and due to a non-standardized digestion and filtration process of gastrointestinal contents and cut-off size for microplastics, we cannot really compare quantities of microplastic ingestion in fish from marine areas around the world (Jovanovic, 2017). In this context, researchers from Italy, France, Spain and Greece in the framework of the European Interreg MED project "MEDSEALITTER" are elaborating a standardized method of analysis for microplastic in fish to be implemented in all the Mediterranean Basin. When validated, this standardized method could be disseminated at a large spatial scale.

**Habitat and trophic information.** The home range of the species is an important information as it determines the spatial scale of monitoring. The habitat gives also information on the feeding behaviour of the species (planktivorous, omnivorous, detritivores etc.) and the potentiality of exposition to the ingestion of microlitter. Guvet et al., (2017), do not find correlation between the number of microplastic ingested and the food web. Javanovic (2017), instead underlines that the type of habitat (pelagic fish vs benthic fish) influences the quantity of ingested microplastics. Filter feeders as anchovies are potentially exposed to the ingestion of microlitter (Collard et al., 2015; Fossi et al., 2017). Opportunistic feeders (e.g. bluefin tuna, albacore) or which feed on the seafloor (e.g. red mullet) can be exposed to the risk of litter ingestion (Fossi et al., 2017).

**Sensitivity.** For the choice of species, the behaviour to a regular plastic consumption should be considered. The frequency of litter ingestion within species and the amounts of plastic found in individuals' stomachs, should be high enough to allow detection of trends over time and local differences related to spatial variations in litter concentrations. Anastasopoulou et al. (2013) showed that elasmobranchs might consume litter more frequently than bony fish (*Pteroplatytrygon violacea* 50 % of ingestion). Avio et al. (2015) reported 44 % frequency of ingestion for *Squalus acanthias* and Neves et al. (2015) 67% for *Scyliorhinus canicula*. Among demersal bony fish, Nadal et al. (2016) suggested Boops boops as a good indicator, since it shows a very high occurrence frequency of microplastics ingestion, with values ranging from 42 % to 80 %, according to different locations. Another demersal species with high sensitivity is *Mullus barbatus*; some results confirming the interest of this species as indicator show 66% (Guven et al., 2017), 64% (Avio et al., 2015) and), 18.8 % (Bellas et al., 2017) frequency of ingestion. The high frequency of microplastic ingestion by demersal fish may be due to their livelihood in more direct contact with the seafloor, considered the ultimate sink for plastics in the marine environment. *Mullus barbatus* swallow sediment together with the prey, after identifying them with their barbels, and expelling the sediment through their gills, which may increase the risk of accidently ingesting plastic. Among benthopelagic fish, *Mugil cephallus* is considered to be a good indicator species, being cosmopolitan in coastal ecosystems. The high incidence of plastic ingestion (73 %) may be due to their mode of indiscriminate benthic feeding. It has not been found differences in plastic ingestion relative to fish size (Naido et al.2016). Guven et al. (2017) concluded in their studies that pelagic fish ingested higher amount of microplastic than demersal species: *Scomber japonicus* (71%), *Sardina pilchardus* (57 %), *Trachurus mediterraneus* (68 %). are pelagic species to be taken into account as indicators.

**Wide distribution.** Due to the fact that monitoring programs are performed at National level, but their results must be comparable at Regional scale (Barcelona Convention, OSPAR and HELCOM) or among European seas (MSFD), samples should be available in a wide area and possible in the Mediterranean Sea and Atlantic Ocean.

