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**Review Article in Marine Pollution Bulletin special issue "6IMDC"**

**Title: Tools and constraints in monitoring interactions between marine litter and megafauna: *insights from case studies around the world***

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# Revised text

## 1. Context and objectives

### *Monitoring marine megafauna*

Marine litter is a critical issue for all major marine taxa, with well-documented adverse impacts on more than 1,400 species, including marine megafauna (defined here as vertebrate species i.e. fish, birds, sea turtles and mammals). The primary impacts include ingestion and entanglement (Butterworth et al., 2012; Gregory, 2009; Kühn et al., 2015. Wilcox et al., 2015) though there is increasing concern about chemical contamination via ingestion (e.g. Fossi et al 2018a).

Over the last few decades, numerous survey approaches and monitoring programs have been developed and implemented around the world. These "observation platforms" collect data and observations about marine megafauna, especially marine mammals, sea turtles and sea birds (e.g. Casale et al., 2010; Danil et al., 2010; Ullmann & Stachowitsch, 2015). Such observation platforms and their corresponding networks have primarily been developed as means for acquiring knowledge on species biology, assessing threats to and trends of populations, and to rescue and rehabilitate marine fauna. They may focus on monitoring breeding activities of colonies and populations of birds, seals and/or turtles, or on providing care through rescue centers and stranding networks. The latter often involve volunteer engagement, including citizen scientist or layperson observations. These rescue centers and stranding networks also extend monitoring activities such as performing external and internal examinations of wildlife, found deceased or alive. Generally, such networks collect data such as species identification, biometric measurements, health status, circumstances of finding, gut contents, causes of morbidity and mortality which can be used to address a variety of questions (e.g. Duguy et al. 1998; NOAA, 2009; Claro & Hubert, 2011; Carreira, 2015; Goldberg et al., 2016a; 2016b).

Due to the increased concern as about marine resources and their continued health and welfare, environmental monitoring programs have been implemented to evaluate ecosystem health. Multiple approaches may be employed within such programs, including aerial surveys, at-sea data collection efforts and coastal data collection platforms (for example COASST, 2018). Such programs are broad and varied: they may aim to provide data about parameters such as species distributions, bycatch frequency and interactions between marine megafauna and anthropogenic activities (Leeney et al., 2008 ; Lauriano et al., 2011; Koch et al., 2013; Rodriguez et al., 2013; Lauriano et al., 2014; Peltier et al., 2014, Peltier & Ridoux, 2015). In some cases, multiple data sources are used. Furthermore, development and improvement of monitoring programs is often an iterative process, taking into account initial assessment data and approaches as goals are refined (e.g. Pibot & Claro, 2011).

One significant challenge, is how to best analyze data based upon multiple and variable sampling approaches. Given that different programs often have different objectives, goals or foci (and these may change through time), data collection approaches may change and there is rarely large-scale synoptic harmonization amongst programs or geographic regions. This means evaluating species status at regional or global scales is difficult; pointing to a clear need to harmonize procedures and protocols. This challenge has been commented on previously, and there is increasing focus on indicators evolving towards coordinated and common approaches (Backer, 2008; Markus et al., 2011; OSPAR, 2012; Rapport & Hildén, 2013; see also section 3.). In Europe for example, the Marine Strategy Framework Directive has defined a complex process and designed different tools to assess the efficiency of measures taken for reducing marine litter in the environment and mitigating the impact of marine litter on the biota (Galgani et al., 2014; Fossi et al., 2018b; Markus et al., 2011). Such tools include sampling devices/ data collection platforms, and indicators/criteria for evaluation (see section 3.1), based on a minimal constraint approach (Markus et al. 2011; RAC/SPA, 2017).

***The importance of testing species and methodologies***

The impact of marine litter on biota is a question which research groups around the world are working to address based on scientific evidence. It is a complex issue, in part because of the likely propensity of non-lethal impacts, difficulties in observing lethal impacts (most deceased animals are likely to remain unobserved), and those that are observed provide a biased sample. Currently, there is a focus on identifying appropriate indicator species that represent ecosystem health, as well as appropriate methodologies to identify and monitor across both time and space.

Marine megafauna such as the northern fulmar *Fulmarus glacialis* (Van Franeker & Law, 2015) and the loggerhead turtle, *Caretta caretta* (Galgani et al., 2013) are already used as ecological indicators of ecosystem health. This is due to their size and geographic distribution, and the preexistence of dedicated observation networks including stranding and rescue of marine mammals and turtles, fisheries observer campaigns, etc. Seabirds and sea turtles are recognized as appropriate indicator species for monitoring the impact of marine litter ingestion, and several methods are already available for better understanding the interactions between these taxa and marine litter (e.g. Van Franeker et al., 2011; Galgani et al., 2013; Van Franeker et al., 2015; Galgani, 2018). For other taxa such as cetaceans and sharks, research is ongoing or is needed, to better understand and document the direct (i.e. pathology, mortality) and indirect (i.e. physiological, ecotoxicological) impact of interactions between these taxa and marine litter (Fossi et al., 2014 ; Werner et al., 2016 ; Fossi et al., 2018a; 2018b; Lusher et al., 2018).

The application of relevant criteria during the process of selecting species is considered of major importance to understand the impact of marine litter on marine biota populations (Fossi et al., 2018b). However, better knowledge, improved tools and the ability to conduct experiments would all aid in advancing our ability to assess the impact of marine litter on marine biodiversity.

***Objectives***

During the Sixth International Marine Debris Conference[1] (San Diego, California, USA, 12-16 March 2018), a session was dedicated to the tools and constraints in monitoring interactions between litter and megafauna. The session's objectives were to share lessons learned from existing monitoring initiatives

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[1] http://internationalmarinedebrisconference.org

at national and regional scales; to share results from recent research; to discuss methods, indicators and technical tools and to engage with a community of practice aimed at standardization of approaches and cooperation among research groups in the international community. Furthermore, the community strived to identify knowledge gaps and tools required to understand the impact of anthropogenic debris on major marine taxa whilst identifying practical recommendations to fulfil these gaps.

Herein, we summarize case studies which discuss entanglement and ingestion including macro- and micro-debris in several taxa and across multiple geographic regions (Fig. 1).

## A. Entanglement

- Case study 1: Stranding and photo-identification network as a tool for monitoring interactions between debris and cetaceans
- Case study 2: Using drones to obtain prevalence counts for fur seals entangled in marine debris
- Case study 3: Feasibility study of an "Entanglement" indicator for monitoring marine litter impact on biota in North-East Atlantic, Baltic and Mediterranean areas

## B. Ingestion

### *Macrodebris*

- Case study 4: FT-IR spectroscopy combined with debris characterization as a tool for studying impact of debris preferentially ingested by megafauna
- Case study 5: Analysis of stomach content for assessing marine debris interactions in Brown boobies (*Sula leucogaster*) and Masked boobies (*Sula dactylatra*) in dead specimen and nests on Clipperton Atoll
- Case study 6: Plasticizers as tracers of plastic ingestion through a non-invasive sampling method in oceanic manta rays from the Mexican Pacific Ocean

### *Microdebris*

- Case study 7: A simple and effective method for monitoring microplastics ingested by marine vertebrates.

Based on the last communication of the session entitled *« The effect of Marine Litter on the Mediterranean Marine Biota: the development of a monitoring strategy for IMAP Candidate Indicator 24"*, and other integrated approaches, the paper then discusses the interest of such tools and the importance of standardizing methods for assessment and management purposes, in the context of international environmental policies and marine litter strategies.

