CleanAtlantic

Tackling Marine Litter in the Atlantic Area

WP 5.3: Indicators for ingestion and entanglement

DELIVERABLE 5.3. Strategy and constraints to support monitoring of Marine Litter Harm: Towards a protocol for the observation of marine organisms entangled/strangled/covered by marine litter during ROV operations

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In the North East Atlantic, marine litter remains a critical problem because of its large quantity and effects on marine fauna. To deal with this problem, the OSPAR Regional Action Plan (RAP) adopted a plan 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. One of the steps identified in the RAP was linked to the implementation of Monitoring, including the regular monitoring of harm.

During the last couple of years, the number of species evidenced to be affected by marine litter has significantly increased, being the numbers of entanglement and ingestion cases for all species of marine megafauna recorded in a number of studies (Kühn *et al.,* 2015; Kühn and van Franeker 2020). Accordingly, marine litter affected 914 species through entanglement and/or ingestion, while Schultze and Werner (2020) indicated that 1 055 species were affected by ingestion or entanglement.

Until present, OSPAR assesses beach litter, seabed litter and plastic particles in fulmar and turtle digestive tracts indicators (Schultze and Werner, 2020). Whereas the fulmar indicator is related to the North Sea, the turtle indicator, proposed first by France within RAP action 44 and developed with the support of the European project INDICIT (https://indicit-europa.eu/), is related to the Bay of Biscay, Iberian Coast and Macaronesia (OSPAR regions IV and V).

While the approach monitoring marine litter ingestion by fulmars and marine turtles is consistent and compatible with the whole set of identified biological, methodological, environmental, logistical and ethical constraints, the use of other species as indicator species will only be considered when scientific background will be sufficient to enable regular sampling and relevant interpretation of trends and patterns. Although protocols for monitoring litter ingestion by marine species have long been implemented in other marine regions, work is still required to identify the relevant species for developing an adequate monitoring programme that effectively portraits the issues of litter ingestion by different organisms. Micro-plastic ingestion by fishes or invertebrates presents an opportunity to develop such monitoring programmes, which. OSPAR and CleanAtlantic project are currently exploring towards the development of new indicators of ingestion of plastic particles by fish.

As for entanglement in marine debris, the proportion of seabirds impacted by Marine Litter ranges from 25% (Kühn et al.,2015) to 36% (Ryan, 2018). Furthermore, according to UNEP (2016, a & b), entanglement in marine debris leads to wounds or death for a large number of other taxa, including 192 species of invertebrates and 89 species of fish. In a more recent review, entanglement was reported in 418 species from reef systems across eight taxa, also evaluating their major conservation implications (Carvalho-Souza *et al.,* 2018).

A reduction in food intake is one of the most frequent consequences of entanglement, as well as, for mobile species, limitations in movements and thus in escaping from predators (Kühn *et al.,* 2015). Entanglement also leads to wounds susceptible to secondary infections and sometimes amputation after constriction (NOAA, 2014 a & b). Although entanglement has been documented in many different types of debris, most records involve Abandoned, Lost, or otherwise Discarded Fishing Gear (ALDFG), with strong geographic variations in incidence, type and quantity of marine litter but with monofilament fishing lines being the most dangerous kind of litter, as they represent a large part of entanglement records (Consoli *et al.,* 2018).

Existing data in the North East Atlantic is inadequate, since different methodologies are used. For this reason, a specific strategy for monitoring of entanglement in marine litter must be defined. Monitoring entanglement should be organized by ecosystem compartments. Observations can be recorded at the level of the coastline (via marine organisms stranding networks) and the surface (e.g. during oceanographic campaigns or through observer programmes) (Galgani *et al.,* 2018). With respect to the methods used, a certain number of elements are needed to set up a monitoring programme. Data collection scheme, standardized protocols, and knowledge of the seasonal variations in the abundance of litter and target species are critical points that need to be taken into account (RAC/SPA, 2017). It is only recently that scientific and public attention has been focusing also on injuries to, and interactions with benthic organisms caused by anthropic litter accumulated on the seafloor.

All plastics and microplastics in the North East Atlantic could be potential carriers of harmful alien species and 'invasive' species representing all taxonomic groups, such as unicellular organisms, filtering organisms (polychaetes, bryozoa, hydras, and barnacles), detritus-eaters (crustaceans), molluscs, echinoderms and algae. In terms of impact, the diversity of mechanisms that preside over the transport of species by litter makes it difficult to carry out in regular monitoring, unless considering a database recording all species and new species rafted or settled on debris, having the potential for developing original impact indicators. This is not considered for now within OSPAR and structuring a monitoring network for these species still lacks scientific and technical bases, subject of much research work before being considered. 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.