Table 5 summarizes the ingestion prevalence of microlitter in individuals of fish species, present both in the Mediterranean Sea and in the NE Atlantic, according to the main studies published. Guven et al. (2017) and Neves et al. (2015) investigated the same species with the following percentage occurrence of microplastic ingestion: *Argyrosomus regius*, 75 % within the Mediterranean Sea, 60 % within the NE Atlantic; *Mullus surmuletus*, 49 % within the Mediterranean Sea, 100% within the NE Atlantic; *Scomber japonicus*, 6,71 % within the Mediterranean Sea, 31 % within the NE Atlantic. Data are not easily comparable due to different sampling sizes and methods used for isolating microplastics. A minimum sample size is necessary to accurately evaluate litter ingestion. Regarding the species Boops boops, Nadal et al. (2016) sampled 337 individuals and Neves et al. (2015) sampled 32 individuals, and they respectively found 57.8 % and 9 % of microplastic ingestion. The number of individuals in each study can partly cause this difference. Regarding the species *Merlucius merlucius*, Neves et al. (2015) sampled 7 individuals with 29 % of microplastic ingestion and Avio et al. (2015) sampled 3 individuals with 100 % of microplastic ingestion. In this last case, the difference is also probably linked to the protocol of isolation of the microplastic. The authors here optimized a new protocol allowing an extraction yield of microplastics from fish tissues ranging between 78 % and 98 % depending on the polymer size, while in both studies total samples number was very low.

**Commercial importance.** Species with commercial importance are needed for two reasons. The first reason is the optimization of monitoring samples. As discussed in this paper, dedicated sampling catch are preferable, anyway for the best cost-benefits ratio it could be useful combine regular fish monitoring programmes of commercial fish with dedicated sampling catch. The Data Collection Framework, performed in compliance with UE Common Fisheries Policy, can provide a useful context for the implementation of a possible monitoring at European scale for the indicator. Fisheries-independent data are yearly collected by means of bottom trawling surveys.

The second reason is that commercial fish are linked to the health of consumers, using commercial target species helps to estimate the potential risk to transfer microplastics from sea food to humans.

**Table 5.** Percentage of microplastic ingested by common species collected in the NE Atlantic and the Mediterranean Sea.

| Species | % with microplastic | Geographical area | Author |
|---|---|---|---|
| ***Argyrosomus regius*** | 75% | Mediterranean Sea | Guven et al., 2017 |
| | 60% | NE Atlantic Ocean | Neves et al., 2015 |
| ***Boops boops*** | 57.8% | Mediterranean Sea | Nadal et al., 2016 |
| | 9% | NE Atlantic Ocean | Neves et al., 2015 |
| ***Brama brama*** | 0% | Mediterranean Sea | Anastasopoulou et al., 2013 |
| | 33% | NE Atlantic Ocean | Neves et al., 2015 |
| ***Caranx crysos*** | 100% | Mediterranean Sea | Guven et al., 2017 |
| | 0% | NE Atlantic Ocean | Miranda et al., 2016 |
| ***Helicolenus dactylopterus*** | 0% | Mediterranean Sea | Anastasopoulou et al., 2013 |
| | 0% | NE Atlantic Ocean | Neves et al., 2015 |
| ***Merluccius merluccius*** | 0% | Mediterranean Sea | Anastasopoulou et al., 2013 |
| | 100% | Mediterranean Sea | Avio et al., 2015 |
| | 16.7% | Mediterranean Sea | Bellas et al., 2016 |
| | 29% | NE Atlantic Ocean | Neves et al., 2015 |
| ***Mullus surmuletus*** | 49% | Mediterranean Sea | Guven et al., 2017 |
| | 100% | NE Atlantic Ocean | Neves et al., 2015 |
| ***Polyprion americanus*** | 0% | Mediterranean Sea | Anastasopoulou et al., 2013 |
| | 0% | NE Atlantic Ocean | Neves et al., 2015 |
| ***Sardina pilchardus*** | 57% | Mediterranean Sea | Guven et al., 2017 |
| | - | Mediterranean Sea | Torre et al., 2016 |
| | 19% | Mediterranean Sea | Avio et al., 2015 |
| | 0% | NE Atlantic Ocean | Neves et al., 2015 |
| ***Scomber japonicus*** | 6.71% | Mediterranean Sea | Guven et al., 2017 |
| | 31% | NE Atlantic Ocean | Neves et al., 2015 |

**Abundance of samples availability:** For several years the abundance of demersal fish stocks is evaluated by regular fish monitoring programmes, some of these still operating.