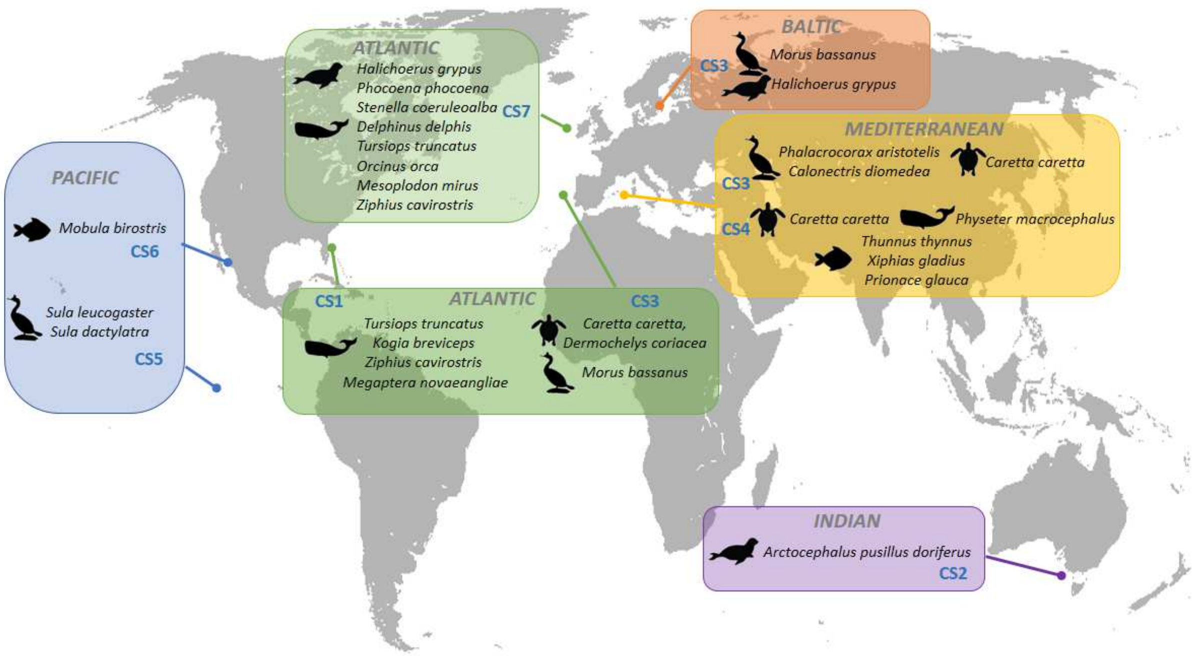


**Figure 1:** Assessing the effect of marine litter on megafauna around the world: distribution of present case studies and related representative species.

# 2. Case studies:

## A. ENTANGLEMENT

### Case study 1: Stranding and photo-identification network as a tool for monitoring interactions between debris and cetaceans

**Specific context**

A 25 year monitoring effort in South Carolina, USA has used strandings and photo-identification to develop an empirical baseline on the occurrence of marine debris entanglements and ingestion in marine mammals, particularly bottlenose dolphins (*Tursiops truncatus*). Data from this region have been collated and included in the United States National Marine Mammal Database (USNMMD)[2]. However, due to inconsistent reporting to the database itself, a potentially significant but unknown number of dolphins sighted at sea photographed with entangling marine debris (Fig. 2) have not appeared in the database. This identifies the issue that estimates are biased toward strandings, with entanglements being underreported.

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[2] https://www.fisheries.noaa.gov/national/marine-life-in-distress/national-stranding-database-public-access

**Figure 2:** Bottlenose dolphin calf entangled in marine debris at sea and not recorded in database. Photo credit: NOAA/NOS.

## Characteristics of the tool:

The South Carolina Marine Mammal Stranding Network (SCMMSN) is a network representative of the observation platform tools dedicated to collect data through a local network integrated in a national program.

It was officially organized in 1991 with a Stranding Agreement between the South Carolina Department of Natural Resources and the National Marine Fisheries Service (NMFS). In 2008, the Stranding Agreement was transferred to Coastal Carolina University in Conway, South Carolina with oversight between NMFS and the National Ocean Service in Charleston, South Carolina. Approximately 20 volunteers respond to strandings along the 300 km coastline of beaches, barrier islands, and tidal marshes. Funding for the SCMMSN has been consistent but is dependent on yearly funds provided by the Prescott Funding for stranding networks, although there have been a few years where funding was not secured. At the minimum, basic data (Level A data) are collected on each stranded animal and recorded digitally in the USNMMSD.
As an example of the utility of such long term networks from repeated surveys that took place between 1992 and 2016, it has been observed that for bottlenose dolphins, 72.2% of entanglements were from rope, 16.7% from monofilament line, and 11.1% from derelict traps. Incidence of entanglement overall, however, appears to be low (2.7%; Fig. 3).

In total four (of 27 marine mammal species in the area) species have been documented as having ingested marine debris: bottlenose dolphins (*Tursiops truncatus*; *n*=3), pygmy sperm whales (*Kogia breviceps*; *n*=6), Cuvier's beaked whale (*Ziphius cavirostris*; *n*=4), and humpback whale (*Megaptera novaeangliae; n*=1). Ingestion of marine debris (mainly plastic garbage bags) appears to be more common in pelagic species such as *K. breviceps* (7.6% occurrence) and *Z. cavirostris* (30% occurrence). Given that most deceased marine mammals die unobserved, it is likely that ingestion is far greater than reported.

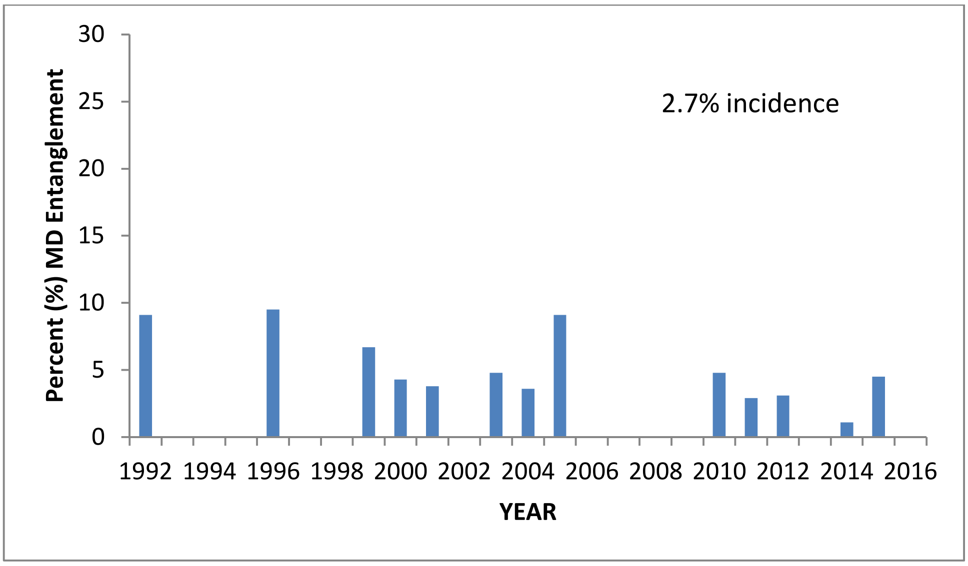


**Figure 3.** Percent of stranded bottlenose dolphins involved with marine debris entanglement from 1992-2016 in South Carolina, USA. Overall incidence was 2.7%. MD= marine debris.

**Constraints**

Generally, the impact of entanglement and ingestion of marine litter is inhibited because of inconsistent record keeping from various marine mammal stranding networks, which focus on inadequate population assessments of many species. Furthermore, there are some difficulties in discerning abandoned, lost, or otherwise discarded fishing gear (ALDFG) which are true debris, from actively fished gears which mean that animals have been incidentally caught and released with a piece of gear, which is not considered as debris.

**Perspective**

Mitigation efforts with marine mammal stranding networks and photo-identification study personnel is needed to consistently define marine debris and record marine debris entanglements and ingestion on existing data forms. The Marine Mammal Health and Stranding Response Program of NOAA have recently made available a human interaction form that does provide a check box for debris which should aid researchers in their assessments of marine debris occurrence.

## Case Study 2: Using drones to obtain prevalence counts for fur seals entangled in marine debris

**Specific context**

In seals, marine debris entanglement is expected to result in death of those affected because they cannot remove the entanglement themselves (Fowler, 1987; Hanni and Pyle, 2000; Campagna et al., 2007). Materials entangling Australian fur seals (AFS) *Arctocephalus pusillus doriferus* are varied but predominantly consist of commercial and recreational fishing material (Lawson et al., 2015; McIntosh et al., 2015). There are more than 10 methods of estimating fur seal rates of entanglement in the literature and many do not provide a true prevalence estimate, confounding comparisons between studies (McIntosh et al., 2015).