Johnson (2008), as cited by Schultze and Werner (2020), presented a framework for biodiversity monitoring and assessment for OSPAR. He indicated that biological indicators should be scientifically sound, easily understood, variable over time, sensitive to the change that they are intended to measure, measurable and capable of being updated regularly, and based on readily available data and information.

He stated that relevant marine litter indicators should be typical of its source, relatively common in the survey area, easy to identify, easy to find, and easy to count. Similarly they should enable us to differentiate between acute and chronic effects on biota, and the spatial extension should be documented. Consideration of the costs of monitoring is desirable, as well. All the recommended approaches should also allow acquiring better information in order to support the implementation of reduction measures, also defining or updating monitoring strategies.

This report is the result of the research performed within CleanAtlantic, taking advantage of the work done previously by OSPAR, UNEP MAP (Barcelona convention), EU MSFD Technical Group on Marine Litter (TGML) and some EU projects like INDICIT (EU wide), Actions for Marine Protected Areas (AMAre -Mediterranean Sea, https://amare.interreg-med.eu), and Plastic Busters in Marine Protected Areas PBMPA -Mediterranean Sea, https://plasticbustersmpas.interreg-med.eu). The CleanAtlantic project main aim is to protect biodiversity and ecosystem services in the Atlantic Area by improving capabilities to monitor, prevent and remove (macro) marine litter. The project is also contributing to raise awareness and change attitudes among stakeholders and to improve marine litter managing systems. The objective of the present report is to review the possible strategies and constraints for monitoring the impact of marine litter in the OSPAR region and to assess the potential of a new indicator of entanglement, since observations have so far been poorly described, which restricts the development of the corresponding monitoring networks.

I.1 Ingestion of marine litter

As described by RAC/SPA (2018), the ingestion of litter by a large range of species of whales and dolphins is acknowledged (Jacobsen *et al.,* 2010; NOAA, 2014a). However, recent analysis of data from stranding of several thousand individuals (whales, dolphins) in the Atlantic shows a low incidence of litter ingestion of these animals (in the order of one per cent)(Pibot and Claro*,* 2012). In some cases, like sperm whales (*Physeter macrocephalus*), litter ingestion is likely to have happened accidentally while these animals were feeding on marine beds. Moreover, 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. On the grounds of these, it seems difficult to integrate marine mammals as indicator species for pollution by marine macro-litter as part of a regional monitoring. In addition to those facts, 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 logistical difficulties linked to the size of some species. In the case of seals, the Atlantic populations are extremely localized and very scarce, which restricts the potential for monitoring these species and acquiring sufficient data for regional long-term monitoring.

Birds are the most studied species as regards ingestion of litter. In some regions, over 50% of the species ingest litter (NOAA, 2014a). Some species are abundant and show high rates of ingestion, which makes them *a priori* interesting candidates to be indicators for monitoring. The most important limitations are the geographical distribution of these species and their mobility, since migratory movements can limit the significance of the data measured. In the Atlantic, work has unfortunately been restricted to some areas and focused on fulmars. Unlike other birds, fulmars, keep part of the ingested debris in their gizzards and are probably more affected by marine litter due to obstructions and ulcerations due to prolonged retention of these foreign elements (NOAA, 2014a; Van Franeker *et al.,* 2011). Plastic particles in fulmars is now a common Indicator for OSPAR with strong scientific evidence, research background and knowledge, available and harmonized protocols, and long time series of data to support it. Thresholds, as defined by the Ecological Quality Objective EcoQO, have been set, and in some EU member states, enshrined in the law as the Good Environmental status for MSFD

(https://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000039130954&categorieLien=id). Some other seabird species have been tested in the southern part of the OSPAR region but their relevance to monitoring is limited to local interest only (Codina-Garcia *et al.,* 2013).

All the species of marine turtle ingest litter (Kühn & van Franeker, 2020), with plastic constituting the main type of litter ingested (NOAA, 2014) and with these animals being extremely vulnerable to this type of human impact. Plastic bags are the main marine litter category ingested by sea turtle species, as they are easily mistaken for jellyfish or other gelatinous prey. The loggerhead *Caretta caretta,* for example, is very sensitive to marine litter and one of the most studied species of marine turtle. In the Mediterranean sea, loggerhead necropsies showed that in 80% of cases the litter was plastic, and in less than 2% it was paper, metal or glass, without difference observed between the litter found in stranded marine turtles when necropsied, and that excreted by animals kept in care centers (Camedda et al., 2014).