For the North Sea a list of surveys is available at http://www.cefas.defra.gov.uk/publicationsand-data/fishdac.aspx, other data may be found at www.ices.dk, including Baltic surveys.

The MEDITS programme, aims at conducting coordinated surveys from bottom trawling in the Mediterranean Sea http://www.sibm.it/SITO%20MEDITS/ (Fig.5).

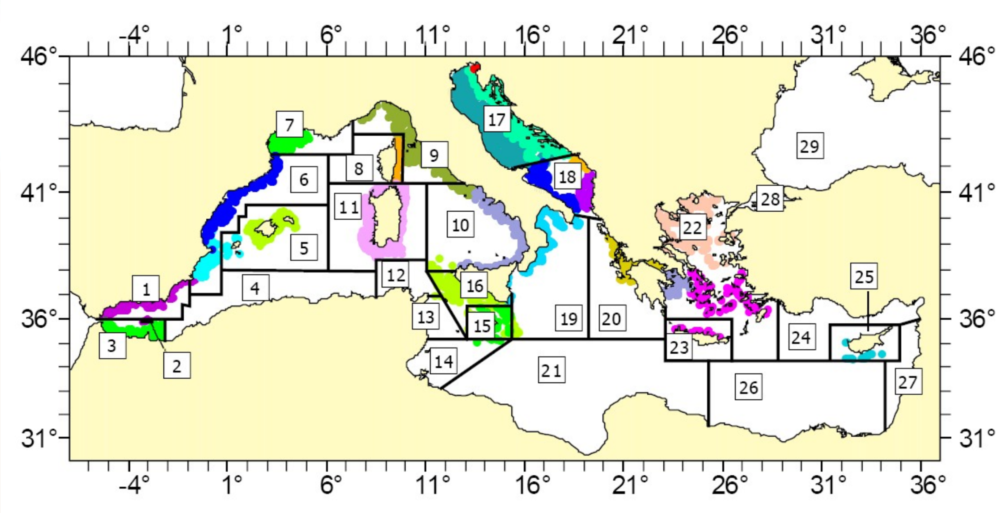


**Fig 5.** MEDITS programme surveys from bottom trawling in the Mediterranean Sea

The surveys intend to include as much as possible all the trawl able areas over the shelves and the upper slopes from 10 to 800 m depth off the coasts of the partner countries. Fish samples can be easily obtained from these, with the limits imposed by the used fishing gear and the distance of the hauls from the coast. However, the bias due to the use of this typology of catches was documented previously in this report. Dedicated sampling catch could be performed with different nets or traps, in shallow water along the coast, near or far from a specific hotspot (river mouth, harbour, city, etc.).

### b) "Microplastic debris ingested by sea turtles"

Direct consumption of plastic has been documented in all marine turtle species (e.g. Carr, 1987; Bjorndal et al., 1994; Hoarau et al., 2014). In the recent years, the ingestion of microplastics has also been described, but most of the studies concerning the impact of marine debris on sea turtles concern only macro-debris (>5mm) ingestion. Currently, and based on the MSFD Directive, researchers have adapted their methodologies to analyse ingested debris up to 1mm size for marine turtles (Casale et al., 2008, 2016; Lazar & Gracan, 2011; Cameda et al., 2014; Nicolau et al., 2016). Nevertheless, the majority of studies did not separate micro-debris (< 5mm) from macro-debris, based on the hypotheses that small pieces of debris may derive from the fragmentation of the macro-debris within the digestive tract.

