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Agenda item 9: Other Specific Issues

Defining the most representative species for IMAP Candidate Indicator 24

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DEFINING THE MOST REPRESENTATIVE SPECIES FOR IMAP CANDIDATE INDICATOR 24

January 2017

Photographs by:
Figure 1: (A) Cestmed, (B) Huvet, IFREMER). (C) J.Van Franeker, IMARES). (D) COB-IEO/Spain. Figure 2 : (A) M. Matiddi/Ispra, after Werner et al., 2017, (B) F Galgani / Ifremer, (C) M. P. Salinas, d'après Comenero et al., 2017, (D) Alnitak. Figure 3 : (B) Cadiou/ Bretagne vivante

This report must be referenced as follows:
Specially Protected Areas Protocol Regional Activity Centre (Barcelona Convention), 2017, Defining the most representative species for IMPA common indicator 24, by François Galgani, SPA/RAC, Tunis, 37 pp.

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LIST OF ABREVIATIONS / ACCRONYMS

GES	Good Environmental Status
MSFD	Marine Strategy Framework Directive
ICC	International Coastal Cleanup
MIO-ECSDE	Mediterranean Information Office for Environment, Culture and Sustainable Development
NGO	Non-Governmental Organisation
GAP	Global Action Programme
UN Environment	United Nations Programme for the Environment
RPML	Regional Plan on Marine Litter

Analytical summary

In the Mediterranean, marine litter pose a critical problem because of its great quantity and effects on marine fauna. To deal with this problem, the UN Environment/Mediterranean Action Plan Barcelona Convention adopted the first ever legally binding Regional Plan on Marine Litter Management in the Mediterranean (Decision IG.21/7[1]). The Regional Plan on Marine Litter (hereafter referred to as RPML), adopted by the Contracting Parties to the Barcelona Convention during their 18th Meeting in Istanbul in 2013, entered into force in 2014; envisages a series of prevention and reduction measures, including a specific work plan and implementation timetable. Its overall scope is to anticipate and reduce the effects of litter on the coasts and in the marine environment in the Mediterranean.

One of the steps identified in the Regional Plan was linked to the implementation of the Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coasts and Related Assessment Criteria (IMAP) and its 10th Ecological Objective i.e. Marine Litter, 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*".

This study's main aim is to improve knowledge on the impact of marine litter in marine fauna and also to assess the IMAP Candidate Indicator 24. This particularly involves continuing the work of selecting the most representative species to be used for the development and assessment of the IMAP Candidate Indicator 24 (deliverable 1, 4.14 of the Marine Litter MED Project).

The main results of the study can be resumed as follows:

- Marine litter affects various compartments of the marine environment and monitoring its impacts on marine organisms is of growing importance.
- Whatever temporal and spatial scale is considered, marine litter (mainly plastics) interact with a vast range of marine species. The different types of impact of marine litter on these organisms can be classified according to the modes of action such as entanglement, ingestion and transportation of species that may be colonised on them.
- Until now, no monitoring has been implemented to assess the impact of marine litter on marine organisms in the Mediterranean; but we have good scientific and technical bases to start doing so.
- On the basis of the available information, the approach that uses monitoring of the ingestion of marine litter by marine turtles is consistent and compatible with the whole set of the identified biological, methodological, environmental, logistic and ethical constraints. The target species for the IMAP Candidate Indicator 24 and also for monitoring at basin scale are the marine turtles species, which are most commonly found in the Mediterranean, i.e. *Caretta caretta. Caretta caretta* has a wide distribution throughout the Mediterranean Sea and a great deal of information is already available. The potential for developing a monitoring network corresponds to the needs expressed by the Contracting Parties.
- The use of cetaceans as indicator species can only be considered on an opportunistic basis, and at the initiative of each Contracting Party that has pre-existing stranding monitoring networks.
- Although protocols for monitoring the ingestion of marine litter by seabirds have been used for a long time in other marine regions, work is still required to identify the most representative species for developing a monitoring programme on the impact of marine litter on seabirds in the Mediterranean. A pilot monitoring programme of marine litter in cormorants' nests is recommended, on the initiative of the Contracting Parties.
- Monitoring the ingestion of micro-plastics by fishes or invertebrates presents a strong potential for developing monitoring programme on the ingestion of litter by marine organisms

[1] https://wedocs.unep.org/rest/bitstreams/8222/retrieve

in the Mediterranean. Supplementary work is however necessary to complete a rigorous protocol which eliminates any risk for contamination of the samples examined and thus of false positives due, for example, to the presence of natural fibres. For these pilot studies deeper research work should be considered with priority to common fish species with wide distribution and easily fished fish species, which are sensitive to microparticles. The selection of necto-benthic fishes, already identified as being the most affected (i.e. *Boops boops*), of important commercial interest (i.e. *Mullus sp*.), or of farmed molluscs such as the mussel *Mytilus edulis*, could facilitate the monitoring approach.

- Concerning the entrapment/entanglement of marine species, observations have so far been poorly described, which restricts the development of corresponding monitoring networks. Carrying out coordinated pilot experiments based on a strategy of improved data collection, seems to be the most suitable preliminary step before envisaging developing regional monitoring. Work should focus on the prevalence of entrapment/entanglement of Mediterranean species, the identification and mapping of risk areas (presence of active or ghost fishing gear, distribution of susceptible species, probability of encounters between susceptible species and marine litter, etc.), and the rationalization of observation procedures on the basis of existing arrangements (stranding networks, Marine Protected Areas observation networks, opportunistic analyses of diving using submersibles or ROVs/Remotely Operated Vehicles).

All the recommended approaches should permit: i) acquiring of better information to support the implementation of reduction measures, and ii) defining of an RPML-friendly monitoring strategy.

Introduction and general context

In order to implement the UN Environment/Mediterranean Action Plan, Regional Plan on Marine Litter Management in the Mediterranean and achieve Good Environmental Status (GES) for the Mediterranean Sea, the Marine Litter MED Project aims in supporting the Contracting Parties to the Barcelona Convention in the Southern Mediterranean and the neighbourhood of the EU, by stressing the five most common marine litter measures suggested by the updated National Action Plans (NAPs). One of the measures identified is linked to the implementation of the Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coasts and Related Assessment Criteria (IMAP), Ecological Objective 10 i.e. Marine litter and particularly related to 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*".

The main objective of this component of the Marine Litter MED Project is to improve knowledge and assessment for the IMAP Candidate Indicator 24; in particular to continue the work related to the identification and selection of the most representative species to develop a monitoring strategy that is best suited to the Mediterranean context.

In this context, the implementation of the Marine Litter MED project will focus on:

i. Defining the most representative species for the IMAP Candidate Indicator 24 (deliverable 1, 4.14 of the Marine Litter MED Project);
ii. Defining a specific protocol and enhancing monitoring capacities, particularly for marine turtles, to ensure that methods and data collection dovetail;
iii. Assessing the available data to suggest thresholds and targets of Good Environmental Status (GES) for the IMAP Candidate Indicator 24;
iv. Crafting an operational monitoring strategy for the IMAP Candidate Indicator 24; and
v. Setting up a Mediterranean network for the IMAP Candidate Indicator 24 to support the exchange of best practices.

The approach also takes into consideration the existence of ongoing scientific projects such as the University of Sienna's Plastic Buster Project on marine litter on a Mediterranean scale, and the INDICIT European research project (DG ENV 2017-2018), coordinated by the CNRS in France to support the harmonizing of marine litter impact monitoring in the Mediterranean.

In this context, this report aims at identifying and suggesting the most pertinent and representative species in the Mediterranean to assess the amount of marine litter ingested by marine species and measure the rates of entanglement/strangling for marine species. It will support the identification of the candidate species to developthe IMAP Candidate Indicator 24.

1. Marine litter in the Mediterranean

Litter is composed of objects and fragments of objects made or used by man, thrown away or deliberately abandoned at sea or on coasts, or litter brought down by larger or smaller rivers, water treatment plants, storms or wind. Their impact is usually chronic and linked to their persistent nature (Gregory, 2009). The most recent work (Gall and Thompson, 2015) shows about 700 species of invertebrate, fish, bird, marine turtle and cetacean that have been impacted, mainly by entrapment/entanglement and by ingestion.

Because of the diversity and complexity of sources and the means by which litter is brought down to sea, the problem is hard to handle. Moreover, the Mediterranean context is special: big human population in the coastal area, vast daily addition of plastic litter to the sea estimated at over 700 tonnes a day (UNEP/MAP, 2015), small exchange of water with the Atlantic and limited escape of litter by the Strait of Gibraltar, intense maritime traffic (about 30% of world shipping traffic) and, lastly, insufficient infrastructure to process the waste. The consequence of these peculiarities is the fact that in the Mediterranean one may find densities of marine litter that is among the greatest in the world

(Table 1), that can reach over 100,000 items per square kilometre of seafloor (UNEP/MAP, 2015a), and over 64 million particles per square kilometre in the case of floating micro-plastics (Van der Hal, 2017).

Table 1: Summary of the abundance of marinelitter in the Mediterranean (source UNEP/MAP, 2015, updated)

	MIN	MAX	NUMBER OF STUDIES	LOCATION	REFERENCES
Beaches	30/km	36000/km	13	Western, eastern and central basin, Adriatic Sea	UNEP/MAP, 2015a; Martinez *et al.* 2009
Floating litter	1,98/km2	45/km2	10	Throughout the basin	UNEP/MAP, 2015a
Sea bed litter	24/km2	120000/km2	37	Western, eastern and central basin, Adriatic Sea	Ioakeimidis *et al.*, 2014; Angiolillo *et al.*, 2015
Floating micro-plastic (average per study)	115000/km2	1518 340/km2*	9	Throughout the basin	UNEP/MAP, 2015a, Van den Hal *et al.* 2017
Micro-plastic on beaches	10/m2	920/m2	3	Spain, Greece and France	UNEP/MAP, 2015a

*Maximum per sample at 64,800,000 particles per sq.km in the eastern Mediterranean

Water circulation is the main driver of marine litter in the Mediterranean. However, the role of currents can be very complex intervening also on the distribution of species that may be affected by marine litter. The main patterns of aggregation observed are characterised by areas with high density of marine litter, that is starting to be fairly well described, as well as the phenomena of strandings, surface transport or accumulation of litter at sea (Mansui *et al*., 2014; Pham *et al*., 2014).