At Seal Rocks, Victoria in Australia, researchers from Phillip Island Nature Parks have identified 455 individual entangled AFSs between 1997 and 2017. However, these data provide an underestimate because fur seals flee into the water upon approach and cannot be counted with confidence (McIntosh et al., 2015). Understanding reliable population level effects of entanglement has been recently prioritized since reduced pup numbers were identified between 2007 and 2013 and the contributing factors are uncertain (McIntosh et al., 2018).

**Characteristics of the tool**

Using Remote Piloted Aircraft (RPA) or drones, we can obtain data with greater precision and accuracy than through ground-based methods. With a small and cost-effective RPA (DJI Phantom 4), we can determine the total number of individuals present at a point in time and the associated prevalence of those entangled (McIntosh et al., 2018). RPAs can be used with increased frequency and less disturbance than typical methods, improving the robustness of the datasets. Because the RPA method is obtained at a single point in time, survey effort is standardized and a simplified measure of prevalence is suitable: that being the number of individuals entangled divided by the total count of fur seals. Additionally, rescue efforts are improved because we have prior knowledge of those entangled onsite.

We visited five breeding colonies across Bass Strait, Australia during January 2018. Ground counts of entangled seals were performed and compared to seals counted using a web-based portal of RPA images 'SealSpotter' (http://natureparksresearch.com.au/sealSpotter/). The same individual (RM) performed all counts for consistency. RPA images with entangled seals were individually validated by classifying the image as 25, 50, 75 or 100 percent confident on being entangled. Validated RPA image counts of entanglements were higher than those obtained on the ground at all sites (Table 1).

**Constraints**

Constraints include the short flight endurance of the <2kg Phantom Pro 4 and the large file size for image storage and processing which uses digital space and time. Also, identifying entanglements includes a potential bias towards larger and more colorful material such as green trawl net compared to embedded recreational fishing line (Fig. 4). If seals are being rescued and released, it can be difficult to separate scars from active entanglements, larger RPAs with higher resolution cameras may enable this. Both these identification biases may exist regardless of method. Finally, there may be localized legislation relating to drone use that constrains this methodology.

**Perspective**

Seals provide an excellent taxonomic group for evaluating marine debris interactions with wildlife, because unlike many marine species, they breed and rest on land in large aggregations where they can be observed. This means that entanglement in particular can be readily observed. To date, however, methods of calculating entanglement prevalence and rates for seals have not been standardized. This lack of congruence makes it difficult to compare studies both temporally and spatially. The effort spent (time or number of observers) and the number of entanglements observed are positively correlated, exacerbating the lack of standardization (McIntosh et al., 2015). Using RPAs to survey fur seals is both cost effective and time efficient and causes fewer disturbances. Also, archived images can be revisited for future research and shared. This method is a great improvement, providing standardized estimates of prevalence that are more accurate and reliable for examining trends.

**Table 1.** Prevalence ($P = n_e/c$) of entangled Australian fur seals (*Arctocephalus pusillus doriferus*) at five sites over the 2017 breeding season determined from Remote Piloted Aircraft (RPA) images. All counts were obtained by one researcher to reduce

bias. Ground count also provided and validated RPA image counts of entanglements for comparison. The total count (c) includes adults and juveniles, not pups.

| Site | Date | Ground count | RPA survey | | | |
|---|---|---|---|---|---|---|
| | | Entangled | Entangled counted | Validated image counts $n_e$ (> 75% certainty) | Total Count c | Prevalence $P = n_e/c$ |
| The Skerries | 16 Jan 2018 | 3 | 12 | 12 | 5,300 | 0.002 |
| Seal Rocks | 28 Dec 2017 | 5 | 13 | 8 | 4,928 | 0.003 |
| Deen Maar Island | 5 Jan 2018 | 1 | 3 | 2 | 2,033 | 0.001 |
| Rag Island | 26 Jan 2018 | 0 | 6 | 6 | 2,221 | 0.003 |
| Marengo Reef | 3 Jan 2018 | 0 | 2 | 1 | 1,225 | 0.001 |


**Figure 4.** Example image from SealSpotter web portal (http://natureparksresearch.com.au/sealSpotter/) showing two Australian fur seals entangled in marine debris (circled in red) at The Skerries breeding colony, Australia. Images to the side of the portal show a zoom (below right) to help identify seals and example images (top right). Green dots identify counted pups, red - adults and juveniles and blue - entangled fur seals.

## Case study 3: Feasibility study of an "Entanglement" indicator for monitoring marine litter impact on biota in North-East Atlantic, Baltic and Mediterranean areas

## Specific context

Indicators are essential tools for environmental policies. As a first step, a state of art approach involving the compiling of published and grey literature as well as expert knowledge is fundamental to support the feasibility and the definition of new indicators and/or criteria.
While an indicator of debris ingestion is already being considered by some environmental policies (Matiddi et al., 2017; Van Franeker et al., 2011; Directive 2008/56/EC of the European Parliament and of the Council; Commission Decision (EU) 2010/477/EU; EIHA 16/05/13 (0513); UN Environment/MAP SPA/RAC, 2018), no indicator related to entanglement has been defined to date for long-term monitoring programs, although recently proposed by EU (Commission Decision (EU) 2017/848). The European project INDICIT and a national French IFREMER-MNHN team evaluated the feasibility of an indicator "Entanglement with debris by marine biota" in EU, OSPAR, HELCOM and Barcelona conventions areas. The method included developing a survey involving stranding or rescue networks and biologists from eight countries (21 questionnaire responses) and performing a literature review (57 publications). Targeted species were cetaceans, sea birds, marine turtles, fish and benthic invertebrates.

## Characteristics of the tool

The tool is a base document which includes both published knowledge and information collected from experts working on the field. This document focuses on providing the existing information and identifying which knowledge is lacking for defining a new indicator (e.g. sensitive species, occurrence, factors for spatio-temporal variability), and for assessing the feasibility of monitoring in the frame of policies' long term monitoring programs (e.g. observation platforms, existing methodologies). It finally proposes recommendations for further development.

Claro et al. (2018)'s state of the art approach and feasibility study documented that 26 megafauna taxa were reported as sensitive to entanglement in marine litter in the Mediterranean, Baltic and North-East Atlantic, with a variable prevalence (Table 2). For each taxon, these authors noticed that the occurrence, circumstances and spatio-temporal variability of entanglement as well as factors for sensitivity were partially documented from the literature and from surveys. Five species were found to represent possible relevant indicator species for marine litter pollution in the target area, which populations are already monitored by existing observation platforms. Information was provided on the distribution of megafauna species, which may be wide (e.g. *Caretta caretta*) to restricted (e.g. *Halichoerus grypus*) within the target areas; this information is useful in order to determine the geographical scale of indicator application (Table 2).

**Table 2:** Prevalence of entanglement of marine megafauna with marine debris in Europe, sampling platforms and major constraints linked to their use as indicator species for monitoring the impact of debris on sealife (after Claro et al. 2018).

| Taxa | Mean Prevalence | Main constraints | Main existing observation platforms per oceanic compartment | | | Species distribution |
|---|---|---|---|---|---|---|
| *MAMMALS* | | | *COASTAL* | *SURFACE* | *COLUMN* | |
| seals | 0.25-6.5% | M | RC,SN | | | restricted |
| cetaceans | 0.1-30% | M,B | SN | AES, ASC | ASC | species dependant |
| *BIRDS** | 0-20% | M**,B | SN, NN | RC, ASC | | species dependant |
| *SEA TURTLES**** | 0.1-58% | M,B | RC, SN, NN | | RC, ASC | wide |

M= methodological (no standard method and/or typology); B= biological (e.g. migratory and diet traits); RC= rescue centers (include fishermen accidental capture alerts); SN=stranding networks (include fishermen accidental capture alerts); NN= nesting monitoring networks ; AES= aerial surveys (include observation of fisheries) ; ASC= at sea campaign. **P. aristotelis, M. bassanus, C. diomedea*; **for non nesting observations; ****Caretta caretta, Dermochelys coriacea*.

Among the different kinds of constraints examined for each taxa (biological, methodological, environmental, logistic, regulatory), the biological and methodological constraints were found to be the most influential, and the main source of bias was linked to the detectability of samples, like already identified by RAC/SPA (2017). The main constraint for using vertebrates, in particular marine mammals and turtles, as indicator species was linked to the difficulty to distinguish entanglement caused by active gears or by "ghost fishing" material.