Concerning microplastics, a potential issue for carnivorous sea turtle species, such as kemp's ridley, olive ridley, loggerhead and leatherback turtles, is the indirect ingestion of microplastics through consumption of contaminated preys, such as cnidarians, molluscs and crustaceans (Parker et al., 2005; Casale et al., 2008, Cole et al., 2013; Wright et al., 2013). This indirect ingestion may lead to sub-lethal effects that are difficult to identify, quantify and attribute to plastic ingestion as opposed to other water quality issues (Baulch and Perry, 2014; Vegter et al., 2014; Gall and Thompson, 2015).

Currently, a big concern to understand the real impact of marine debris on marine biota is to analyse the effects of microdebris ingestion; and the first step in megafauna species, as marine turtles, is to identify if micro-debris derives from macro-debris fragmentation or by other bias, such us trophic transfer.

Related with methodologies, manual extraction by visual observation of gut content extracted from the digestive tract is the most commonly used technique (Tomas et al., 2002; Santos et al., 2015; Fukuoka et al., 2016; Nicolau et al., 2016), including both macro and microdebris. Nevertheless, this technique is not adequate for microplastic collection, due to the difficulties to detect smaller debris (< 5mm), which could remain camouflaged into the organic content. For this rason, several researchers conducted chemical or enzymatic digestion of the digestive tract, where each section (esophagous, stomach and intestine) is cut into small pieces that are digested by chemicals (KOH 10% or H2O2) (Lusher et al., 2016; Ostiategui-Francia et al., 2016) or enzymatic solutions (Duncan et al., 2016; Caron et al., 2016). In this way, all organic tissues are degraded and only inorganic materials remain in the sample.

Until now, only two studies included smaller and independent sieves to analyse debris according to size. Lusher et al. (2015) used 1 mm, 0.5 mm and 0.118 mm mesh sieves to analyse debris ingestion on beaked whale (Mesoplodon mirus). Pham et al. (2017) used 5 mm and 1 mm mesh sieves to analyse separatelly macro and microdebris ingested by 24 loggerhead turtles accidentally captured by long line fisheries in the North Atlantic gyre (Azores, Portugal). In this study, microplastics were found in 58 % of the turtles and classified as: 87 % plastic fragments, 8% sheets fragments and 5 % industrial pellets, concluding that some of the micro plastics found could derive from macrodebris fragmentation into the digestive tract, but an important part of them were probably arrived there by trophic transfer (Pham et al., 2017).

In relation with the implementation of the indicator, more samples are required. Using several sieves (5 mm, 1 mm, 0.5 mm, etc.) during the gut content lavages is a easy and effective method to be implemented by researchers, allowing to increase the sample size and identifying the negative effects of microplastics ingestion on megafauna species, such as marine turtles. INDICIT project established only two mesh sieves (5 mm and 1 mm) to study microdebris ingestion by marine turtles, in order to avoid the contamination concern that could be involved with smaller litter sizes, and because most part of stakeholders do not have the adequate infrastructures to obtain data on < 1mm microdebris.

One important issue to take into account on microdebris ingestion studies is to identify the potential relation between microdebris found in the digestive track and the animal diet (e.g. prey ingested by the turtle). The use of several sieves allows diet sampling in the biggest sieves (5 mm), while smaller ones could undergo digestion process (H2O2, KOH, enzymatic digestion, etc.) to avoid that microdebris remain camouflated within the organic material.

One of the most serious constraint in microdebris ingestion analysis is to avoid contamination. Nevertheles, exhaustive protocols to avoid contamination will be followed:

• Intensive lavages of digestive tract before opening to extract gut content;

• Intensive lavages of instrumental (scisors, petri disks, plates, sieves, etc.) before using

• Working on a airflow cabinet or laminar flow hood to identify and classify the microdebris

• (Alive turtles) Intensive lavages of the turtle before introducing into the tank

• (Alive turtles) Filtering systems in the water circuit before entering into the tank.

• (Alive turtles) Eviscerate food before supplying to the turtle.

• (Alive turtles) Take samples on empty tanks to identify air contamination.