Marine litter in the Mediterranean can have a great variety of shapes, colours and material composition. Besides the cigarette butts found on beaches, the most common marine litter items in the Mediterranean are plastic packaging, bottles and caps, and to a lesser extent metal cans and glass bottles. The objects found indicate a predominance of land-origin waste resulting mainly from leisure/tourist activities (ARCADIS, 2014) and increase markedly during and after the tourist season. However, out at sea and on the seabed, certain parts of the Mediterranean are more affected by fisheries related marine litter, constituting a major risk of entrapment/entanglement for marine fauna.

Marine litter can persist for long periods in the marine environment due to the slow decomposition rate, based on processes of abrasion (mechanical), heat, chemical and biological photo-degradation that can slow down at sea in the dark, and where there are low levels of oxygen. Until now, there is little data available on the degradation and standard tests are still necessary. One of the more common phenomena for degradation is fragmentation, leading to the presence of micro-plastics (smaller than 5mm), even potentially of nano-plastics less than a micron in size. Little is known about the impacts of plastic little particles on marine organisms, and the rate of entanglement of marine species in these particles seems small; however their ingestion could be the source of more substantial effects that should be taken into consideration when monitoring. So far, there is a lack of validated research methodologies and data to assess their concentration levels and the real environmental impacts, particularly related to the smaller litter particles.

Fishing gear (mesh nets, trammel nets, ghost nets, pots and traps) when damaged or used may be thrown away or abandoned by fishermen, or may broke up and thus being dispersed by storms. Some of this fishing gear may continue to catch and kill marine organisms (fishes and crustaceans, of commercial value or not, birds, mammals and marine turtles) for months, even years, until they totally degrade (UNEP/MAP, 2015b). The results of a recent regional survey carried out in 12 non-European Mediterranean countries by UNEP/MAP/MEDPOL (UNEP/MAP, 2015b) indicate that abandoned ghost nets and fishing gear are considered as a problem by 71% of fishermen, skippers and sailors, who are also aware of the environmental harm and specific impacts they engender. Although the rates of gear loss are low, the risk of impact still remains significant, with mesh net fishing being very common throughout the Mediterranean basin with over 20,000 boats involved in this kind of fishing (http://firms.fao.org/firms/fishery/761/en.).

Marine litter affects marine organisms at different levels of biological organisation, via entrapment/entanglement, ingestion, contamination, acting as a vector of species and by harming assemblages of different species (Werner *et al*., 2017). The study of these impacts should consider the different trophic levels. A summary of the literature published between 1986 and 2014 on the interactions between plastic litter and marine organisms shows that 134 species from different taxa can be affected (Deudero and Alomar, 2015). In the context of monitoring, only a few species present an interest, particularly those that are of special concern for biological reasons. Thus, marine turtles' inability to distinguish gelatinous prey from certain transparent plastic packaging, the fact that some birds can accumulate litter in the gizzard, the presence of certain species (plankton, fishes) in the upper layers of the water where micro-plastics proliferate, the high rates of filtration in species like filtering cetaceans and molluscs, or the diet of other detritus-eating species that means they ingest micro-plastics present in the sediment, are biological features that deserve special attention when selecting target species for monitoring. Similarly, the information gathered can be of interest in the field of health, such as the ingestion of micro-plastics by commercially important species consumed by humans. In the case of entanglement, species with vulnerable conservation status, at risk of extinction, or scarcity should also be considered with attention because of their heritage interest.
So far, over 80 studies have dealt with interactions between marine organisms and marine litter (mainly plastics) in the Mediterranean basin (reviewed in Deudero & Alomar, in CIESM, 2014; Galgani *et al*., 2014; Deudero and Alomar, 2015; UNEP/MAP, 2015). These studies cover a wide range of depths (0 to 850 metres down), an extended timescale (1986-2017), and identify a vast range of species affected by marine litter from invertebrates (polychaeta, ascidians, bryozoa, sponges, etc.) to fishes, reptiles and cetaceans. The effects identified in these studies concern entanglement, ingestion, and to a lesser extent colonization by and transporting of species.

2. Ingestion of litter

2.1. State of the art

It has been estimated that over 62 million marine litter items are floating around the Mediterranean (Suaria and Aliani, 2014) and can affect marine organisms by indirect effects on their health, particularly after ingestion. Moreover, certain species can also ingest litter directly from the seabed where they feed. Above and beyond the direct impact on survival, the ingestion of litter provokes sub-lethal effects linked, for example, to the proportional dwindling of natural food within the stomach, or to the ingestion of toxic substances absorbed on or directly freed by the litter when this is made of plastic (Gregory, 2009). These substances can act as endocrinal disturbers and thus affect the development and state of health of individuals (Teuten *et al*., 2009). Over 180 marine species have been listed as ingesting plastic litter, among them different species of seabird (Van Franeker *et al.*, 2011), fishes (Boerger *et al*., 2010), marine mammals (De Stefanis *et al*., 2013), and many invertebrate species including plankton species (Cole *et al*., 2013; UNEP, 2016b). It has also been remarked that all the species of Mediterranean marine turtle, listed as vulnerable or even endangered at world level (IUCN), ingest litter. Except in the case of occlusions (marine turtles, mammals etc.) or storing by some species (Procellariforms), particles that cannot be digested are usually excreted in the faecal matter by all sorts of organisms. The most serious direct effects of ingestion of litter are occlusion of the digestive tract and internal lesions from sharp objects that can result in death (Katsanevakis, 2008).

The sub-lethal effects caused by the ingestion of litter can not only affect individuals but also populations in the long term. When a large amount of litter occupies the stomach of an organism like a marine turtle, the sensation of satiety is distorted and the appetite declines. The nutritive elements, diluted in a mass almost exclusively made up of artificial matter, are not sufficient for the organism to develop and continue its vital functions. Several harmful consequences of this state of malnutrition may follow: a drop in growth rate for juveniles, lower reproductive performance for adults, a state of weakness making the individual less mobile and more vulnerable to predators, and thus a lower survival rate at both individual and population level (McCauley and Bjorndal, 1999). These sub-lethal effects of marine litter and its impacts at population level must be better understood as well as for micro-plastics (GESAMP, 2015), the absorption of great amounts of which could also have effects on energy reserves, feeding behaviour, movement, growth and reproduction (GESAMP, 2015; Sussarellu *et al*., 2015).

According to their feeding behaviour and strategy, the modalities and consequences of the absorption of litter by predator marine organisms, plankton-eaters, filterers, detritus-eaters etc. are variable. Food chains can be long or very short, as is the case for plankton-eating filtering cetaceans, which when they absorb large amounts of water filter many micro-plastics (Fossi *et al*., 2014). The organisms' litter excreting capacity has been documented by several studies recently carried out *in situ* in care centres (turtles) or in laboratories (Cole, 2013; Camedda *et al*., 2013; Van Cauwenberghe *et al.*, 2013; Darmon *et al*., 2014). The results of these works show that the average duration of retention of litter in the digestive tract varies according to species from several hours (for plankton) to several days (for filtering molluscs) and several weeks or months (for marine turtles). For turtles, the duration of retention depends on many factors (temperature of the environment, state of health, type of food, characteristics of the litter) and certain litter can be excreted whereas other litter will remain longer in the digestive tract. For mussels, it has been possible to measure the rate of retention, which is about 0.013% (Van Cauwenberghe, 2013). These bits of information encourage us to think that the risk of the litter being transferred within a food chain is less high than had been feared, due to the direct excretion capacity of litter ingested by marine organisms, and to the excretion at every trophic level of the litter ingested by predators. In the light of the present data, accumulation of litter at the end of the food chain does not appear probable; so far, in any case, it has not been demonstrated. When ingested litter is monitored, the interpretation of the data must bear in mind the potential distance travelled by an individual during the digestive transit in order to avoid any error as to the geographical origin of the litter. The issue of trophic transfer does however remain for the smallest particles, some nanometres or hundreds of nanometres in size (nano-plastics). Indeed, if these particles exist at sea, it is possible that their minute size allows them to traverse the intestinal wall and be present in the tissues of the organisms that ingested them.

3.2. Bio-indicator species of ingestion of marine litter

Marine mammals

The ingestion of litter by a large range of species of whales and dolphins is recognised (De Stephanis *et al*., 2013; Jacobsen *et al.,* 2010; NOAA, 2014). The published work concerns dead, stranded or accidentally caught animals. A recent analysis of data of strandings in the Atlantic (Pibot *et al*., 2012) on several thousand individuals (whales, dolphins) shows generally low rates of incidence of the order of one per cent. In some cases, these were animals that accidentally ingested litter when feeding on marine beds, such as, for example, the sperm whale *Physeter macrocephalus*. In the Mediterranean, a young sperm whale was found dead in 2011 off the Greek island of Mykonos with 100 plastic items in its stomach, and in March 2014 the autopsy of a sperm whale stranded in the south of Spain showed that it had ingested 59 bits of plastic. Usually, the diagnosis of the cause of death is difficult, and the ingestion of litter has only rarely been formally identified as the cause of death.