The ingestion of litter by fishes is not as well described than litter ingestion by birds or marine turtles, but a recent report by FAO has documented their presence in 220 different marine species (Lusher *et al.,* 2017).

Plankton-eating fishes feed in areas where both prey and plastics are common. Predator fishes can also confuse litter with prey, and rates of ingestion can be over 50% for some individuals (Anastasopoulou *et al*., 2013). Fishes, however, seem to be more selective than turtles or plankton. Rates of ingestion seem to be linked to feeding behaviour, aggregation of litter and distribution constraints (currents, advection). Studies using large samples of individuals (Neves *et al.,* 2015; Bella *et al.,* 2016) confirm the potential use pf *Boop boops*, *Trigla lyra* as indicator species for monitoring programmes. Among the widely sampled species, some species like the sardine (*Sardina pilchardus*) present very variable ingestion rates, making it less appropriate as a litter ingestion indicator. *Scyliorhinus canicula* (spotted dog fish), *Merluccius merluccius* (hake) and *Mullus barbatus* (striped mullet), on the other hand, have sufficient prevalence to consider them as indicators for monitoring. Finally, recent studies performed within CleanAtlantic (Gago *et al., 2020;* Maes *et al.,* 2020), describe other fish species like the lancet fish (*Alepisaurus ferox*) or sharks that could be of particular interest for monitoring marine litter ingestion and contamination levels.

Moving to micro-plastics, various studies have highlighted the ingestion by several taxa of benthic invertebrates like annelids (*Arenicola sp.*), molluscs (Mytilidae, Ostreidae, Veneridae, Pectinidae), crustaceans and echinoderms (GESAMP, 2015 & 2016; Wesch *et al.,* 2016). The data is scarce concerning pellagic species, but ingestion has also been observed in jellyfish (Paradinas, 2016) and some crustaceans (copepods Calanideae, Euphausiaceae). Generally, the sedentary species that feed on detritus or filter water for feeding (e.g. *Mytilus sp.*, sea cucumbers, *Talitrus saltator*) are more subject 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 *Mytilus sp.*, amounts of micro-plastics ranging from 0.04 to 0.34 particles per individual have been observed (Van Cauwenberghe *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). For these species, and for copepods, it was also observed that in strong concentrations the ingested micro-particles affect fertility and feeding (Cole, 2013; Sussarellu *et al*., 2016).

The ingestion of micro-plastics has also been observed in different other macroinvertebrate carnivores such as crabs (Wesch *et al.,* 2016), the shrimp *Crangon crangon* (Cole *et al*., 2013) and the lobster *Nephrops norvegicus* (Murray and Cowie, 2011). Despite some studies suggest a trophic transfer in the laboratory, most indicate its absence.

I.2 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 by marine litter ingestion, including by micro-plastics.

RAC/SPA (2018) reviewed the various constraints and strategies to monitor harm. Identifying interactions between marine litter and fauna relies, in a large extent, on data collection methods. Data is often 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, are hard to be assessed. An unquantifiable proportion of dead marine animals always remains unknown and cannot be taken into account (death at sea, being eaten by predators, advanced stage of decomposition of a carcass, etc.). Thus, there is an urgent need for relevant and unbiased strategies that enable systematic monitoring approaches. The existing approaches and the setting up of monitoring networks are subject to a certain number of biological, methodological, environmental, logistical and ethical requirements (Table 1).

Table 1: Identification of the main features and requisites to be considered for the implementation of monitoring of litter ingested by marine organisms (modified from RAC/SPA, 2018)