In birds, the indicator "entanglement in nest" was identified as possibly overcoming this issue in certain species such as *Phalacrocorax aristotelis* and *Morus bassanus* where interaction caused by debris may be identified (Cadiou et al., 2011; Werner et al., 2016). Ryan (2018) underlines that tracking changes in plastic use in bird nests (e.g. Hartwig et al., 2007 *in* Ryan, 2018, Votier et al., 2011) is a useful tool to monitor entanglement risk and a non-destructive method to estimate encounter rates with plastic debris, particularly among colonial species where large numbers of nests can be sampled in the same location. However, Ryan (2018) addresses that comparisons need to control for local conditions, because the incidence of plastic in nests reflects not only the local availability of plastic (e.g. Bond et al., 2012 *in* Ryan, 2018), but also the availability of natural nesting material (e.g. Witteveen et al., 2017 *in* Ryan, 2018).

In the conclusion, Claro et al. (2018) identifies gaps of knowledge for potential megafauna indicator species, in particular about the spatio-temporal variability of entanglement and factors for sensitivity. The study underlines the need for standard methodologies and provides recommendations in the perspective of developing an entanglement indicator and a long term monitoring program. The interest of an alternative approach relying on an indicator per oceanic compartment (coastal, surface, column) rather than an indicator based on species, as proposed by RAC/SPA (2017) and Galgani et al. (in press) was confirmed and included in these recommendations.

**<u>Constraints</u>**

The state of the art approach and feasibility study constraints are linked to several factors such as time, language and accessibility of either data/ information and experts/networks. In the case of Claro et al. (2018), the duration dedicated to the survey was short (6 months) for dissemination of the questionnaire, receipt of responses, sending of reminders, planning of complementary interviews etc. Some experts and network representatives did not speak English, and translation skills were necessary for obtaining and analyzing grey literature (e.g. activity reports) and interviews. In our case, the questionnaire was sent to experts and networks from eight countries, through the project partners who translated the questionnaire into the national language. Some experts and network representatives, in particular rescue centers whose activity is mainly in the field, were not easily accessible through e-mail which impacted the rate of response to the survey. Furthermore, due the time requested for extracting data, financial means were requested by the respondents.

**<u>Perspective</u>**

While the state of the art approach is commonly used before starting any study, literature often mentions observations of species and specimens, but lack practical and operational information from the observation platforms. This information is very useful for evaluating the feasibility of indicators and long term monitoring at national or regional levels, in particular in the frame of policies. The development of a methodology and an evaluation of time and financial budgets should be integrated as a next step before routine application of the tool.

# B. INGESTION

## *Macro-debris*

### Case study 4: FT-IR spectroscopy combined with debris characterization as a tool for studying impact of debris preferentially ingested by megafauna

**Specific context**

Despite an increase in the number of studies in recent years, information on marine debris ingestion in Mediterranean organisms remains very poor and inconsistent (Fossi et al 2018a; 2018b). It is difficult to compare among different species and studies on amounts and types of ingested debris because data are not standardized. This case study aims to apply and implement a standardized protocol for quantification and characterization of marine debris in five Mediterranean megafauna species belonging to four taxa. The specific objectives of the study were to: evaluate occurrence of individuals that ingested marine debris, obtain information on abundance and weight of marine debris ingested, collect data on category, colour, polymer type of plastics ingested. Gastro intestinal content of 85 bluefin tuna *Thunnus thynnus*, 84 swordfish *Xiphias gladius*, 95 blue shark *Prionace glauca*, 76 loggerhead turtle *Caretta caretta* and 13 sperm whale *Physeter macrocephalus*, were collected along the Italian coast.

**Characteristics of the tool:**

The first step of the tool consists for the analysis of ingested marine debris through examination of gastro-intestinal contents, following the MSFD Descriptor 10 standard protocol developed for sea turtles (Matiddi et al., 2018). The second step is to analyze the polymer composition using the Fourier transform infrared (FT-IR) spectroscopy technique (Hummel, 2002). Combining the polymer analysis to the characterization allows to understand the composition and origin of the debris ingested.

Marine debris were found in the gut of all the five species with a different percentage of occurrence: swordfish (9.5%), bluefin tuna (21.18%), blue shark (25.26%), loggerhead (68.82%) and sperm whale (77%). Loggerhead sea turtle and sperm whale were the species most affected by marine debris ingestion both as regards the occurrence of individuals affected, and the mean number and weight of MD ingested (Fig. 5). The polyethylene and polypropylene sheet like user plastics, widely used as packaging material, are the most ingested debris in all species investigated. The characterization of marine debris and the analysis of polymers of plastic items found in the gastrointestinal tract provide useful information about the sources to implement future mitigation actions.

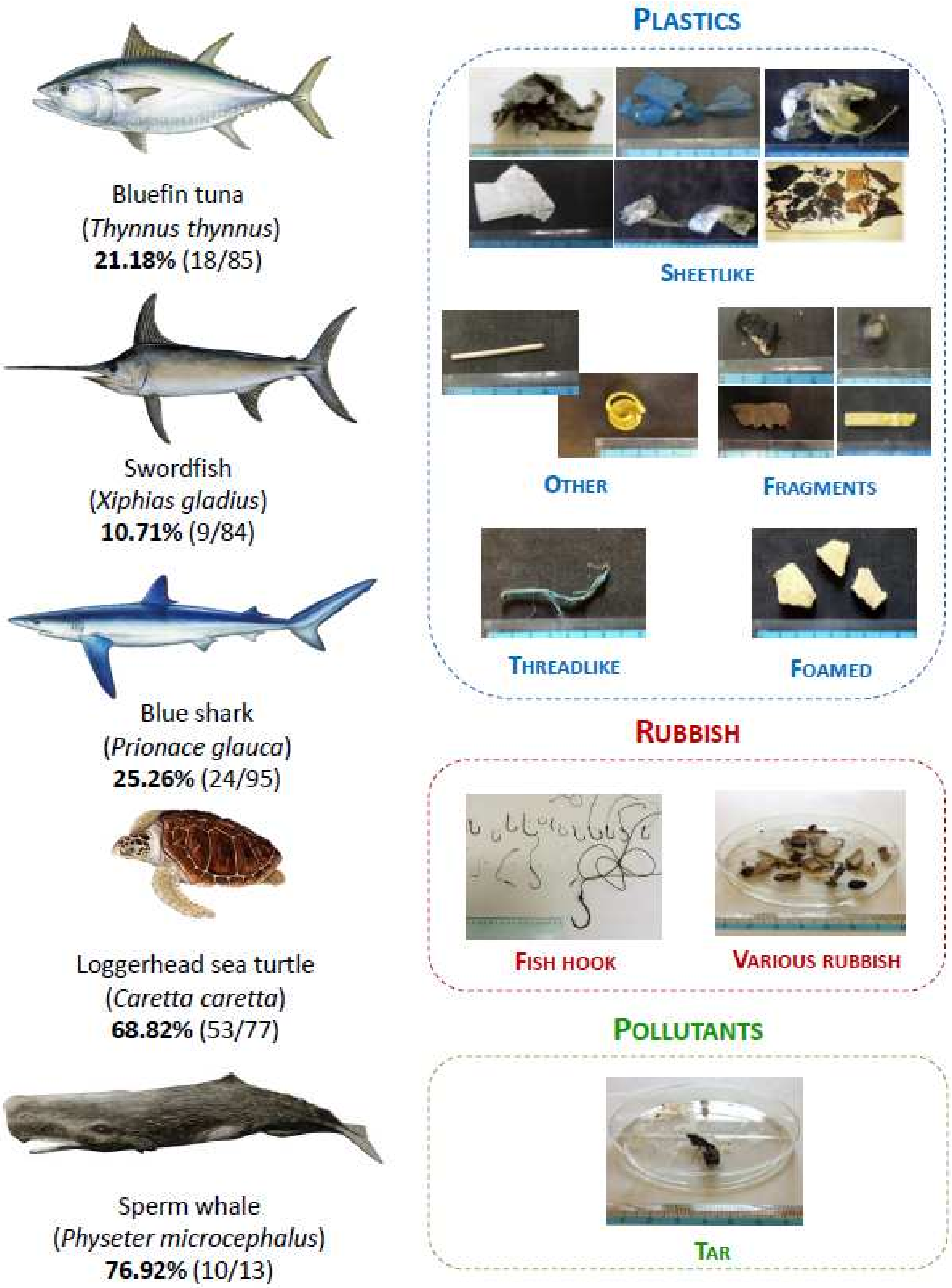


**Figure 5.** Marine debris percentage of occurrence in different megafauna species; photos of items found in the gastrointestinal tract subdivided in the different categories.