Work on the humpback whales *Megaptera novaeangliae,* rare in the Mediterranean, have shown in the digestive tract the presence of polyethylene, polypropylene, polychlorovinyl PVC, terephtalate polyethylene and nylon of size varying from 1 mm to 17 cm (Besseling *et al*., 2015). The big marine organisms that live in the Mediterranean and are relatively abundant, like the fin whales, also feed by filtering. Due to the vast amounts of water filtered with every mouthful (about 70,000 litres of water for the fin whale *Balaenoptera physalus*), these organisms could be exposed to risks caused by the ingestion and degradation of micro-plastics. Indeed this is what is suggested by the presence of plastic additives (e.g. phtalates) in the tissues of stranded animals and in skin samples taken by biopsy from animals at sea (Fossi *et al*., 2012).

Despite these observations, it seems difficult to integrate marine mammals as indicator species for pollution by marine macro-litter as part of a regional monitoring. Monitoring the ingestion of litter by cetaceans is difficult because of the small number and heterogeneous distribution of the stranded animals, as well as the logistic difficulties linked to the size of some species. In the case of seals, the Mediterranean populations are extremely localised and very scarce, which restricts the potential for monitoring these species and acquiring sufficient data for regional long-term monitoring.

Birds

Birds are the species most studied as regards ingestion of litter. In some regions, over 50% of the species ingest litter (NOAA, 2014). Some species are abundant and present high rates of ingestion, which makes them interesting *a priori* candidates to be indicators for monitoring. There do however exist a great diversity of behaviours and the choice of suitable indicator species integrates many criteria. The most important of these criteria are the geographical distribution of these species and their mobility. It is important to take this feature into account since their sometimes migratory movements can limit the significance of the data measured. The Procellaria (albatrosses, fulmars, puffins) have a special habit of keeping part of the ingested debris in their gizzards and are probably more affected by marine litter due to obstructions and ulcerations that can follow prolonged retention of these foreign elements (NOAA, 2014; Van Franeker *et al*., 2011). These species mainly feed out at sea, on the surface, and constitute good indicators since they more significantly reflect the state of pollution of the sea than those that also feed on land (e.g. gulls). The litter ingested by seabirds are micro-plastics, also meso-plastics (of between 5 and 25 mm in size). Although in appearance the ingestion of litter by birds can not be a problem for managers, work has however shown that the amounts ingested can be high in proportion to the bird’s size (about 0.6 g per bird weighing on average 1 kilo in the case of the *Fulmarus glacialis* fulmars of the North Sea) (Van Franeker *et al*., 2011), and the physiological state of these birds is weakened.

In the Mediterranean, work has unfortunately been restricted to some rare studies. Except for a video observation of the ingestion of plastics in the Aegean Sea by a falcon (*Falco eleonorae*, Steen *et al*., 2016), a single study has gone into the ingestion of plastics by seabirds in this region (Codina *et al*., 2013). The results of the work done on 171 individuals of 9 species of bird accidentally caught by longlines in the western Mediterranean between 2003 and 2010 show very significant differences in rates of ingestion, without a difference in the features of the plastic ingested or between the sexes. The puffins *Calonectris diomedea*, *Puffinus yelkouan* and *Puffinus mauretanicus* present the highest occurrence of litter ingestion (70-94% of individuals according to species) and the greatest number of tiny particles of plastic per affected bird. Yet these species have a restricted distribution in the Mediterranean. The other species, like the Audouin’s gull and the yellow legged gull (*Ichthyaetus audouinii*, *Larus michahellis*), labbes (*Catharacta skua*), and northern gannets (*Morus bassanus*) are less affected (10-33%). The kittiwake (*Rissa tridactylus),* with an ingestion rate of nearly 50%, represents a locally interesting target species but its distribution in the Mediterranean remains fairly restricted.

Marine turtles

All the species of marine turtle ingest litter; plastic constitutes the main type of litter ingested (NOAA, 2014). According to Norton (2005), turtles’ long life expectancy and late sexual maturity (25-35 years for loggerheads) mean that these animals are extremely vulnerable to human impacts.

In the Mediterranean, the loggerhead turtle (*Caretta caretta*) is the most abundant of marine chelonians (Casale and Margaritoulis, 2010). Among the litter ingested by the species are plastic bags that it mistakes for jellyfish and other transparent gelatinous prey when feeding in neritic habitats and out at sea. The loggerhead is very sensitive to marine litter and one of the most studied species of marine turtle in the Mediterranean. Although the species is able to ingest all sorts of litter (Figure 1), plastic objects are more often ingested (Lazar and Graçan, 2011; Campani *et al*., 2013; Camedda *et al*., 2014; Darmon *et al*., 2016). In France, for autopsies of loggerheads having ingested litter, Darmon *et al*. (2016) showed that in 80% of cases the litter was plastics, and less than 2% of ingested litter was paper, metal or glass. No difference is observed between the litter found in stranded marine turtles when autopsied and that excreted by animals kept in care centres (Camedda *et al*., 2014), for an analysis shows homogeneity as regards total abundance, weight and composition of the litter whether the animals are living or dead.

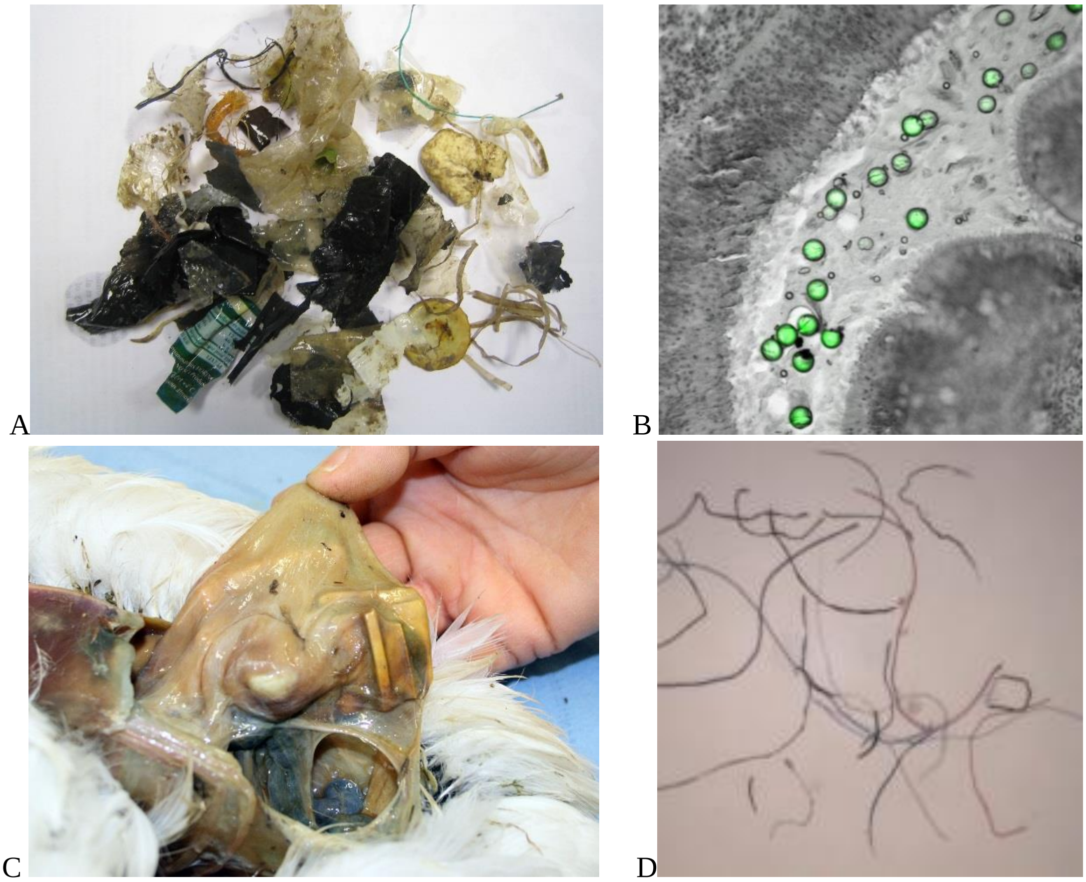


Figure 1: Litter ingested by Mediterranean marine organisms. (A) Litter excreted by a turtle in a care centre in the French Mediterranean (species Caretta caretta, care centre CESTMed, credit CESTMed). (B) Micro-plastics highlighted by fluorescence during an experimental study of the (in vitro) transit of micro-plastics in the digestive gland of an oyster (Crassostrea gigas) (credit A. Huvet, IFREMER). (C) Litter ingested by a North Sea northern fulmar (Fulmaris glacialis, credit J. Van Franeker, IMARES). (D) Fibres ingested by an individual of the fish species Boops boops sampled in the Balearic Islands (credit COB-IEO/Spain).

The effects of the presence of litter in the digestive tract may be direct or indirect, shorter or longer term.

Fragments of plastic and other human-origin material can be directly responsible for the death of marine turtles by occlusion of the digestive tract or by lesions in the digestive mucus membrane (UNEP/MAP, 2015a) when the volume and nature of the litter are such as to block all transit and/or perforate the digestive wall. In the long term, as described in Paragraph 3.1, the presence of litter ingested by turtles can have a certain number of consequences for their health, i.e. growth, capacity to move about, and this can have repercussions on their migratory aptitude and chances of escaping from prey, and lastly on reproduction. Teuten *et al*. (2009) estimate that the long period of retention of plastic litter in the digestive tract can provoke the liberation of toxic chemical substances (e.g. phtalates, PCBs) that can act as endocrinal disturbers and thus compromise the health of individuals and their various endocrinal functions. Thus we can grasp to what extent ingestion of litter can affect

both individual survival and that of populations of marine turtle, whose conservation state is already highly precarious due to other human-origin and natural threats.
Marine turtle species have ways of life that vary according to the different phases of their development. So they can frequent different fields where they feed on epipelagic or benthic prey in areas that are oceanic and neritic. In the early phase of life, marine turtles are probably essentially inactive, and on the surface, but in the adult phase, according to species, they can exploit the seabed and the water column to feed (Casale *et al*., 2012; Lazar and Graçan, 2012). It has been remarked that certain adult loggerheads were faithful to their neritic feeding areas that can be those where they were recruited in the juvenile phase (Casale *et al*., 2012); they are thus likely to ingest litter in different types of habitat over the course of their lives. Passage from the pelagic phase to the neritic phase happens in the Mediterranean when the curved length of the shell is about 40 cm (Darmon *et al.*, 2014). Although certain studies have reported that young oceanic turtles are more likely to ingest litter than big turtles, most of the results obtained in the Mediterranean have shown that the adult loggerheads presented higher amounts of marine litter than juveniles (Campani *et al*., 2013). Adult individuals are able to distinguish colours to find food, but adults and young alike ingest plastic matter 'taken' on the surface or sub-surface of the sea. The loggerhead turtle *Caretta caretta* presents a great tolerance for ingestion of human-origin litter and the species is usually able to excrete these objects (Casale *et al*., 2012; Frick *et al*., 2009). Camedda *et al*. (2014) remarked that although marine turtles excreted human-origin litter in the faeces after over one month of hospitalisation, most of the litter was expelled in the first two weeks. Studies on the duration of transit of the substances in the gastro-intestinal tract of loggerhead turtles have shown that the materials (like polyethylene globes) are expelled in about ten days (review in Darmon *et al.*, 2014). Consequently, the authors conclude that given the average distance covered in ten days by *C. caretta*, the litter excreted in pools during hospitalisation is likely to have been ingested at a distance of less than 120 km (Camedda *et al*. 2014).