FEATURES & REQUISITES	
BIOLOGICAL	Target species must have a wide distribution to enable a comparison between sites on a large scale.
	The species must be sensitive to litter and ingest significant and sufficient amounts of it for measurements to be comparable.
	Harm must be understood (duration of intestinal transit, nature of ingested objects, ingestion/age or size relationship, sensitivity at different stages of development, etc.) to enable a rational interpretation of the results and an optimization of protocols.
	The movements of the animals (particularly migratory species) must be limited for the spatial relevance of measurements.
	Sampling must be clearly defined and pertinent (whole animal, entire digestive tract or parts such as stomach, intestine, etc.)
	Taking excreta into account can be a good strategy, especially for animals kept in rescue centers.
	Scientific information must be accessible and accepted/recognized by the scientific community.
METHODOLOGICAL	Relevant protocols are available
	Protocols have been referenced, tested, compared and validated by the community of specialists.
	The existence of bias in the measurement (natural fibers, contamination during the processing of samples) must stop the use of a protocol (example of low sized microplastics).
	Conservation procedures (freezing, fixing, eliminating the organic elements in the samples, etc.) must not be destructive to the plastics.
	Data is collected according to recognized and validated procedures.
	Reproducibility and representatively must be guaranteed by adopting standard operational procedures with quality assurance and common methodological guides.
	Standardization must be reached for regular monitoring.
ENVIRONMENTAL	The data must be representative of the state of the environment and of Good Environmental Status (GES).
	It must be possible to establish a diagnosis for deaths, pathologies and the physiological state of the affected individuals.
	The results must enable areas to be categorized according to their level of pollution
	The results must allow different types of objective to be met according to the type of litter (specific measurement on a particular type of litter).

LOGISTICAL	Regular monitoring can only be envisaged if its cost is reasonable and the sampling conditions the simplest possible.
	Opportunistic approach using existing monitoring networks can be an attractive alternative.
	Accessibility is an important requisite for monitoring, and the choice of a very accessible species is important.
	The existence of rescue centers makes available living individuals for in-deep veterinary analysis.
	The existence of good logistical practices and common approaches must encourage the comparability and harmonization of results.
ETHICAL	It is not relevant to consider the ingestion of litter by rare species, even with a narrow distribution and with small numbers of individuals.
	The protection status of the species must be examined before including them in a monitoring programme. Sampling by destruction of protected species is prohibited.

With the current knowledge, 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 relying on the monitoring of litter ingestion by fulmars and marine turtles is consistent and compatible with the whole set of existing requirements. In the North East Atlantic, the two most common species of marine turtle, i.e. *Caretta caretta* and *Dermochelys coriacea,* with have a wide distribution and for which enough information and certain monitoring structures are already available (stranding networks and rescue centers) (INDICIT consortium, 2019).
The use of stranded cetaceans can only be considered on an opportunistic basis and dependent of the initiative of each Contracting Party that possesses existing networks for monitoring stranded animals. Such monitoring strategy must consider the limitations imposed by the low number of stranded organisms, the small rates of litter ingestion, and the impossibility of keeping wounded animals in rescue centers.
There are also protocols suited to the monitoring of litter ingestion by birds. Since these protocols are being used in the North Sea on the species of this northern region (Van Franeker *et al*., 2011), detailed work is still necessary for its further development in the South OSPAR area.

The ingestion of micro-plastics by fishes or invertebrates represents an opportunity for the development of new approaches and indicators for the monitoring of litter contamination and ingestion within the OSPAR area. At the current stage of development, we suggest pilot experiments to rationalize a standard method relying on ingestion by selected taxa to assess litter contamination in the marine realm.
For pilot studies or in-depth research work, common species with a wide distribution, that are easily fished and sensitive to micro-plastics, must be given priority. Among these species we can mention the most affected necto-benthic fishes (*Boops boops*) or those that present an important commercial interest (*Mullus sp., Dicenthrarchus labrax*) and the pelagic species *Scomber sp* as particular species of interest. The possible use of lancet fish or sharks to monitor ingestion (Gago *et al.,* 2020; Maes *et al.,* 2020) still requires additional work to develop appropriate, harmonized and intercalibrated protocols. The search for other species must not be neglected, but their application to monitoring must go through the diverse stages of validation, especially for fast moving fishes.

Existing monitoring infrastructures should encourage the development of networks and take advantage of regular scientific cruises to collect samples and analyze stomach contents that are already in place in certain countries. An alternative solution, after some work of validation, is to take advantage of networks that are measuring chemical contamination using mussels ('mussel watch'). These arrangements could provide the necessary samples for a regular and organized monitoring of ingested micro-plastics. Within OSPAR, the existing monitoring approaches could lead to more specific strategic choices. To give an example, the choice to monitor the impact of litter in nectobenthic species in the deeper part of the shelves would require the choice of suited species. In this case, existing programs of trawling for demersal species (International Bottom Trawl Survey - IBTS for example) would be a suitable solution, sampling common species on a regular basis, together with litter data. This would enable, to cross map the distributions of both litter and species, and support a risk assessment for both the state of the environment and, possibly, human health.