## Constraints

Monitoring activities on commercially harvested species are logistically and normatively simple, as specimens and samples can be easily accessed through fishing activities. However, if sepcies are protected, threatened or endangered, special permits are required for specimen transport and necropsy, and it is advantageous to involve regional or national networks to maximize sample retrieval. Another important constraint is related to the size of the isolated marine litter particles: through this protocol it is possible to highlight the ingestion of particles larger than 1 mm. Smaller items should be isolated applying specific methods as those described afterwards in CS7.

**Perspective**

It will be essential to harmonize methods across research teams and laboratories and to extend the analysis to other megafauna species in the Mediterranean Sea and worldwide to get reliable data on changes in quantities and types of ingested debris.

## Case study 5: Analysis of stomach content for assessing marine debris interactions in Brown boobies (*Sula leucogaster*) and Masked boobies (*Sula dactylatra*) in dead specimen and nests on Clipperton Atoll

**Specific context**

Clipperton is an isolated atoll in the Pacific Ocean, 768 nautical miles South of Cabos San Lucas, Mexico. Once home to the largest colony of Masked boobies (*Sula dactylatra*) in the world, with a population estimated at 110,000, follow up counts have revealed this number is drastically decreasing (Pitman et al., 2005). The overwhelming abundance of plastic on the atoll could be causing these birds to die off or relocate. While logistic conditions did not allow a survey of the entire area, there was a clear evidence of many bird carcasses including that of Brown boobies (*Sula leucogaster*) on the atoll and our aim was to investigate links between the deaths and the plastic pollution.

**Characteristics of the tool:**

Digestive content analysis of the dead birds found during opportunistic surveys were investigated. The case study was conducted on 33 fresh carcasses of boobies. While for every one bird sampled, it was estimated that there were three in the immediate vicinity, these were not examined since they were dried carcasses that could have been scavenged and were exposed to the elements for a prolonged period of time. Of these 33 stomach contents, one contained visible plastics (Fig. 6). To understand and explain why the ingestion of plastic was so low, while the volume of marine debris was so high, Sulid's evolutionary traits, such as highly developed eyesight and their plunge-diving feeding technique, as well as environmental factors, such as healthy fish populations and water clarity, were considered as likely variables.

To determine if these seabirds were integrating plastics into their nest structures, visual analysis and photo documentation was conducted on over 50 nests, in several areas of the atoll. Plastics were found in many of the nest structures, not only as practical construction materials, but also in what appear to be aesthetic decisions. Several nests included monochromatic color schemes, with blue plastics appearing most frequently (Figure 6).

**Figure 6.** Interactions of the Brown boobie *Sula leucogaster* with marine debris in Clipperton Atoll. Left. Removal of a plastic debris (12" backpack strap) from stomach. Right. Nest with blue/green plastics.

**Constraints:**

Due to permitting limitations, it was not possible to take blood and tissue samples from live or deceased seabirds. To confirm the presence of toxins from plastic pollution, future trips should request permits that include the taking of blood and tissue samples.

**Perspective:**

Due to the volume of plastic pollution on Clipperton Atoll, and research conducted on seabirds in similar living conditions, visual analysis of stomach contents was expected to be the best method for evaluating plastic ingestion, and interpret the dangers of living on a landmass so polluted. This determination may not be that simple, as birds do not appear to be ingesting the plastic and it is possible that invisible bio-accumulative toxins in the food web may be affecting the diminishing bird colony, and contributing to the numerous deceased birds found.

## Case study 6: Plasticizers as tracers of plastic ingestion through a non-invasive sampling method in oceanic manta rays from the Mexican Pacific Ocean

**Specific context**

Recently, a growing concern has been raised about the impact that micro-plastics can have on filter-feeding megafauna (Fossi et al., 2014; Germanov et al., 2018). These animals are protected in many countries and the collection of stomach content for plastic ingestion analysis is usually restricted to stranded animals or bycatch. As an alternative to this invasive methodology, plasticizers and flame retardants have been used as tracers of plastic ingestion in different tissues. Through the chemical analysis of samples collected from dead or alive animals (biopsies), this indirect tool has allowed to investigate species that cannot be fished or hunted, such as the basking shark, the whale shark and the fin whale (Fossi et al., 2014; 2017).

**Characteristics of the tool**

Non-lethal biopsies taken from free ranging wildlife are a useful tool for plastics impact investigation (Fig 7), but it can represent a challenge once in the laboratory due to the small sample size. Plasticizers are trace pollutants that can be present in animal tissues at concentrations lower than 1 ng/g. To allow the detection of very low concentrations in animals, the minimum weight of tissue that undergo chemical extraction should be at least 0.5g wet weight (w.w.). Depending on the species, the biopsy can include skin, blubber (marine mammals) and sometimes muscle (manta rays). To minimize the risks for the animals, the biopsy tips used are often small, so that the animals show no or very little reaction to the tissue sampling. This way, the amount of tissue to be analyzed, can be very limited. In our case with oceanic manta rays (*Mobula birostris*) from the Mexican Pacific Ocean, biopsies ranged between 0.1 and 1.1 g w.w. Once lyophilized, the weight of the dermis ranged between 2.3 and 50.7 mg dry weight (d.w.), while the muscle ranged between 0 and 120 mg d.w.

**Constraints**

Due to the small size of some biopsies, not every sample that was taken from the animals could be used for chemical analysis of plasticizers in manta tissue.

**Perspective**

One solution to the problem of low sample size might be taking a double biopsy from each animal, but in the field with free ranging animals it is not always possible. Using a bigger biopsy tip could harm the animal and lead to infections in deep tissues. Nevertheless, this is a baseline study which presents the possible ingestion of plastics by manta rays and a method which can be applied to the other species of Mobulid rays in the future.

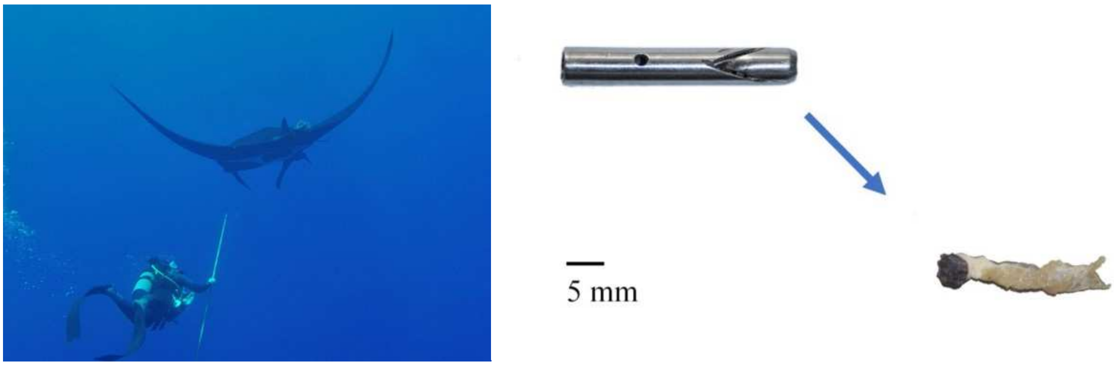


Figure 7. Non-invasive sampling of oceanic manta ray for assessing the impact of marine debris in the Mexican Pacific ocean. Left: use of hawaiian sling with modified tip for sampling during scuba diving. Right: example of biopsy taken with the modified biopsy tip that has enough tissue to undergo chemical analysis for plasticizers.

## *Micro-debris*

### Case study 7: A simple and effective method for monitoring microplastics ingested by marine vertebrates

**Specific context**

Monitoring marine litter in megafauna can be technically challenging. Although the implementation of standardized methods can be improved and developed considerably by utilizing monitoring methods already employed to understand diet and parasitology of individuals. It is therefore essential that a protocol for the separation and identification of marine litter including micro-plastics in marine vertebrates (mammals, birds, turtles) is devised which is easy to follow and adaptable depending on research infrastructure.