The populations of marine turtles studied in the Mediterranean are more affected by ingestion of litter than those in other parts of the world (review in Darmon *et al.*, 2014). Table 2 presents ingestion rates measured in marine turtles in various parts of the Mediterranean. For the autopsied marine turtles, the cases of ingestion of marine litter recorded from the Adriatic Sea (35.2%) seem to be less frequent that in the western (79.7% for Spain) and central (51.1% for Lampedusa) Mediterranean. However, these figures may be biased underestimations, for the number of samples for some studies is relatively low, and, the analysis dating from several years back in certain areas, it is possible that the figures have changed since, and are higher (Table 2). In France, for example, a rise in ingestion rate was observed over time; indeed, analysis done as part of the DCSMM in 2016 revealed ingestion rates that were rising compared to those done earlier (Table 2 and Darmon *et al.*, 2014). Between 2003 and 2008, 35% of autopsied turtles presented litter in their digestive tract, whereas between 2013 and 2016 the figure for the rate of ingestion was 76%.

Table 2: Rate of ingestion of litter by marine turtles (mainly C. caretta) in the Mediterranean (UNEP/MAP 2015a, modified after Darmon et al., 2014). nd=non determined; nc=non communicated

Zone	**Date**	**Size (cm)**	**Nb morts**	**With Litter (%)**	**Nb* litter**	**With litter (%)**	**Total**	**With litter (%)**	**References**
Sardaigne	2008-2012	21-73	30	20	91	12	121	14,04	Camedda *et al.*, 2013
Toscane	2010-2011	29-73	31	71	_	_	31	71	Campani *et al.*, 2013
Adriatique	2001-2004	25-79	54	35,2	_	_	54	35,2	Lazar & Graçan, 2011
Turquie	2001	_	_	_	_	_	65**	5	Kaska *et al.*, 2004
Espagne	nd	34-69	54	79,6	_	_	54	79,6	Tomas *et al.*, 2012 in UNEP/MAP, 2015a
Lampedusa	2001-	25-80	47	51,5	33	44,7	79	48,1	Casale *et al.*, 2008

	2005								
Malte	1988	20-69	_	_	99	20,2	99	20,2	Grammentz, 1988, in UNEP/MAP, 2015a
France	2003-2008	nc	20	35	225	0,02	245	0,04	Claro & Hubert, 2011
France	2011-2012	nc	2	0	54	20,4	56	19,6	Dell'Amico & Gambaiani, 2012; UNEP/MAP, 2015a
France	2013-2016	25-65	23	75,6	36	41,7	59	54	Darmon & Miaud, 2016
Baléares	2002-2004	36-57	19	37,5	_	_	19	37,5	Revelles *et al.*, 2007
Linosa	2006-2007	26,7-69	_	_	_	_	32	93,5	Botteon *et al.*, 2012, in UNEP/MAP, 2015a
Italie/Espagne	2001-2011	_	_	_	155	50	155	50	Casini *et al.*, 2012, in UNEP/MAP, 2015a

***Observations and care centres; ** Both living and deadFishes**

The ingestion of litter by fishes is less well described than for birds or marine turtles. Plankton-eating fishes feed in areas where both prey and plastics are common. Boerger *et al*. (2010) have shown that the nature of the litter ingested by fishes corresponds in 35% of cases to the litter present in their feeding areas. Furthermore, Carson *et al*. (2013) showed that predator fishes can also confuse litter with prey, especially if the objects are long and blue. Rates of ingestion can be over 50% for individuals, and such rates are observed even in areas where there is not much litter, like the deeper parts of the Mediterranean (Anastasopoulou *et al*., 2013). In the case of micro-plastics, it seems that omnivorous species present higher rates of ingestion than herbivores or carnivores (Mizraji *et al*., 2017). Fishes however do seem more selective than turtles or plankton and rates of ingestion seem to be linked to feeding behaviour, the aggregation of litter and distribution constraints (currents, advection), although this must be confirmed. It also seems that the larger the size of the fish, the greater the selectivity of litter and the less passive or accidental the ingestion. Sharks are among the species of fish affected by litter. But only a low occurrence, under 0.5% of 15,600 individuals of 14 species studied in the global ocean (Gregory, 2009), is found. The litter ingested is mainly fragments of plastic and objects from fishing.

In the Mediterranean, the impact of litter on fishes varies considerably according to the ecological compartments they exploit. Rates of ingestion also vary according to species (Table 3). Typically, *Boops boops*, the Myctophidans, *Schedophilus ovalis* and *Naucrates ductor* are among the most affected species (Deudero and Alomar, 2014; Nadal *et al*., 2016). But the possibility of sampling these species is not homogeneous in the monitoring context. Recently, Teresa *et al*. (2015) showed that the Mediterranean bluefin tuna (*Thunnus thynnus*) and swordfish (*Xyphias gladius*) were also affected and ingested micro- (<5 mm) and meso-plastics (5 to 25 mm) as well as, in over 18% of samples, bigger plastics (>25 mm). Similarly, sharks can ingest macrolitter (NOAA, 2014) and even micro-plastics in the case of basking sharks (*Cetorhinus maximus*) that feed by filtering (Fossi *et al*., 2014).

Table 3: Ingestion prevalence of macro and micro-litter in individuals of various fish species present in the Mediterranean according to studies published between 1998 and 2016.

SPECIES	HABITAT	PREVALENCE (%)	REFERENCES
Balistes carolinensis	nectobenthic	14	Deudero, 1998
Boops boops	nectobenthic	29	Deudero and Alomar, 2014
Cetorhinus maximus	pelagic	83	Fossi *et al.*, 2014
Coryphaena hippurus	pelagic	6, 7	Deudero, 1998; Massuti, 1998
Etmopterus spinax	benthic	6, 8	Madurell, 2003; Anastasopoulou *et al.*, 2013
Galeus melastomus	nectobenthic	3, 13	Madurell, 2003; Anastasopoulou *et al.*, 2013
Helicolenus galepterus	nectobenthic	2	Madurell, 2003
Mullus sp. (Portugal)	nectobenthic	64-100	Neves *et al.*, 2015
Myctophum punctatum	pelagic	100	Collignon *et al.*, 2012
Naucrates ductor	pelagic	18	Deudero, 1998
Polyprion americanus	pelagic	55	Deudero, 1998
Pteroplatytrygon violacea	nectobenthic	50	Anastasopoulou *et al.*, 2013
Sardina pilchardus	pelagic	0-19	Neves *et al.*, 2015; Avio *et al.*, 2015;
Schedophilus ovalis	pelagic	50	Deudero, 1998
Seriola dumerilii	pelagic	2	Deudero, 1998
Squalus blainville	pelagic	1	Deudero, 1998
Thunnus alalunga	pelagic	32,4	Romeo *et al.*, 2015
Thunnus thynnus	pelagic	12,9	Romeo *et al.*, 2015
Trachurus sp.	nectobenthic	1	Anastasopoulou *et al.*, 2013
Xyphias gladius	pelagic	12,5	Romeo *et al.*, 2015

A study, using a large sample of individuals, was done in the Atlantic on species of fishes that were also found in the Mediterranean (Neves *et al*., 2015). The results confirm the interest as indicator species of *Boop boops* (9% of 32 individuals affected) and to a lesser extent *Trachurus trachurus* (7% out of 44 individuals). Moreover, the species *Scomber sp*. (31% of individuals affected), *Scyliorhinus sp*. (12% out of 17 individuals affected) and *Trigla lyra* (19% of individuals affected) present significant rates of ingestion, highlighting their potential as indicator with a view to monitoring. Among the widely sampled species, some like the sardine (*Sardina pilchardus*) present very variable ingestion rates.

More recently, Bella *et al*. (2016) measure the rates of ingestion of micro-plastics by demersal species in the Atlantic and the Mediterranean. Out of 212 individuals belonging to the three species *Scyliorhinus canicula* (spotted dog fish), *Merluccius merluccius* (hake) *and Mullus barbatus* (striped mullet) the prevalences are respectively 15.3%, 16.7%, and 18.8%; the size of the micro-plastics ingested varies from 0.38 to 3.1 mm. These species, regularly used as bio-indicators, could be the subject of a data collection based on commercial and/or scientific fishing.

Work by Neves *et al*. (2015) shows a prevalence of litter ingestion of 64 to 100% according to species of the genus *Mullus sp*. Similarly, work done in the laboratory shows that a species like *Dicentrarchus labrax* is likely to ingest litter (Peda *et al*., 2016), but no study has been done *in situ* on this species. Because of their commercial interest and their wide Mediterranean distribution, the two last species deserve more attention with a view to monitoring.