I.3 Entanglement and strangling

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 a & b), the latter also occurring on beaches during the egg laying period. According to the UNEP (2016a & b), 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).
ALDFG are associated to 72% of all observations of entanglement. They 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 (MacFayden *et al*., 2009).

As described in RAC/SPA for cetaceans (2018), the factors that may contribute to organisms being entangled in or strangled by ALDFG 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 inability 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 deformations due to the constriction of parts of the body in the case of individuals in their growing phase (see for example Gregory, 2009; Claro et al, 2018).

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 a & b; Claro et al, 2018). Certain marine organisms, when caught in active fishing gear (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. In this case, it is not easy for the observer to make out whether the animal was entangled in an existing bit of litter or in an initially active piece of fishing gear.

The incidence of entanglement can vary strongly according to the geographical 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 strategies with which these birds interacted but also according to age, with immature birds apparently being more sensitive than adults. Birds can be caught by the beak, wings or claws, what restricts their agility, their ability to fly and their ability to feed. Similarly, some fish species, particularly sharks, are also very sensitive to this type of impact (NOAA, 2014 (a & b), as they often become unable to open their jaws and feed due to entanglement. Less visible but equally exposed to marine litter, 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 (Kuhn *et al*., 2015).

Litter contamination in nests is another problem that can be used as a proxy to monitor entanglement of . As an example, the work by Votier *et al.* (2011), has led to the currently ongoing drafting of master guidelines for monitoring litter in nests of seabirds as a potential source of entanglement for fledglings. Even if additional research is needed to gain knowledge on the reproductive seasons, the types of litter brought to the nests by seabirds and the behaviour that leads to this phenomenon, species like the shag (*Phalacrocorax aristotelis*) are promising indicators for the North East Atlantic (Cadiou and Fortin, 2015). This species is very common throughout the sea with nesting occurring in the coastal areas of most EU countries. This approach consisting of recording data on litter brought by seabirds to their nests has already been used in many sites all over the world. Athough it remains in an experimental stage, it presents a strong potential for setting up future monitoring protocols and strategies (Figure 1). Another new protocol, also under development (Schultze and Werner, 2020), is being applied in breeding colonies of gannets on the rocks of Helgoland (Dürselen et al., in preparation). This protocol relies on observations of entangled victims and marine litter as nesting material and will use the TGML joint list as reference to categorize the types of litter ítems that are nested.

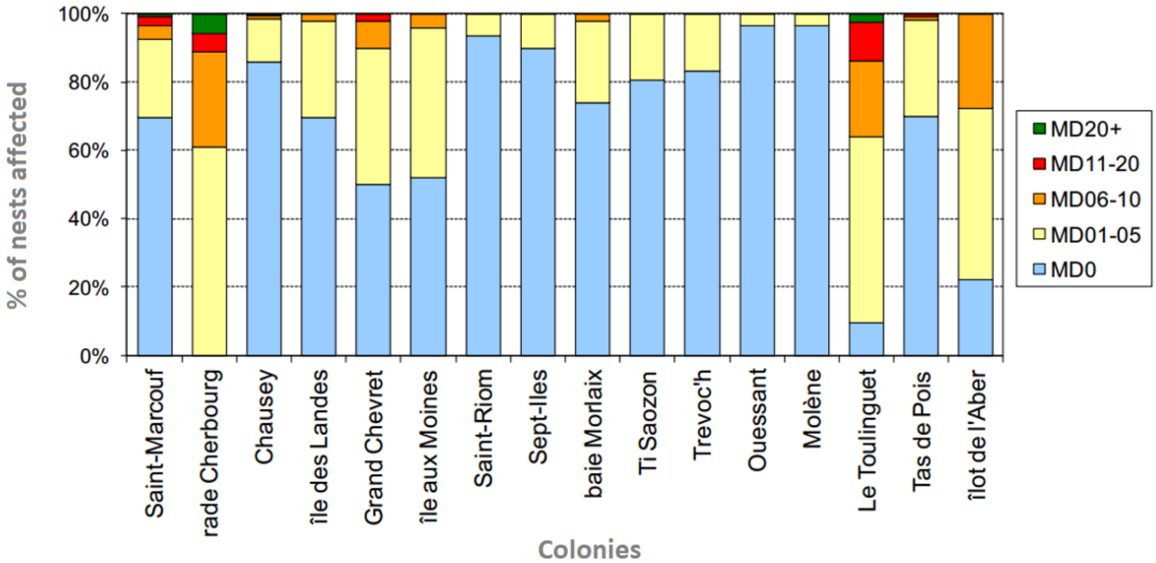


Figure 1 : *Presence of litter (and associated risk of entanglement) in nest from the species Phalacrocorax aristotelis (Shag) collected in various areas in Normandy and Brittany (France). The towns of Cherbourg (Normandy) and Le Toulinguet (Brittany, entry of the Bay of Brest) were the most affected areas with more than 80% of the nest with litter and some with more of 20 items each. As a counterpart, islands (Molène, Ouessant) were the less affected areas, with less than 5% of the nests with litter. MD: marine debris; % des nids: % of nests (Cadiou & Fortin, 2015)*