# V – Conclusion and recommendations

## Conclusion

Monitoring the ingestion of litter is a complex task. The literature on this issue is growing but collection of standardized data is necessary to develop knowledge. This is also needed to improve the comparability among studies, with reference to the protocols applied in laboratory to isolate and quantify the plastics, but also regarding sampling techniques.

The variability in microplastic ingestion by fish is very high (Tab.6). The reason for this variability is not strictly linked to the quantity of microlitter in the environment, but it could also depend on the heterogeneity in methodologies to assess microplastic ingestion. Choosing different microplastic size classes, by using different mesh of sieves, and variation in the number of individual sampled can lead to a different representation of the incidence of marine microplastic ingestion in fish. It is thus very important define a common protocol in order to establish the minimum value (size of particules and number of samples).

The prevalent type of plastic identified by the different studies is fibers. The high percentage of fibers can be related to sampling and analysis contamination or anthropogenic waste.

**Table 6.** Summary of main data from the literature analysis of this report (details in Tab.3)

| Microplastic (< 5mm) ingestion | Range (literature analysis of this report) |
|---|---|
| % of fish affected by microplastic | 1.9 - 77% |
| Number of debris items per fish | 0.03 - 56 |
| Size of microparticels | 0.09 - 5mm |

**Sampling methods.**

Fish: Regarding methodologies for sampling, the commonly used methods are those coming from routine monitoring surveys programs for fish stocks or from fish markets. In both cases, methods of catch increase the interaction between the fish and the net and can increase the feeding of microplastics during capture. Moreover, fish that spend longer in nets are subject to additional stress increasing regurgitation and/or stomach inversion, the contact time between microplastics and fish could facilitate ingestion and adhesion of particles.

Turtles: few researchers have used methods to analyse micro and macro debris ingestion separately on marine turtles, but shows that an important part of microplastics were probably arrived by trophic transfer and their effect need to be investigated. A unified methodology is required to identify differences between areas, animal sizes and stages, species, etc.

**Isolation.**

Another important issue to be optimized is the protocol for the digestion of the organic material to isolate the ingested microplastics. Strong oxidizing acid (e.g. HNO3) with high digestion efficacies can destroy fibers and microbeads, with an underestimation of microplastics. Strong bases (e.g. KOH, 10%) also can be used to remove biological material, but the time required for digestion is too long (2-3 weeks). New studies demonstrate that 10 % KOH, at 40 °C, for 48-72 h, with NaI extraction is a highly efficient protocol. Hydrogen peroxide (H2O2, 15%) is an efficacious digestant and it has no impact on PE or PS microspheres, but excessive foaming might obscure samples.

**Minimising contamination.** Contamination must be taken into account during the process, both during field and laboratory sampling. Different methods to avoid contamination both in the field and in laboratory were presented in this report.

**Implementation of the indicator.** The choice of a good target or indicator species is a major element when developing a monitoring strategy. Several factors are needed before choosing a target species: the species must be sensitive to litter, samples should be available in a wide area and the species should be commercialized (for fish).

## Recommendations

### Sampling methods.

Fish: The collection from commercial fish markets or fisheries cruises is not ideal; there is no control on the method of capture and the handling conditions on transport. It could be better to operate with fishermen to ensure fish are sampled appropriately. Specimens should be rapidly transported to the laboratory avoiding egestion in transit. Some sampling gears (bottom trawl or manta net) can produce overestimated results. These fishing gears collect more microplastics than others and consequently fish during capture in nets feed them. Purse seine, gill net, longline could prevent the artificial inflation.

Turtles: Using two mesh sieves (5mm and 1mm) during the gut content lavages conducted on debris ingestion studies will be the most simple and effective method to identify the effects of macro and micro debris ingestion in marine turtles. Manual extraction by visual observation in the 5mm sieve could be conducted, instead of the digestion process (with $H_2O_2$, KOH or enzimes) required for the sample collected in the 1mm sieve.