Invertebrates

Micro-plastics are ingested actively or passively. Fishes and certain invertebrates seem to actively ingest micro-plastics because they fail to distinguish them from plankton or food. Laboratory work (Cole *et al*., 2013) has shown that active catch was, however, rare for plankton organisms, for these in fact ingest particles of micro-plastic passively, as do filtering cetaceans.

Most of the impacts from litter on invertebrates have been demonstrated in the laboratory, sometimes with high doses that do not automatically correspond to concentrations found in the natural environment.

Various studies have highlighted litter ingestion for several taxa of benthic invertebrates like annelids (Arenicola), molluscs (Mytilidae, Ostreidae, Veneridae, Pectinidae), crustaceans and echinoderms (GESAMP, 2015; Wesch *et al.,* 2016). The data is scarcer concerning high sea and surface species, but ingestion was also observed for jellyfish (Paradinas, 2016) and some crustaceans (copepods Calanideae, Euphausiaceae). Generally speaking, the sedentary species that feed on detritus or filter food (*Mytilus galloprovincialis*, sea cucumbers, *Talitrus saltator*) are more exposed than others to the ingestion of litter. These present, therefore, a certain interest for a better grasp of the harm suffered by invertebrate species by ingestion of litter. The high filtration rates can typically explain why we see high rates of ingestion of micro-plastics in these species. Thus, in the case of *M. galloprovincialis*, amounts of micro-plastics ranging from 0.04 to 0.34 particles per individual have been observed in the Mediterranean (Van Cauwenberghe *et al*., 2015; Vandemeersh *et al*., 2015). Similarly, species of commercial interest like oysters or mussels are important because they enable us to measure rates of ingestion on farmed species and assess the risks for human consumption. In the laboratory, the size of the micro-particles ingested by molluscs is of the order of 80 µm, but it is much lower in the natural environment (Wesch *et al.,* 2016). When the micro-particles are bits of polystyrene, a rise in energy expenditure can be seen (Van Cauwenberghe *et al*., 2015). For these species, and for copepods, it was also observed that in strong concentrations the ingested micro-particles affect fertility and feeding (Wegner *et al*., 2012; Cole, 2013; Sussarellu *et al*., 2015).

Sensitivity to the ingestion of micro-plastics is high in the case of scavengers that ingest a lot of sand particles (Graham and Thompson, 2009). The ingestion of micro-plastics was also demonstrated for different carnivores that are sometimes present in the Mediterranean, such as crabs (review by Wesch *et al.*, 2016), the shrimp *Crangon crangon* (Cole *et al*., 2013; Devriese *et al*., 2015) and the langoustine *Nephrops norvegicus* (Murray and Cowie, 2011). Despite these studies suggesting a trophic transfer in the laboratory (Farrell and Nelson, 2013), this mechanism remains hypothetical in the natural environment.

3.3. Monitoring the ingestion of marine litter by marine organisms

Monitoring the ingestion of litter is a complex task, with ever more important stakes, partly because of the ever-growing quantity of waste at sea, and partly because recent results show that a large number of species is affected, including by micro-plastics.

Identifying interactions between marine litter and fauna depends to a great extent on data collection methods. Most of the data on fishes, turtles and cetaceans is provided by analysis of the digestive contents of stranded or accidentally caught individuals, but this reflects only a small part of the real interactions that may occur. The rate of interaction between marine organisms and marine litter and the impact on populations of marine species is hard to be quantified. Generally speaking, in the literature, one finds percentages of animals that ingested marine litter compared to the number of specimens that could be autopsied, but still remains an unknown proportion of dead marine animals that cannot be taken into account (death at sea, being eaten by predators, advanced state of decomposition of a carcass, etc.). Thus, there is an urgent need for new methods to be developed to assess, in an unbiased way, the death rates and the effects on the dynamics of populations of the affected species. The existing approaches and the setting up of monitoring networks are subject to a certain number of constraints that are biological, methodological, environmental, logistic and ethical.

Biological constraints

The choice of a good target or indicator species is a major element when developing a monitoring strategy. This choice depends on various factors that can be extremely constrictive:

a) The chosen species must have a wide distribution to enable a comparison to be made between sites on a large scale. In order to facilitate interpretation of the results of the monitoring, a species with wide Mediterranean distribution is thus necessary.
b) The species must be sensitive to litter and ingest significant and sufficient amounts of it for measurements to be comparable. Low rates of ingestion and small amounts of ingested litter make sampling and counting difficult.
c) Ingestion and impact mechanisms must be known. It is, for example, important to possess basic knowledge such as duration of intestinal transit, nature of ingested objects, etc., to enable a rational interpretation of the results and an optimization of protocols. Interpreting data collected only on individuals that are found dead or have been placed in a care centre (turtles) can constitute bias. Indeed, these turtles are stranded because of an acute or chronic pathology, linked or not to direct or indirect interaction with litter (disorders of the pica-appetite in animals that have been suffering for a long time).

Table 4 sums up the main constraints recently identified with a view to a pertinent definition of Good Environmental Status (GES) for marine turtles (Claro *et al*., 2014).

Table 4: Pertinent biological constraints and parameters for defining Good Environmental Status (GES), concerning the ingestion of litter by marine turtles (adapted from Claro et al., 2014)

Parameter/ Constraint	Pertinence	Considerations	Possible bias	Need for knowledge
Sex	?	Possible differences of feeding diet according to sex and reproductive phase (before or after egg-laying, etc.)	Not much known on the influence of sex at the level of ingestion of litter (2 studies)	Yes
Size, development phase, population of origin	Yes	The level of ingestion depends on the mode of feeding which is linked to size, itself dependent on the individual's origin (Atlantic, Mediterranean)	According to the structure and origin of the sampled population	Yes
Habitat	Yes	According to development phase, habitat and resources available, the individuals feed in a neritic or pelagic habitat (or both) with variable levels of concentration of litter	The value of the indicator is affected by the habitat exploited by the turtles sampled in a given region	Yes
State of health	Yes	Possible differences of ingestion between individuals that died suddenly (collision, by-catch) and stranded individuals	If the animals were ill for a long time before the stranding they may have excreted all or part of the ingested litter, whereas animals that died suddenly had not the time for this	Yes
Capacity for	No	Ingestion is subject to the	Possible error of	Characterisation

moving/duration of digestive transit		amount of litter present in the living areas or those crossed during migrations. The movements of the turtles (speed, distance travelled) and the duration of intestinal transit are not constant .	interpretation if the scale for measuring the impact of litter by ingestion is not correct	needed of biological distribution areas and migrations

In an in-depth study on the whole set of published work based on analysis of dead and living stranded specimens, Casale *et al*. (2016) argue that rates of ingestion are subject to many factors such as area of origin, date of stranding, state of health or duration of residence in captivity. In these conditions, the authors suggest that the aggregation of data engenders a loss of homogeneity of data that must be taken into account. For these authors, monitoring should only consider individuals that lived in natural conditions (feeding, etc.) in order to facilitate the interpretation of the results and trends.

d) Other basic data must be available to make clearer the sensitivity of species and the conditions of interpretation of the measurements, like for example the 'ingestion/age' or 'ingestion/size' relationship, sensitivity at different stages of development, etc.
e) The movements (if these exist) of the animals (particularly migratory species) must be limited for the spatial scale of the measurements to be precisely grasped.
f) The target elements of the sampling must be clearly defined and pertinent. In the case of small species that ingest micro-plastics, for example, these elements can be the whole animal, the entire digestive organ, or elements of the digestive structure (oesophagus, stomach, intestine, etc.).
g) Taking excreta into account can be a good strategy, especially for animals kept in care centres or pools.
h) Scientific information must be accessible and accepted/recognised by the scientific community.

Methodological constraints

The choice of suitable protocols depends on several constraints:

a) The availability of dependable protocols is essential.
b) Developing inter-calibrated protocols can take years and this limits the development of harmonized monitoring. It is necessary to possess protocols that have been referenced, tested, compared and validated by the community of specialists.
c) The existence of bias in measurement must stop the use of a protocol. The example of micro-plastics is important here. There are many studies that show very variable results according to the size of the particles considered. In some samples, organic non-human-origin natural fibres have been confused with micro-plastics because of the impossibility of confirming the plastic nature of certain little particles, or because of the possible contamination of samples by fibres during packaging (GESAMP, 2016). These works show the limits of the development or validation of protocols suited to measuring micro-plastics ingested by various species. Only the taking into account of big particles that can be chemically characterised should be considered in the current context of knowledge.
d) Conservation procedures (freezing, fixing, eliminating the organic elements in the samples, etc.) must not be destructive to the plastics.
e) Common banking of the data according to recognised and validated procedures must be organised.
f) Reproducibility and representatively must be guaranteed by adopting standard operational procedures with quality assurance. Generally speaking, these standard approaches are not very well developed for harmonizing the monitoring of ingestion. Similarly, reference documents and methodological guides are not yet sufficiently widely circulated and used.
g) Standardization, the final stage when developing a protocol, is an aim in the context of monitoring the indicator EI 18.

In a recent analysis of work published between 1949 and 2015 on the ingestion of litter by macrofauna (Provencher *et al*., 2017), the importance of standardization of methods was noted. Although the number of studies differs according to the target species considered, the metrics used are common. Frequency of observation of ingestion, called frequency percentage or prevalence, is the most commonly used approach. For all groups, the number of ingested objects and their mass are also used, with a recent tendency to assess average values of density or weight for litter ingested. Colour and size of objects are however less considered. For these authors, necropsies on stranded animals, collected for other work, found dead and accidentally caught are the most frequent methods of collection. For turtles, retention in a care centre is a significant source of data.