It is extremely hard to observe the interactions of larger marine organisms with micro-plastics. However, utilizing knowledge of common standard protocols, a protocol was devised to allow researchers to collect as much information as possible to benefit the study of marine litter ingestion, but also parasitology and diet analysis. This protocol is proposed as an operation tool for monitoring and management, which can be added to current monitoring procedures

**Characteristics of the tool**

This tool has been developed alongside routine monitoring programs of stranded animals in Ireland (Lusher et al., 2018). It is envisioned that monitoring programs of stranded or bycaught vertebrates can utilize this method alongside routine monitoring to acquire a much more in-depth knowledge of vertebrates diet and ingestion of marine litter (including macroplastics and microplastics).

Organisms which have stranded or been by caught should be monitored according to standard protocols and target tissues (stomach, intestines) collected and stored for analysis. Furthermore, pellets and regurgitates can also be collected from beaches (Lusher and Hernandez-Milian, accepted). In short, the method utilizes potassium hydroxide (KOH, 10%) as an effective way to analyse samples for microplastics. KOH is a cheap, effective and simple alkaline digestant that allows extraction of plastics from the sample matrix. Once microplastics are extracted they can be further analyzed by way of chemical characterization to which allows size, shape, color and polymer of each particles to be ascertained.

By following dissection protocols which have been optimized to reduce contamination, this protocol allows researchers to identify microplastic presence in a range of large marine organisms. We highlight that this can be used for not only the intestines of marine mammals (Lusher et al., 2015; Lusher et al., 2018), but also scats, regurgitates and pellets (Lusher & Hernandez-Milian *in press).*

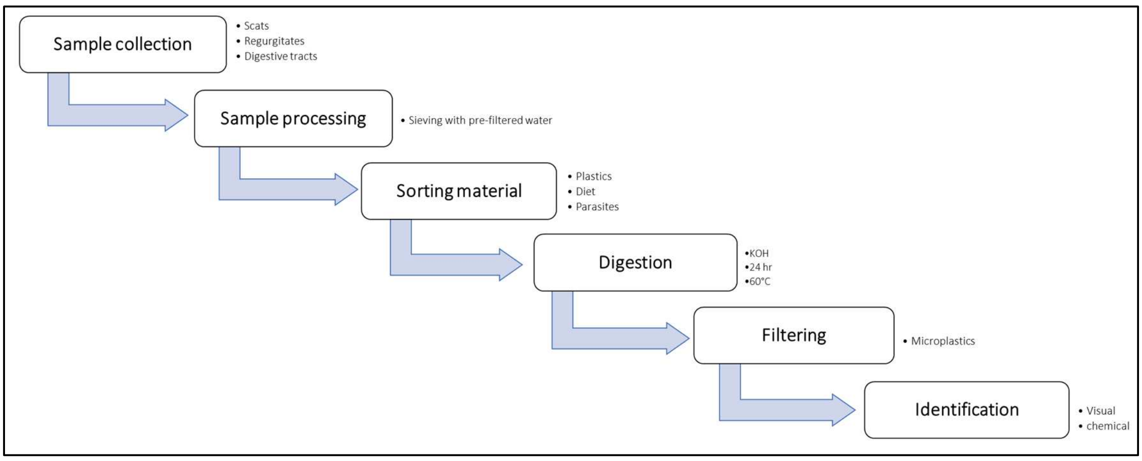


Figure 8. Steps in standardized protocol for the extraction of microplastics from marine mammal samples, including digestive tracts, scats and regurgitates (adapted from Lusher and Hernandez-Milian, *in press*)

**Constraints**

This method limits the number of constraints as the method can be simple, or complex depending on the needs of the individual or research group. Microplastic identification will require more advance analytical techniques but this method allows researchers to carry out contamination specific protocols so that microplastic samples can be analyzed at a later date.

**Perspective**

This protocol is also unique in that it allows researchers from different backgrounds, with different infrastructures to collect data that can be stored and maintained for future analysis or for distribution within the wider research community. This protocol has been harmonized with other protocols for the collection of different samples (e.g., diet, parasitology, pathology). The implementation of this protocol at different levels of economic and/or laboratory resources make information on microplastic incidence available to the entire research community.

# 3. Results and discussion

## 3.1. Tools

There are many different reasons and approaches to monitoring marine megafauna. However there is some overlap in the types of tools required for monitoring. The following are the components suggested for best practices and complementarity, regardless of the question or approach employed:

- *Training programs*, whether for capacity building in working with volunteer citizen scientists or for ensuring quality control in any data collection effort;
- *Defined scales* (both temporal and spatial) for survey efforts;
- *Data collection standards/methods* suitable to the question being asked and with validation processes for quality assurance/quality control;
- *Database* (for storage and to extract data for analysis);
- *Analytical capacity* for robust analysis to identify trends and patterns;
- *Identify and leveraging sources of funding* or *developing a framework for if/when funding lapses*.

Together, with forethought and careful consideration, these components can be used not only to establish or determine a baseline, but also to identify whether trends and patterns emerge (with continued sampling effort through time and space).

The definition of "tools" may differ depending on the socio-professional category which needs and uses them for carrying out its activities. The following items may be considered as tools for data producers and environmental policies:

T1. Observation platforms (networks/ programs) for collecting samples and data
T2. Standard procedures and storage mechanisms (databases)
T3. Defined scales (temporal and spatial) for collecting data and for evaluating status
T4. Basic knowledge
T5. Indicators with minimum constraints, for evaluating status and trends
T6. Initial assessments/ baselines/ thresholds (or norms) for interpretation, and targets values for defining precise objectives within a program of measures

The case studies (CS) presented here provide new insight regarding T1, T2 and T4 types of tools (Table 3).

In megafauna, operational monitoring through stranding networks (CS 1 & 7) have existed since a long time in many countries, especially for emblematic species such as marine mammals, birds and sea turtles. Since documenting interactions with marine debris was not their primarily focus, some of these networks need to revise database structuration in order allow specific data processing and analysis. Such opportunistic observation platforms are sometimes considered as incomplete tools for providing indicators of litter impact mainly because all specimens impacted by this pressure are not detectable (see RAC/SPA, 2017 for detailed argumentation). Aerial drones (CS 2) represent a promising tool for better detection of entanglement in seals, especially for areas difficult of access, and for reducing the cost of surveys. Other tools (CS 4 & 7) facilitate the analyzis of debris from digestive contents and excreted feces, which is currently very time consuming. However their application will be chosen according to the equipment that is available.

CS 6 provides a minimum-invasive method to indirectly characterize the impact of marine litter ingestion in live organisms instead of dead individuals. Following the threefold monitoring approach proposed by Fossi et al. (2018b), this tool allows not only the measurement of visible marine litter items extracted from the digestive tracts and feces, but also its effects on specimens, tissues, cells, and molecules.

The scale at which tools may be applied is one of the important characteristics to document before choosing which one will better fit with the scale of management measures (e.g. Regional Management Units for conservation of mobile animal population conservation, Basins and sub-regions for Regional Sea conventions Parties etc.). Four of the tools presented in this paper may be applied at the global scale (Table 3). However, while CS 1, 2 and 6 presented local application, they are already applied (stranding networks) or applicable in other areas (drones).

Constraints were identified in all case studies. Apart from the legislation, which limits sampling in protected species, methodological constraints were most frequently identified (Table 3). Avoiding disturbance may represent a constraint for sampling wild animals, and the development of non-invasive methods is crucial, like those proposed in rays by CS 6, are interesting. In opportunistic or routine surveys of bird nests (CS 5), the time of observation campaigns should be carefully chosen, to avoid disturbance of breeding colonies (see Ryan, 2018 for references). Detectability is a limitation which was cited in four CS. Difficulties in discerning the material (debris from active fishing gears) responsible for entanglement of megafauna was a constraint highlighted in CS 1, 2 and 3. For laboratory tools, detectability concerned the size of debris items (CS 4 to 7).

Even though some tools are routinely used on a local scale, authors considered that most of them require further testing and development (acquiring knowledge, methods development, wider scales) before being submitted to validation and integration in the frame of monitoring programs. Nevertheless, it has to be emphasized that policies like MSFD and UN Environment/ MEDPol program consider criteria as "candidate" or "pilot scaled" (OSPAR candidate indicator "debris ingested by sea turtles" and IMAP 24 for UN Med Action Plan, see section 3.3), with protocols evolving progressively along with the implementation of monitoring programs.