I.4 MONITORING THE ENTANGLEMENT/STRANGLING OF MARINE ORGANISMS BY MARINE LITTER

Monitoring the 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 affected at local levels, particularly in areas of intense fishing activity, strong presence of litter or significant abundance of sensitive species (i.e. turtles' egg-laying areas, or protected areas with high diversity (MFSD TSGML, 2013; Claro *et al.,* 2019).

As indicated above, the monitoring of entanglement and strangling of marine organisms by marine litter demands an in-depth analysis of the existing work and substantial developments before an optimal strategy is defined (Claro *et al.,* 2019). Moreover, while ingested litter is based on monitoring indicator species, entanglement and strangling, is very often species selective and monitoring strategies must consider several zoological groups (cetaceans, birds, reptiles, fishes, and invertebrates) and be organized by compartments (Claro *et al.,* 2018, 2019). Observations of various entangled species and specimens can indeed be recorded at the level of 1) beaches via stranding networks (Claro *et al.,* 2018), 2) the surface during oceanographic campaigns, and 3) the seabed, thanks to underwater means of observation like divers for shallow areas, or submersibles/ROVs (Remotely Operated Vehicles) for deep water areas (Consoli *et al.* 2018, Galgani *et al.,* 2018).

According to the approach 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, with the specific issue of impossibility to account for decomposing/ disappearing dead animals to solve. Moreover, entanglement is more often linked to the impact of ALDFG, which constitute a special category of marine litter. On the seabed, the potential to use invertebrates as an entanglement indicator is interesting because of the possibility of significant observations at all depths on the sea floor (RAC/SPA, 2018).
In the present stage of development, the identification of the main constraints inherent in a possible monitoring of the entanglement/strangling of fauna by marine litter is a prerequisite (Table 2).

Table 2: Identification of the main features and requisites to be considered for the implementation of monitoring of entanglement (Modified from RAC/SPA, 2018)

FEATURES & REQUISITES	
BIOLOGICAL	Entanglement can involve a small number of target species or every one of the species listed exhaustively.
	A complex life cycle can induce a great variability in prevalence depending on the phases of life, behavior, development stage, size and associated feeding behavior, sex, migration, etc.
	The probability of species being impacted by litter is affected by the biological cycle.
	Knowledge of the prevalence or rate of entanglement (proportion of entangled individuals in a sample) is an important prerequisite.
	Knowledge of pathologies to describe exactly the impact of the entanglement of marine animals in litter (wounds, strangulation, amputation etc.) and criteria for diagnosis are essential.

	A background knowledge on the biology of species must be available.
METHODOLOGICAL	Data collection is organized.
	The protocols currently available are scarce, badly described, or need further development
	Criteria that allow entanglement/strangling due to litter to be distinguished from active fishing gears to enable a correct interpretation of the results are lacking (or are needed).
	Factors that can interfere with the results (movement of living individuals after entanglement, decomposition of dead animals, etc.) must be known to correctly interpret the results.
	Proper knowledge of the seasonal variations in the presence of litter (fishing activity, tourist season) and species (migration) must be taken into consideration when organizing data collection.
ENVIRONMENTAL	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.
LOGISTICAL	The cost of the monitoring, the accessibility of samples and data (stranding networks, observation and monitoring by diving, etc.) are essential and must be widely taken into account.
	Strandings networks enable to overcome seasonal variations but are unpredictable. Information sharing of pre-existing campaigns of observation by diving, will guarantee data that would be less random
ETHICAL	Assessment of entanglement is made by non destructive observation.

Existing data on strangling and entanglement of marine species are still limited and insufficient for assessing harm on a regular basis and a systematic monitoring. The strategy recommended at this stage is to organize coordinated data collection and pilot experiments that will enable to define scientific and technical baselines. The work should focus on: the prevalence of entanglement/strangling, the identification of priority areas (presence of fishing gear, distribution of sensitive species, probability of encounters between sensitive species and litter, etc.) and the rationalizing of existing data collection systems and protocols (stranding networks, networks for observing Marine Protected Area - MPA).