Environmental constraints

a) The data must be representative of the state of the environment and of Good Environmental Status (GES).
b) The significance of the results is important. It must be possible to establish a diagnosis for deaths, pathologies and the physiological state of the affected individuals to avoid merely counting ingested litter without information on the lethal or sub-lethal effects associated.
c) The results must enable areas to be categorized according to their level of pollution.
d) The results must allow different types of objective to be met according to the type of litter. Thus, categorizing litter and the choice of an indicator species will differ according to the size of the litter in which we are interested (micro-plastics or macrolitter) and according to its nature (plastic, metal, etc.). This constraint is particularly important in the perspective of defining measurements to be taken on a particular kind of litter in the context of reduction measures envisaged by the Regional Plan on Marine Litter. In this case, the strategy will aim at choosing a suitable target species (turtles/plastic wrappings; filterers/micro-plastics, etc.).

Logistic constraints

The logistic aspects and existing infrastructures must not be neglected, for to a great extent the development of monitoring depends on them.

a) The cost: deep sea sampling of species with a narrow distribution can be very expensive. Although the data obtained during the monitoring can be of scientific interest, the perpetuating of data collection can only be envisaged if its cost is reasonable and the sampling conditions the simplest possible.
b) An opportunistic approach using existing monitoring networks can be an attractive alternative. For example, the systematic sampling of fish stocks associated with a regular analysis of the stomach contents of species of commercial interest offers an attractive opportunity for monitoring the ingestions of micro-plastics by marine species. In the same way, the existence of strandings networks and structured observation, where samples on dead specimens of turtles, birds or cetaceans are collected in a simple, routine way, constitutes a favourable opportunity for monitoring litter ingested by marine fauna.
c) Accessibility is an important constraint for monitoring, and the choice of a very accessible species can prove judicious. It is preferable to encourage sampling in an area very much affected by litter, and/or with a lot of species that are litter-sensitive. Sampling on beaches, for example, appears to be a simple approach, either for monitoring the ingestion of litter by species that get stranded, or monitoring the effects of micro-plastics for species that depend on this environment (Ugolini *et al*., 2013).
d) In the case of marine turtles, the existence of care centres makes available living individuals that are the subject of in-depth veterinary analysis (radiology etc.) and excreta of litter that can be analysed. This is a complementary approach to data collection from dead animals.
e) At logistics level, the existence of good practice and common approaches must encourage the comparability and harmonization of results.

Conservation and regulatory constraints

The interest of monitoring can coincide with managers' conservation objectives and must not be neglected.

a) It is perhaps interesting in the context of continuous monitoring on a Mediterranean scale to considered the ingestion of litter by rare species, even with a narrow distribution and with small numbers (the monk seal *Monachus monachus,* for example). In fact, opportunistic analysis of dead individuals can bring useful data to monitor population trends over time and be representative of a specific sub-region. In these conditions, the monitoring modes must be adapted (duration, assessment of trends) and considered over a very long term.
b) The protection status of the species must be examined before including them in a monitoring programme. In the case of protected species, sampling by the destruction of individuals is prohibited and intervention on living specimens (including autopsies) may or may not be the subject of exemptions, according to the regulatory provisions taken at national level.

3.4. Selecting approaches and species for monitoring ingestion

In the present state of knowledge, and if only the Mediterranean is being considered, it is recommended to choose different approaches according to species, compartment of the marine environment, or nature of litter considered.

- On the basis of accessible expertise and available information, the approach that uses the monitoring of litter ingestion by marine turtles is consistent and compatible with the whole set of existing constraints. It also corresponds to the approach recently chosen in the southern OSPAR zone. In the Mediterranean, the target species is the most common species of marine turtle, i.e. *Caretta caretta*, with its wide distribution around the Mediterranean Sea and for which a lot of information and certain monitoring structures are already available. The potential for developing a monitoring network corresponds to the needs expressed by the Contracting Parties. Also, it is a good idea to use *Caretta caretta* as the most representative specie to monitor in the framework of the the Candidate Indicator 24 concerning litter ingestion for a basin-wide monitoring.

- The use of stranded cetaceans can only be envisaged on an opportunistic basis and at the initiative of each Contracting Party that possesses existing networks for monitoring stranded animals. These indicator species cannot be adopted as part of a voluntary monitoring approach throughout the Mediterranean basin because of the low number of stranded organisms, the small rates of litter ingestion recorded so far, and the impossibility of keeping the wounded animals in care centres.

- There exist protocols suited to the monitoring of ingestion by birds. Since these protocols are being used in the North Sea (Van Franeker *et al*., 2011) on the species of this northern region, work is still necessary for development in the Mediterranean. The indicator species that are abundant on a Mediterranean scale for which protocols could be implemented remain to be identified before any consideration of these species with a view to monitoring.

- Monitoring the ingestion of micro-plastics by fishes or invertebrates presents a big potential for the development of monitoring of litter ingestion in the Mediterranean. This requires, however, supplementary work to perfect a rigorous protocol that will eliminate the risks of contamination and false positives, such as the presence of natural fibres. The existing monitoring infrastructures should encourage the development of networks and take advantage of the regular campaigns to analyse stomach contents that are already in place in certain countries bordering on the Sea, or again of the existence of networks for measuring chemical contamination using mussels ('mussel watch'). These arrangements could provide the necessary samples for a regular and organised monitoring of ingested micro-plastics. At this stage of development, we shall encourage the implementing of complementary work to rationalise a method of measuring litter that is suitable and standardized. For pilot studies or in-depth research work, common species with a wide distribution, that are easily fished and are sensitive to micro-plastics, must be given priority. Among these species can be mentioned the most affected necto-benthic fishes (*Boops boops*) or those that present an important commercial interest (*Mullus sp.*, *Trigla sp.*, *Dicentrarchus labrax*) and the pelagic species *Scomber sp*. The mussel *Mytilus galloprovincialis*, a benthic species that feeds on food in suspension, a bio-indicator traditionally used for monitoring, must be the subject of complementary studies so that scientific and technical bases are provided for it to be used as part of an operational monitoring.

In conclusion, the search for other species must not be neglected, but their application to monitoring must go through the diverse stages of validation.

Moreover, a particular specific need of one or many of the Contracting Parties can lead to more specific strategic choices. To give an example, the choice to monitor the impact of litter in deep sea environments will require the choice of suited species. In this case, existing programmes of trawling for demersal species would be a suitable solution.

4. Entanglement, strangling

4.1. State of the art

In 2015, 340 original works were published recording the interactions between organisms and marine litter corresponding to entanglement (Gall and Thompson, 2015). Birds represented nearly 35% of entangled species, followed by fishes (27%), invertebrates (20%), mammals (almost 13%) and reptiles (nearly 5%). Among the species described, pinnipeds and marine turtles were the species on which the occurrence of impacts was the highest (NOAA, 2014), the latter being on the beaches during the egg-laying period.

According to the UNEP (2016), entanglement incidents lead to wounds or death, with a declining order of species affected per taxon, for 192 species of invertebrate, 89 species of fish, 83 species of bird, 38 species of mammal and all species of turtle (7).

Dolphins and other cetaceans are often caught by the neck and fins when they get tangled up in marine litter (Kuhn *et al.*, 2015). More generally for the cetaceans, the factors that may contribute to organisms being entangled in or strangled by abandoned fishing gear or litter include 1) the presence of organisms in or near the nets, 2) water turbidity, making the litter and gear less visible, 3) ambient noise in the marine environment that can hide or distort the echoes produced by fishing gear, and 4) the ability to detect nets by echolocation. Furthermore, the lack of experience of juvenile or immature individuals can make them vulnerable to being caught in mesh nets.

In certain cases, entanglement can lead to deformation (constriction of part of the body for growing individuals, for example; Gregory, 2009). Birds are caught by the beak, wings or claws, which restricts their agility, and their ability to fly and to feed. Some species, particularly sharks, also very sensitive to this type of impact (NOAA, 2014), can no longer open their jaws.

Benthic organisms can also be caught in traps or objects on the seabed. Typically, crabs, octopus, fishes and many small invertebrates are taken in traps on the seabed and die of stress, wounds, or prolonged fasting (review in Kuhn *et al*., 2015).

Abandoned mono-filament fishing lines are perhaps the most dangerous kind of litter, for they can represent up to 45% of entanglements observed (http://www.monachus-guardian.org/mguard21/2121covsto.htm). Indeed, abandoned fishing gear, including fishing lines, nets, *orins* and traps and pots for crab/lobster/fishes represent 72% of all observations of entanglement. Lost gear can have an impact on the environment in many different ways, including i) the continuing catch of target species, ii) the catching of non-target fishes and crustaceans, and of all other species, iii) the entanglement of turtles, mammals, seabirds and fishes in lost nets and litter, and iv) the physical impact of gear on the benthic environment (Ayaz *et al*., 2006; MacFayden *et al*., 2009). Factors that complicate the analysis of entanglement data were described in the FANTARED Project (mentioned in UNEP/MAP, 2005a; Table 5).

Table 5: Factors influencing the analysis of trends of entanglement of fauna at sea (adapted 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 dying 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
	Little data available before the 1980s

In the Mediterranean there is a general lack of data. Entanglement has been described for cetaceans, pinnipeds, marine turtles, birds, fishes including sharks and for many invertebrates (Galgani *et al.*, 1996; UNEP/MAP, 2005a; Cedrian, 2008; Rodriguez *et al.*, 2013; Bo *et al.*, 2014; Tubau *et al.*, 2015; Colmenero *et al.*, 2017). As recent work has shown, lost gear or litter in general can also harm benthic organisms and habitats, including deep sea Mediterranean species like sponges, gorgonians, or certain cold water corals (Pham *et al.*, 2014; Fabri *et al.*, 2014).