**Table 3:** Characteristics of showcased tools for characterizing and monitoring interactions between marine debris per megafauna taxon. For types of tools, refer to text. CSx) case study number. Obs= field observation (visual, photo & vidéo). Spectro= spectroscopy. B= birds, T= sea turtles, C= cetaceans, S= seals, F=fish. Entangl.= entanglement ; ingest.=ingestion (micro or/and macrodebris). Geogr.= geographic ; develop= development. ALDFG= abandoned, lost, or otherwise discarded fishing gear.

| Type | Case study | Data/ Samples | Modality of interaction | C | S | B | T | F | Geogr. scale | Stage of develop. | Perspective *(constraints)* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T1 | Stranding/photo-ID network (CS1) | Obs | Entangl. | | | | | | local (survey) | routine | revise database structure (litter) *(method : discerning ALDFG from active gear)* |
| T2 | Drones (CS2) | Obs | Entangl. | | | | | | local (colonies) | experimental | disseminating protocol *(method: detectability, flight endurance, possible legislation)* |
| T4 | State of art/ feasibility study (CS3) | Knowledge (local/general averages and tendencies) | Entangl. | | | | | | global | to be developed | propose a standard methodology, acquire data and confront with expert knowledge *(language, time, data accessibility)* |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T2 | Characterization of litter (visual+spectro.) (CS4) | Tissue, Digestive Content | Ingest. (macro.) | | | | | | global | routine | collaboration for increasing data and species ; harmonization *(legislation, method :debris > 1mm only)* |
| T1 & T2 | Land based survey (beach/nest) (CS5) | Digestive content | Ingest. (macro.) | | | | | | local (survey) | routine | associating to toxicology *(methods: small items undetectable ; legislation)* |
| T2 | Detection of plasticizers in tissues (CS6) | Tissue | Ingest. (micro. & macro.) | | | | | | global | experimental | extend to other Mobulids *(method: size of tissue samples)* |
| T2 | Chemical digestion of microplastics (CS7) | Digestive content | Ingestion (micro.) | | | | | | global | proposed routine | possible delayed analysis (*method)* |

## 3.2. Knowledge and integrated approach for conserving species and environment

Through this session at the 6IMDC we provided new information on the impact of debris on megafauna, and about tools which may help to describe and monitor trends per time. CS 5 provides information about the impact of litter in two sea bird species in remote areas and the possibility to assess debris in nests from non-permanent surveys (Clipperton). O'Hanlon et al (*in press*) mentions that only thirteen quantitative studies on nest incorporation of debris are published, which covered seven (2%) of the world's 361 seabird species, across eight countries, with the majority focused on single species, colonies and years, and with various metrics which lead these authors to recommend a standard methodology for acquiring knowledge.

Acquiring knowledge in order to draw an initial assessment and support the development of indicators is currently considered of major importance in the frame of species and environmental policies. To do this we need to better identify the general context, further advance the work related to the bio-indicator species with a particular focus on ingestion and entanglement, develop and harmonize the monitoring protocols and strategies and well define the related constraints (i.e. biological, methodological, environmental, logistic, conservation and regulatory) (RAC/SPA, 2017). This process requires several years, significant resources and the engagement and input of experts. It will contribute to the aforementioned process in the Mediterranean basin, which will feed and support the development of policy strategies and related action plans.

In Europe, MSFD illustrates the interest of adopting such an integrated approach for addressing the marine litter issue. This approach integrates i) basic knowledge, ii) representative indicators, iii) monitoring program, iv) program of measures (see for example Markus et al. (2011), for MSFD descriptor "marine litter" and Santos & Pierce (2015) for descriptor "biodiversity"). In support, several European Commission and national calls for technical and research projects have been launched in order to acquire the basic knowledge and develop tools. CS 3 was performed under the framework of such a project (INDICIT) which was selected by European Commission and in the frame of a national study, in order to develop indicators of impact of marine litter on biota, in particular Sea Turtles. The main goal of INDICIT is to develop the indicator of marine litter ingestion for the Loggerhead sea turtle *Caretta caretta*, which is considered as an indicator species of impact of marine litter in the subregion Mediterranean by MSFD, and as a candidate indicator by UN Environment- MEDPOL and OSPAR. The InterReg Med Project Plastic Busters MPA is another example of project operated in support to marine policies mainly focused on the impact on biodiversity and in particular on endangered species. Both projects contribute to the integrated approach in compiling existing knowledge, disseminating procedures at European and Regional Sea Conventions levels, and reinforcing capacities (workshops and training sessions).

## 3.3. Strategies and action plans

Marine debris is an issue of concern at the global level with proven negative effect at the ecological, biological and socio-economic levels. This has resulted for marine litter to be highly ranked in the global, regional and international policy agendas and led UN Environment to adopt two Sustainable Development Goals (SDGs) (i.e. 14.1 & 14.2; UN Environment, 2018). The policy approaches, of which some are legally binding, are mostly considering the establishment of different regional and global governance instruments and strategies. The Regional/Action Plans on marine litter that are in place, or are under review and development, around the world (i.e. Wider Caribbean, North-East Atlantic, Mediterranean Sea, Baltic, Black Sea, ROPME Sea, East Asian Seas, North West Pacific, and Pacific) offer a unique tool for coordinated response to combat the different aspects of marine litter and also offering solutions for its effective management (Markus et al., 2011; UN Environment, 2017). The effect of marine litter on biota and the related work for the identification of bio-indicator species to effectively assess the effect, is included in most of the regional/action plans.

The Mediterranean is a region highly affected by marine litter. The densely populated coastline, the numerous big coastal metropolitan cities, the significant maritime and fisheries activities, the contribution of rivers coupled with the hydrodynamics and the geomorphology including the low water flux through Gibraltar strait, comprise a unique combination of factors supporting the generation and accumulation of marine litter around the region.

To deal with this problem, the United Nations (UN) Environment/Mediterranean Action Plan (MAP) Barcelona Convention adopted the first ever legally binding Regional Plan on Marine Litter Management in the Mediterranean (Decision IG.21/7 adopted by the COP 19).

One of the steps identified in the Regional Plan was linked to the implementation of the Integrated Monitoring and Assessment Program of the Mediterranean Sea and Coasts and Related Assessment Criteria (IMAP) and its 10$^{th}$ Ecological Objective (EO10: Marine Litter. This is partly based on the Candidate Indicator 24 "*Trends in the amount of litter ingested by or entangling marine organisms focusing on selected mammals, marine birds, and marine turtles*". Currently, UN Environment/MAP and its Specially Protected Areas Regional Activity Centre (SPA/RAC), in the framework of the EU-funded *Marine Litter MED* project are working to improve knowledge on the impact of marine litter on marine fauna (UN Environment/MAP SPA/RAC, 2017). The overall aim is to develop the IMAP Candidate Indicator 24 and at a later stage to be integrated into the national monitoring programs of the Mediterranean countries. The development of the IMAP Candidate Indicator 24 is based on a strategy comprising of the following steps:

- Improve knowledge on the most representative species to be used for IMAP Candidate Indicator 24, through a regional consultation process;
- Develop a specific protocol with regards to the establishment of a wide-basin monitoring of ingested marine litter by sea turtles;
- Assess available data to propose Good Environmental Status (GES) and related targets for IMAP Candidate Indicator 24;
- Develop an operational strategy for monitoring of IMAP Candidate Indicator 24; and
- Support the creation, or improve the existing, Mediterranean network of rescue centers.

### 3.4. Cooperation and clarity

70 participants from different professional horizons (policymakers, scientists, conservationists) attended the session « tools and constraints in monitoring interactions between litter and megafauna ».

Some links were settled between the speakers and audience, however further connections and exchanges remain to be developed, which could be facilitated by a mandate or a decision at regional or global level. Several regional or global technical groups dealing with marine litter exist, such as the European Technical Group "Marine Litter" (TG ML), or the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) which act as advisory bodies, for respectively the European Commission and the United Nations (UN). Over the last few years, work on marine litter has been moving towards more coordination at the global scale, and discussion platforms have been created at Regional seas levels, and under the political agenda of both G7 since 2015[3], or G20 since 2017[4]. Our session, and the need for more information and tools regarding the impact of marine litter on biota, is one of the steps which could lead to the creation of a group of experts dedicated to the more specific issue of impact on megafauna for advising policy makers and managers in choosing potential indicator species and criteria, helping determine suitable and standardized methods for monitoring and assessments, identifying high risk areas and litter responsible for harm.