### Isolation.

A balance will need to be found between a cost-effective digestive (time consuming) agent, with the capacity to degrade biological material without damaging microplastics, $H_2O_2$ is a good compromise, but it is needed to reduce foaming, avoiding a slow filtration of digestants. When the sampled gastrointestinal tract is heavier than some grams the solvent digestion of organic material could be not completed. Otherwise, incubating fish tissues in 10% KOH at 40 °C and NaI extraction as additional step to separate the digestion resistant materials before the optical examination of particles on the filter membrane it could be a good method.

### Minimising contamination.

During field sampling: cleaning all the equipment before sampling; covering samples and equipment between different uses with e.g., cotton coverage, wearing polymer-free clothing or cotton coveralls, and gloves.

During analysis in the laboratory: petri dishes must be rinsed, dried with compressor air and checked under microscope for any plastic contaminants; cotton laboratory coat must be worn to ensure that synthetic fibers from clothing does not contaminate samples; and pre-filtered (0.45 mm filter) deionised water and hypersaline solution should be used. Exhaustive lavages of digestive track sections will be conducted before opening to extract gut content. All samples processing should be performed in a clean airflow cabinet, or laminar flow hood. Procedural blanks are highly recommended for quantifying contamination and for identifying aspects of the experimental design where contamination can occur.

### Possible candidate indicator species.

Fish: The main criteria for the choice of fish target species are: sensitivity, wide distribution and commercial importance. Also trophic information is important but at the moment there is no a general consensus. Pelagic and benthic ingest different percentages of microplastic types. Nadal et al. (2016) showed that pelagic species ingest more particles and benthic species more fibers. Elasmobranchs may consume litter more frequently than bony fish (Anastasopoulou et al. 2013; Avio et al. 2015; Neves et al. 2015). In order to establish the implementation for the monitoring of the marine litter ingestion by fish, a good compromise it is select at least one pelagic, one demersal bonefish species and one elasmobranch.

Taking into account all species discussed within this report and the 4 criteria for selecting a species to be tested as bio-indicator, possible candidate indicator species are:

**Pelagic**: *Sardina pilchardus* and *Scomber sp.*, are planktivorous, show high occurrence frequency of micro-plastic ingestion, their distribution covers both the Eastern Atlantic and the Mediterranean Sea and they are both commercial fishes. *Sardina pilchardus* could be a good candidate as it is a species typically eaten without removal of the digestive tract so the direct transferig of plastic to human beeing is higher.

**Demersal**: *Boops boops*, *Mullus sp.* and *Mugil sp.*, are omnivorous and cover the three criteria. Their wider diet sources increase the amount of microplastic ingested. *Merlucius merlucius* is another good candidate as the adults live close to the bottom during daytime, but move off-bottom at night, this vertical migration plays an important role in the exposition of microlitter ingestion.

**Elasmobranchs**: *Scyliorhinus canicula,* is a good candidate, although in this paper is reported only for NE Atlantic, it is a species distributed also in the Mediterranean sea, Baltic sea and Black sea. Moreover, it is considered sensitive to ingestion of microplastic and commercialized in all Europe.

There is another species that could be taken into account even if there are no studies related to ingestion, *Raia clavata*. It could be an interesting species to analyse for the variety of its diet, it's feeding in contact with the sediment increases the possible ingestion of microplastic. *Raia clavata* is widespread and abundant, and it is the dominant rajid in commercial landings.

Marine turtle: As was described in the Indicator 1 report, among the different species of marine turtles, the loggerhead turtles is the most common in the European waters, so, is the most indicate specie to be considered. Besides, the loggerhead turtles presents a wide range of distribution, usually uses the main currents to move through the ocean, spends the 95% of their life in the surface of the water column - where plastics accumulate-, and is an omnivorous species which feeds in a wide range of habitats and preys. Also leatherback turtle could be included on the Atlantic European waters, due to their very wide range of distribution and their specific feeding behaviour focused on gelatinous organisms, such us jellyfish.

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