A) B) C) D)

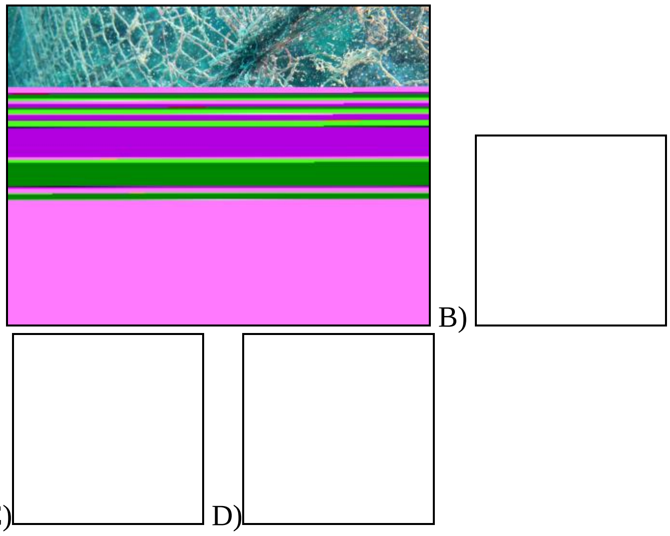


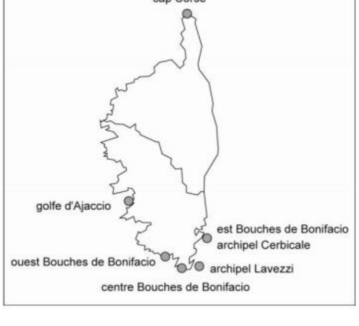
Figure 2: Strangling/entanglement of marine organisms. (A) A Paramuricea sp. gorgonian entangled in a ghost fishing net (credit M. Matiddi, after Werner et al., 2017); (B) An urchin covered with two plastic sheets (credit F. Galgani, unpublished); (C) A Prionace glauca shark strangled by a plastic ring (credit M. P. Salinas, after Colmenero et al., 2017); (D) Turtle entangled in a lost net (credit Alnitak)

The incidence of entanglement can vary strongly according to region and other factors. A study done by Rodriguez *et al*. (2013) on the northern gannets (*Morus bassanus*) showed a different incidence between the Atlantic and the western Mediterranean according to the fishing activities with which these birds interacted, and also according to age, the immature birds seeming to be more sensitive than the adults.

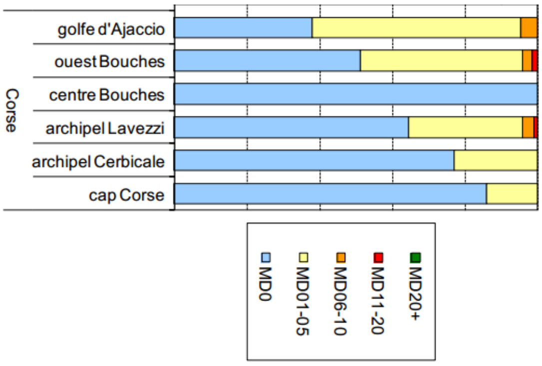
Fasting is one of the frequent consequences of entanglement, as well as the impossibility of moving and thus escaping from predators; it also leads to wounds and secondarily to infections and sometimes amputation when a prolonged constriction prevents the blood supply from reaching the limbs (NOAA, 2014).
Certain marine organisms, when caught in active fishing gear (cordage, nets and lines) can tear it off and attempt to free themselves and so continue to move with bits of gear around their bodies. They can thus carry these bits of gear over considerable distances. It is in this case not easy for the observer to make out whether the animal is entangled in an existing bit of litter or in an initially active piece of fishing gear.

Monitoring the relative impacts of strangling must enable the impact of litter to be distinguished from that of active nets. Current difficulties of data interpretation, the relatively small number of stranded animals currently recorded and the problems associated with wide-scale risk assessment because of the rarity of strandings, clearly indicate that this approach can only be reasonably applied to particular species that can be very locally affected, particularly in areas of intense fishing activity, or strong presence of litter, or abundance of sensitive species (i.e. turtles' egg-laying areas, or protected areas with high diversity (MFSD TSGML, 2013; UNEP/MAP, 2015a).

Scientific research can contribute to the crafting of new, more specific, indicators of entanglement. Work by Votier *et al.* (2011), for example, has led to the currently ongoing crafting of master guidelines for monitoring litter present in the nests of seabirds as a source of entanglement for fledglings, it being impossible that such litter comes from active fishing gear (Van Franeker, *personal contribution*). Even if additional research work is needed to make more clear the reproductive seasons and the types of litter brought to the nests by seabirds, and the description of the behaviour that leads to this phenomenon, species like the Mediterranean shag (*Phalacrocorax aristotelis*) are promising as indicator species for Mediterranean monitoring (Figure 3). This species is very common throughout the Sea and nests in the coastal areas of most of the Mediterranean countries. The approach consisting of recording data on litter brought back to their nests by seabirds is routinely used in many sites all over the world, especially in protected areas. In the Mediterranean, this approach is still experimental but presents a strong potential for setting up future monitoring in the framework of the UN Environment/Mediterranean Action Plan, Regional Plan on Marine Litter Management in the Mediterranean.

A



B



C

Figure 3: Percentage of nests containing litter and average number of bits of litter observed in the nests of Mediterranean shags (Phalacrocorax aristotelis) in Corsica (Work of the Bonifacio Reserve, the Corsican Conservatory of Natural Species, and IFREMER and Bretagne Vivante: Cadiou et al., 2016). The results show the possibility of distinguishing the areas subject to human-origin impact from the areas least affected by pollution. (A) Sites of observations; (B) Nest of Mediterranean shag (credit B. Cadiou) occupied by two fledglings; the litter, here mainly cordage, has been used just like natural materials to build the nest; (C) Number of bits of litter per nest (N=556 sampled nests). Each horizontal histogram represents for each site 100% of nests and each colour the percentage of nests (vertical graduation – 20%) with a variable number of bits of litter (MD) (0.1 to 5.6 to 10.11 to 20 and over 20).

4.2. Monitoring the entanglement/strangling of marine organisms by marine litter

As indicated above, although the monitoring of ingested litter can be envisaged on solid scientific and technical bases, that of entanglement in and strangling by marine litter demands an in-depth analysis of the existing work, to this day greatly insufficient in the Mediterranean, and requires envisaging substantial development work before an optimal strategy is defined.
Monitoring ingested litter is based on monitoring indicator species, whereas monitoring entanglement and strangling, based on arrangements that are very often species selective, must consider several zoological groups (cetaceans, birds, reptiles, fishes, and invertebrates) and be organised by compartments. Observations of various entangled species and specimens can indeed be recorded at the level 1) of beaches via strandings networks, 2) of the surface during oceanographic campaigns, and 3) of the seabed, thanks to underwater means of observation like divers for shallow areas, or submersibles/ROVs (Remotely Operated Vehicles) for deep water areas.

According to the arrangements used, the observations will concern dead organisms, as in the case of most of the strandings, or living organisms out at sea and on the seabed. This last approach is important, for out at sea and on the seabed the dead animals decompose quickly and disappear. It is also important for monitoring the impact of abandoned fishing nets, which constitute a special category of marine litter on the bed. Generally speaking, entanglement/strangling out at sea and on the beaches are found on big organisms, mainly mammals and marine turtles. On the seabed, the potential to use invertebrates as an entanglement indicator is interesting because of the possibility of significant observations at all depths, including the benthic level.

In the present stage of development of thinking on the subject, it appears to be necessary to identify the constraints inherent in a possible monitoring of the entanglement/strangling of fauna by marine litter.

Biological constraints
The biological constraints on monitoring entanglement/strangling integrate several elements:

- The choice of a number of species to monitor: this can involve a small number of target species or every one of the species listed exhaustively

- The life cycle of the different species (behaviour linked to reproduction, to development phase/to the size and associated feeding behaviour, to sex, to migration, etc.); a complex cycle can induce a great variability of sensitivities according to the various phases of the species and thus a strong variability of results
- The probability of encounters between species and litter. An analysis of the risks as defined in recent work on the loggerhead turtle, for example (Darmon *et al.,* 2016) can help, by locating risk areas, in defining priority monitoring areas
- Knowledge of the prevalence or rate of entanglement (proportion of entangled individuals in a sample) is an important preliminary to defining a monitoring programme. High rates, representing a real risk for populations, must be a priority criterion in the decision-making
- From the veterinary point of view, a knowledge of pathologies to be able to describe exactly the impact of the entanglement of marine animals in litter (wounds, strangulation, amputation etc.) and criteria for diagnosis are essential
- Some basic knowledge on the biology of species likely to be the subject of the monitoring must exist and be available.

Methodological aspects
As regards the method used, a certain number of elements are needed to set up a monitoring:

- Organising data collection
- Improving and developing protocols; the protocols currently available are few in number or badly described, or to be developed, whether this involves monitoring by diving, by ROV or submersible, or from the stranding of marine organisms
- Criteria that allow entanglement/strangling due to litter to be distinguished from active fishing gear. This would enable a correct interpretation to be made of data from the point of view of Good Environmental Status (GES). The current absence of criteria is a source of great bias in the case of monitoring carried out by strandings networks
- Identification of factors that can interfere with the results, particularly the possible loss of information due, for example, to the movement of living individuals after entanglement, or the speed at which their tissues decompose at sea when the animals are dead
- Correct knowledge of the seasonal variations in the presence of litter (fishing activity, tourist season) and species (migration) to be taken into consideration when organising data collection.

Once these elements have been acquired, it will be suitable to adopt, on a Mediterranean scale, common, harmonized protocols that are accompanied by quality assurance and banking arrangements and procedures that will guarantee optimal monitoring.

Environmental aspects
At environment level, and from the point of view of GES, the significance and representatively of entanglement/strangling as a pollution indicator have not yet been confirmed. It is necessary for scientists to test already available sets of data before envisaging this kind of monitoring.