With most available records involving fishing gear, especially ALDFG (Angiolillo, 2018), evidences suggest there is a direct link between the occurrence of entanglement in epibenthic invertebrates and the spatial distribution of fishing efforts, which may be of great importance for future monitoring designs and strategies (Consoli *et al.,* 2018). On the seafloor, areas dominated by sessile suspension feeders, such as sponge and coral assemblages, are often called as “animal forests” as they provide shelter and habitat for numerous species. Habitat constructing species are of particular interest for monitoring the impact of litter, as entanglement damages live tissue and can compromise them, with possible cascading effect in the local habitat. Additionally, being sessile and stationary, these organisms present advantages for systematic and periodic monitoring while avoiding constraints and bias from motile taxa (Galgani *et al.,* 2018). This approach should provide consistent datasets and better information to map marine litter trends and support reduction measures to be implemented in the NE Atlantic and in support of the OSPAR Regional Plan.

I.5 RECOMMENDATIONS

Monitoring the impacts of marine litter in marine fauna depends on the availability of indicator species to measure the prevalence and effects of litter ingestion and of entanglement/strangling in litter. Monitoring strategies can be designed with multi-species approach in order to cover the range 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 diversity of life strategies (sedentary, benthic, necto-benthic, pelagic, aerial) and feeding (detritus-eaters, suspension feeders, omnivores, carnivores) of marine species exposed to marine litter. The multiplicity of approaches needs to take this variability into account and requires the use of multiple target species, which is only possible if custom-crafted infrastructures with multiple skills are in place. In the present state of our knowledge, monitoring can only progress gradually, stage by stage, according to the degree of maturity of the indicators.

While the monitoring of litter ingested by fulmars and sea turtles has been implemented in the OSPAR region, it seems reasonable to also consider starting experimental work to test new potential indicator species, mainly to measure the impact of micro-plastics, in other relevant taxa. A particular focus should be given to certain fish species with wide distribution and that have a high rate of ingestion (Neves *et al.,* 2015; Bella *et al.,* 2016; Gago *et al.*, 2020; Maes *et al.,* 2020). Additional consideration should be given to some invertebrate species, particularly the mussel *Mytilus edulis (*Schultze and Werner, 2020), present throughout a vast part of North-East Atlantic.

A consistent monitoring of entanglement/strangling, still requires a compilation of available information and data to better define monitoring procedures and strategies. Mobilizing stranding and observation networks must be considered as a priority by the OSPAR Contracting Parties for experimental monitoring of entanglement/strangling of particularly sensitive species (mammals, birds, turtles) (Claro *et al.,* 2018). The potential of monitoring litter by inspecting seabird nests must be re-examined by experts in order to propose guidelines. To this effect, experimental monitoring programmes should be set up for locally relevant species, using standardized protocols elaborated by the EU MSFD TG ML (MSFD, 2020 under preparation) on the basis of voluntary action by the OSPAR Contracting Parties.

As part of future development, we strongly recommend to consider the potential of surface and underwater observation campaigns as they may provide a unique perspective on the abundance and distribution of litter over time. The potential of monitoring by diving in shallow areas, especially in MPA, and by using submersibles or ROVs for greater depths as tools for collecting observations on entanglement/strangling of the most affected species (invertebrates and fishes) must be considered. 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 the OSPAR RAP.

Carrying out coordinated pilot experiments based on a strategy of improved data collection, seems to be the most suitable preliminary step before considering the development of a regional or sub regional monitoring strategy. Work should focus on the prevalence of events, 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.

II TOWARDS A NEW PROTOCOL TO SUPPORT MONITORING OF THE MSFD CRITERIA D10C4

Studies from Regional Seas Conventions (RAC/SPA, 2018; Galgani *et al.,* 2018 and 2019) have already suggested an approach using benthic invertebrates as an entanglement indicator, since they offer the possibility of monitoring this impact at a wide range of depths. A report from the INDICIT project (Claro *et al.,* 2018) confirmed the potential of epibenthic invertebrates for monitoring entanglement. This group of organisms is widespread, and sufficiently abundant for relevant monitoring programmes.