The continuous exchange between policy makers/stakeholders and experts, most of the latter being scientists, is essential while sometime challenging, to a certain extent because of their respective differences in terminology, goals, and agendas. This could be considered as a specific constraint itself. In Europe, consortiums applying to EU calls dedicated to MSFD such as INDICIT (see section 2.3) are particularly vigilant of this constraint when choosing the most representative advisory board members and coordinating with other projects working on similar or complementary topics.

### 3.5. Standardization of procedures

Our paper presents several procedures at different stages of development for describing the impact of marine litter on megafauna (Table 3). The tools which are used routinely are good candidates for becoming standard protocols, however they have to be proposed and validated in the frame of a structured processes.

Standardization is a key issue in the perspective of settling common approaches and monitoring programs in the field of managing marine litter and its impact on vulnerable species. This issue was addressed at the international level by the Regional Seas Program (RSP) of UN Environment and the UNESCO Intergovernmental Oceanographic Commission of United Nations Educational, Scientific and Cultural Organization, in response to the globally increase in the number and scope of national and international marine litter investigations and assessment programs.

However the standardized operational guidelines for marine litter survey and monitoring programs prepared by a Technical Working Group (TWG) comprising of sixteen "globally spread" experts from various regions and countries of the world and published by Cheshire et al. (2009) did not consider the impact on biota at that time.

At the global scale, new guidelines are currently under preparation by the GESAMP, as presented during the 6IMDC panel discussion "developing guidelines to promote harmonized monitoring and assessment of marine litter" and by Galgani (2018) at the poster session. These guidelines to be published late 2018 by UN Environment, will cover indicators and methods related to the impact of marine litter on biota.

In Europe, Galgani et al. (2013) included in the European Commission guidelines a chapter about the impact on biota, providing procedures for monitoring the ingestion of litter on birds (*Fulmarus glacialis*) and dead and live sea turtles (*Caretta caretta*), as well as the monitoring of debris in bird nests. In birds, the indicator of impact "litter ingested by Fulmar" is considered as an Ecological Quality Objective (EcoQO) by OSPAR (Van Franeker & Law 2015) with a policy target for an ecologically acceptable level of plastic litter defined as less than 10% of stranded Fulmars in the North Sea containing more than 0.1 g of plastic in their digestive system. This tool has been validated and has been enshrined in the law of some countries. The related method for describing this interaction is considered as a reference for monitoring programs in the frame of several Regional Sea Conventions, and of scientific studies worldwide.

In sea turtles, the protocol for monitoring ingestion of litter proposed by the TG ML (Galgani et al., 2013) is currently further developed in the frame of the european technical project INDICIT (INDICIT consortium, 2018.) and although initially dedicated to the loggerhead turtle, is applicable to all sea turtle

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[3] http://www.g7italy.it/sites/default/files/documents/Communiqué%20G7%20Environment%20-%20Bologna_0.pdf

[4] https://www.g20germany.de/Content/DE/_Anlagen/G7_G20/2017-g20-marine-litter-en.html

species. A procedure for describing entanglement is also proposed, as a first experimental step towards the definition of the MSFD entanglement criteria D10C4 (EU, 2017). In order to disseminate widely the protocol and contribute to standardization of sampling, the project also aims to disseminate these procedures at European and Regional Sea Conventions level, and contribute to reinforcing capacities (workshops and training sessions). In the Mediterranean, a procedure for describing ingestion and entanglement in sea turtles is also under preparation by RAC/SPA and Litter MED project, in the frame of Barcelona convention (RAC/SPA, in prep.).

# 4. Conclusion and recommendations

While interactions between megafauna and marine litter have been reported since 1960 (see Ryan, 2015 for references), with a growing level of information about the exposure to this anthropogenic pressure (species, locations, prevalence of entanglement and ingestion, quantities ingested, material responsible for entanglement), information rarely allows comparisons due to variable methodology, and knowledge about the impact remains un-strategic and scarce.

In parallel to the development of the risk approach, which offers interesting methods for assessing this impact (see the report of the 6IMDC session "approaches to ecological and public health risk assessment from marine debris and micro-plastic exposure" in this special issue), efforts must be strengthened in the field of methodology for describing the impact of marine litter on megafauna.

Indicator species or "sentinels" have been used as a tool to communicate the health of ecosystems for decades (Zacharias and Roff, 2001), and when used correctly, they can synthesize large quantities of information on pollution, and other natural and anthropogenic changes including the impact of marine litter. This paper describes several recent studies which describe and diagnose the physical impact and toxicological stress related to litter-associated pressures in several megafauna species. These studies, originating from environments exhibiting contrasting levels of anthropogenic pressure, highlight the diversity and scale of impacts being felt by marine species and the role these organisms can play in our society as sentinels of ocean health.

Many megafauna species investigated in this paper are charismatic and iconic indicators that can serve as flagship species for marine conservation. While umbrella species are useful for directing intervention strategies, flagship species can provide a mechanism for communicating awareness and stimulating action to tackle marine plastic pollution in all the marine ecosystems (Germanov et al 2018). The case studies presented here emphasize the interest to consider megafauna species for providing information not only on marine litter but also on other anthropogenic pressures (by-catch, ship strikes etc.) when developing management plans for the conservation of ecosystems and biodiversity. They also show perspectives of knowledge acquisition, development of new tools and approaches which have to be supported by environmental managers, biologists and species conservationists, in order to find the minimum constraint and most informative approach for monitoring programs.

In this respect, the following recommendations could be considered for further development : i) create a working group of experts dedicated in the impact of marine debris (or as part as all anthropogenic pressures) on megafauna ; ii) organize multidisciplinary workshops for defining the priorities of knowledge acquisition and disseminating targeted information (description of impact of marine debris on biota, description of standard procedures for collecting data, compile and analyzing information for prioritizing impact per category of debris, identify high risk areas, working on proxies and criteria/indicators etc.); iii) create horizontal coordination mechanisms between studies, project and initiatives aiming to coordinate efforts and to maximize results.

# 5. Acknowledgements

CS1: The scientific results and conclusions, as well as any opinions expressed herein, are those of the author(s) and do not necessarily reflect the views of NOAA or the Department of Commerce. The mention of any commercial product is not meant as an endorsement by the Agency or Department.

CS 2: Australian fur seal research was performed by Phillip Island Nature Parks under ethics permit 2.2016 and research permit 100010007974. This project would like to thank the efforts of all volunteers and collaborators, in particular Ross Holmberg (Phillip Island Nature Parks) and Karina Sorrell (Monash University). The research was funded by Telematics Trust, Phillip Island Nature Parks and the Penguin Foundation. BDH was supported by CSIRO Oceans and Atmosphere.

CS 3: was performed by INDICIT consortium partners and IFREMER-MNHN, in the frame of respectively, the DGENV project 11.0661/2016/748064/SUB/ENV.C2, and the convention MNHN SJ 114-13, Contract IFREMER 13/3211941 Avenant 131941-AV1. Special thanks to Gaëlle Darmon for improving Figure 1.

CS 6: Manta rays research was performed under CONAPESCA permit #PPF/DGOPA-133/17. T. Pelamatti (TP) is grateful to all the collaborators of Pelagios Kakunjá and "Proyecto Manta del Pacifico Mexicano" who helped with the collection of biopsies, especially to Dr. Edgar Mauricio Hoyos-Padilla and Iliana Araceli Fonseca-Ponce. TP's research was funded by CONACYT and the field trips were supported by Pelagios Kakunjá, Proyecto Manta, "Ecología trófica de los tiburones en la costa occidental del Golfo de California" [Grant Number SIP 20181417] and the National Geographic Society [grant number WW-263ER-17, 2017]. The participation to the 6IMDC was supported by CICIMAR-IPN through the Fellowship SAI. TP wish to thank Dr. Felipe Galván-Magaña (Instituto Politecnico Nacional) and Dr. Lorena Rios-Mendoza (University of Wisconsin Superior) for their helpful comments regarding this manuscript. TP expresses special thanks to Mexico Liveaboard and Club Cantamar for the logistic support during sampling campaigns.

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