Logistic aspects
At logistics level, aspects linked to i) the cost of the monitoring, ii) the accessibility of samples and data, iii) the prior existence of permanent or seasonal data collection arrangements (strandings networks, campaign for observation and monitoring by diving, etc.) are essential and must be widely taken into account. Continuous monitoring of entanglement/strangling by existing strandings networks would for example enable us to break free from the constraints related to seasonal variations. Sharing pre-existing campaigns of observation by diving, to which an additional monitoring objective is assigned (entanglement), would lessen monitoring costs and guarantee accessibility to data and samples that would be less random than that based on the unpredictable nature of events such as strandings.

To sum up, existing data on the strangling and entanglement of marine species is still too ill listed and insufficient for making analyses concerning impact analyses and justifying the development of permanent monitoring networks. The strategy recommended at this stage is to organise and structure complementary data collection and to do pilot experiments in a coordinated way that will enable scientific and technical bases for monitoring this kind of interaction to be defined, and modes of monitoring that suit the Mediterranean context specified. The work should focus on: the prevalence of entanglement/strangling of Mediterranean species, the identifying and mapping of risk areas (presence of fishing gear, distribution of sensitive species, probability of encounters between sensitive species and litter, etc.) and the rationalising of existing data collection arrangements and procedures (strandings networks, networks for observing Marine Protected Areas, campaigns of diving in submersibles or ROVs (Remotely Operated Vehicles). The entire approach should give rise to better information in support of the measures to reduce marine litter that will be implemented in the future and permit the defining of a monitoring strategy that is suited to the Regional Plan on Marine Litter (RPML).

5. Other impacts

Some marine organisms use litter for shelter, for hanging onto, and for settling in. Since much of the litter is mobile and moves around with the currents, it in fact constitutes a means of transport that helps these organisms move to new territories. This kind of dispersion, now well known on a world scale, presents a problem that is particularly acute in that a recent increase in floating particles, mainly plastics, has been noted. Thus, in the Mediterranean, the some 250 billion bits of micro-plastic measured on the surface and floating in the Sea (Collignon *et al*., 2012) could all be potential carriers of harmful alien species and 'invasive' species. As described by Katsanevakis *et al*. (in CIESM, 2014), the organisms that litter can carry represent all taxonomic groups, such as unicellular organisms, filtering (polychaetes, bryozoa, hydras, and balans) organisms, detritus-eaters (crustaceans), molluscs, echinoderms and algae, whose distribution is affected by many factors such as motion, nature and roughness of substratum, temperature, salinity, abundance of plankton, and concentration of plastics (Carson *et al*., 2013). Floating litter can help carry species outside their natural distribution borders. This role is less well known in the Mediterranean Sea, with the result that marine litter has not so far been included as a potential vector of introduction of alien species in the latest assessments of primary pathways of introduction (Katsanevakis *et al*., 2013). In terms of impact, the diversity of the mechanisms that preside over the transporting of species by litter makes it difficult to carry out regular monitoring. Despite all this, as indicated by CIESM (2014), thirteen species alien to the Mediterranean are known to colonise floating litter elsewhere in the world.

Moreover, for these authors, over 80% of alien species known in the Mediterranean could have been introduced by colonising marine litter or could potentially use litter to extend their geographical distribution (secondary invasion).

At depth, litter potentially provides substrata and new habitats to marine organisms with the result that they can influence the distribution of benthic species (Pham *et al*., 2014).

In both cases, an inventory of species that are fixed onto litter in the Mediterranean or a monitoring of populations that are attached to litter could constitute indicators of impact on biodiversity.

But structuring a monitoring network for these species still lacks scientific and technical bases, and developing operational monitoring must be the subject of much research work before being envisaged. Taking this type of approach into consideration would however make sense in the context of monitoring impacts on fishing, fish farming, tourism, water purification, or the diversity of protected species, particularly in that pathogenic germs can potentially be among the species that are likely to be carried and dispersed by marine litter.

Among the species which use litter as shelter are cephalopods (octopuses). This observation is very common in the Mediterranean, and the phenomenon could be the subject of research work to determine the effects it could have on ecosystem equilibrium, and the potential of these species for developing original impact indicators. Such an approach can only be envisaged within the boundaries of the interpretation of effects and in the wider context of Good Environmental Status (GES).

6. Conclusions, recommandations and prospects

Monitoring the impacts of marine litter on marine fauna depends strongly on the availability of indicator species to measure the prevalence and effects of ingestion of litter and entanglement/strangling in litter. Monitoring these effects can be designed within a multi-species approach in order to cover the field of impacts linked to both the diverse types of litter, of varied size (micro-particles and macro-litter) and nature (plastics, metal, glass, etc.), and also with the varied ways of life (sedentary, benthic, necto-benthic, pelagic, aerial) and feeding (detritus-eaters, suspension-eaters, omnivores, carnivores) of the species that interact with it. The multiplicity of approaches needed to take this variability into account thus requires the use of many target species, and this is only possible if infrastructures crafted using diverse skills are in place. In the present state of our knowledge, monitoring can only be done gradually, stage by stage, according to the degree of maturity of the indicators. For a first stage, it is recommended that a pilot monitoring network be developed based on the use of the *Caretta caretta* marine turtle species, the indicator of ingestion of litter by this species being at the most advanced stage of development.

It seems reasonable to also envisage starting experimental work to test the potential of new indicator species, mainly to measure the impact of micro-plastics, in particular certain species of fish that have a high rate of ingestion and wide distribution (*Boops boops*, *Mullus sp*.) and invertebrates, particularly the mussel *Mytilus galloprovincialis*, present throughout a vast part of the Mediterranean Basin. Table 6 lists the species/taxa already used, or that could be used, as bio-indicators, and their potential for use in the context of monitoring.

Table 6: Selection of indicator species for monitoring ingestion of litter by marine organisms in the Mediterranean

Taxon	Type of litter	Method	Infrastructure	Indicative Species	Priority	Remarks
Birds	macro-litter	Autopsy	Strandings networks,by-catch	To be researched	+	Work needed in the Mediterranean
Cetaceans	macro-litter	Autopsy	Strandings networks,by-catch	All species	+	Small number of species, low rate of ingestion, only opportunistic approach
Cetaceans	micro-plastics	Autopsy / chemical	Strandings networks,by-catch	All species	+	Sampling and measuring difficult
Marine turtles	macro-litter	Autopsy / excreta monitoring	Strandings networks,by-catch , care centres	*Caretta caretta*	+++	Necessity of mastering biological parameters
Necto-benthic fishes	micro-plastics	Stomach contents	Coastal fishing and trawling	*Mullus sp., Boops sp.*	++	Wide distribution of species, easily caught
Demersal fishes	macro-litter	Stomach contents	Scientific and commercial trawling	*Scyliorhinus sp.*	+	Opportunistic collection possible
Pelagic fishes	micro-plastics	Stomach contents	Commercial fishing		+	Opportunistic collection possible
Molluscs	micro-plastics	Stomach contents / chemical	Collection, farming, chemical monitoring networks	*Mytilus sp.*	++	Existing collection networks, concerning public health
Crustacean	micro-plastics	Stomach contents / chemical	Collection		+	Work needed in the Mediterranean

Other invertebrate s	micro-plastics	Stomach contents / chemical	Collection	Holothuries	+	Work needed in the Mediterranean

Concerning entanglement/strangling, it is still necessary, in the present conditions, to organise the collection of information and to define monitoring modes (Table 7). The mobilising of strandings networks must be considered as a priority by the Contracting Parties on a voluntary basis at first for experimental monitoring of entanglement/strangling of the main most sensitive species (mammals, birds, turtles).

Table 7: Monitoring arrangements and indicator species to be tested for monitoring entanglement/strangling in the Mediterranean

ESPECES	TYPES OF LITTER	METHOD	EXISTING NETWORK	SPECIES	PRIORITY	REMARKS
Birds	Fishing gear, macro-litter	Observations , diagnosis	Strandings networks	All species	++	The monitoring must be organised per system with the following priorities: 1) Pilot study concerning opportunistic monitoring by strandings networks 2) Evaluation and tests of video/diving monitoring systems in protected areas 3) Surface observation test
Cetaceans	Lost nets, ghost nets	Observations , diagnosis	Strandings networks and at-sea observation	All species	++	
Turtles	Lost nets, ghost nets	Video monitoring (diving and ROVs)	Strandings networks and at-sea observation	All species	++	
Necto-benthic fishes	Fishing gear	Video monitoring (diving and ROVs)	Video monitoring (diving and ROVs)	All species	+	
Pelagic fishes	Lost nets, surface ghost nets	Observations , fishing	networks of sea observation	Big pelagic sharks	+	
Invertebrates	Lost nets, macro-litter	Video monitoring (diving and ROVs)	Protected area monitoring, scientific campaign	All species	+	
Birds	Meso-/macro-litter	Observation, litter in nests	Nesting monitoring networks	European Shag	++	Indicator of effect partially concerning strangling To be tested on a pilot scale

The potential of monitoring litter in nests must be re-examined by experts in order to propose master guidelines; to this effect, an experimental monitoring should be set up, particularly in the Mediterranean protected areas and on the basis of voluntary action by the Contracting Parties.

As part of future development, we recommend that the potential of surface and underwater observation campaigns (Table 6) be assessed. The interest of shallow diving, especially in Marine Protected Areas, and using submersibles or ROVs (Remotely Operated Vehicles) for greater depths as tools for collecting observations on entanglement/strangling of the most affected species (invertebrates and fishes) must be assessed. This last approach (submersibles/ROVs) should not be dissociated from operations of inventorying or reducing abandoned fishing gear/nets in areas defined as priority areas in the context of the MAP's Regional Action Plan.

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Annexe I **ABREVIATIONS**

GES	Good Environmental Status
MSFD	Marine Strategy Framework Directive
ICC	International Coastal Cleanup
MIO-ECSDE	Mediterranean Information Office for Environment, Culture and Sustainable Development
NGO	Non-Governmental Organisation
GAP	Global Action Programme
UN Environment	United Nations Programme for the Environment
RPML	Regional Plan on Marine Litter