Monitoring activities could be conducted on a regular basis using ROVs, towed cameras or by diving. Monitoring of entangled corals would have to be performed in areas with a rocky substrate and the main constraint of this method could be its cost, which can be highly dependent on the type of vessel used. However, opportunistic approaches such as using regular surveys for biodiversity in MPAs have great potential. Additional records of litter and interactions with marine organisms will provide sufficient information for measuring the 10DC4 indicator that is now mandatory within the MSFD. This approach is well adapted for MPAs that regularly record data on biodiversity (abundance, distribution). As for ROVs, any oceanographic means operated like towed cameras could be useful data collection devices for long-term monitoring.

Within CleanAtlantic project, and building on (*i*) results from IFREMER research in the Bay of Biscay, (*ii*) an initiative of the French Museum of Natural History (Muséum national d'Histoire naturelle, MNHN), and (*iii*) outputs from the INTERREG AMAre Project, also linking with the INTERREG project PBMPA, a protocol was improved and tested during a cruise (RAMOGE Survey 2018) aboard IFREMER's R/V ATALANTE, a vessel deploying the VICTOR 6000 ROV. For technical reasons and due to some opportunistic considerations, it was tested in the Mediterranean Sea, where the RAMOGE institution (http://www.ramoge.org/fr; UN affiliated) aims to protect the maritime area between the French PACA region, the Monaco Principality and the Liguria region in Italy. The cruise was devoted to the exploration of canyons and seamounts, with the CleanAtlantic partner IFREMER in charge of assessing the interactions between litter and marine organisms on the sea floor. Although it had been planned to have some additional experiments, work in the North East Atlantic was eventually cancelled, because of the COVID 19 pandemic and the resulting cancellation of all scientific cruises.

In Europe, the Marine Strategy Framework Directive (MSFD) recognizes that marine litter affects marine life at several organizational levels and their impacts vary depending on species or populations, activity-sources, environmental conditions and the region or country considered. MSFD Descriptor 10 is defined as "the properties and quantities of marine litter do not cause damages to the coastal and marine environment". The pressure corresponding to Descriptor 10 is relative to the litter spill.

For Descriptor 10, the process to review the original decision (2010/477) started in 2015 led to proposals from the European "DG-ENV/MSFD/Good Environmental Status (GES)/Technical Group Marine Litter" (GES TG ML) to modify the nomenclature and indicators. Descriptor 10 is composed of two types of indicators aiming to measure the abundance and distribution of marine litter (D10C1 seafloor litter) and their impacts on the environment and fauna (D10C4 entanglement). In the current version of the GES D10 definition, primary and secondary criteria are now classified depending on whether they fall under pressures (macro-litter or micro-litter) in different compartments of the marine environment (more specifically on beaches, at the surface, in the water column and on the seabed) or under impacts, especially ingestion or strangling/entanglement.

For pressure criteria, the main goals are to determine the spatial distribution, quantities and nature of litter, the anthropogenic activities that are the source of litter, paths of its introduction to the marine environment, dispersal paths and accumulation zones. For impact criteria, the goal is to determine the impact and/or effect of ingestion, entanglement and/or strangling on animals.

10DC1 Criteria – Seafloor litter Indicator

10DC1 corresponds to the criterion "Composition, quantities and spatial distribution of litter on the coasts, at the surface, in the water column and on seabed, at a level that does not affect the coastal and marine environment". This criterion is evaluated by three indicators: litter on beaches, floating litter, and seafloor litter. Currently, France reporting on the GES D10C1 evaluation (Seafloor litter, Gérigny, *et al.,* 2018) essentially relies on data collected by trawling on the continental shelf, operated as part of fishery stocks monitoring surveys (Gérigny, *et al.,* 2019). Nevertheless, these data are collected only on sandy bottom litter on the continental shelf and don't target rocky bottoms (not trawlable). To overcome this issue and obtain data on a wider geographic area, it is possible to use opportunistic data collected on dives with underwater vehicles, such as ROVs or towed cameras. This means that deep-dives in canyons are a potential source of data for the 10DC1 indicator.

10DC4 Criteria – Entanglement Indicator

10DC4 corresponds to the criterion "Number of individuals affected by litter an irreversible way by strangling and/or entanglement or any other type of injuries or mortalities of concerned species". This indicator is still being developed and methods, metrics and units of evaluation have not been defined yet. In the revision of the decision, the GES TG ML group suggested that entanglement/overlaying was taken into account as a new impact criterion due to its significance in certain regions of Europe and for certain species. The consideration of this Entanglement/Strangling indicator in all regions of Europe is highly debated, due to the low strangling rate measured in stranding networks and especially because of the difficulty of interpreting certain measurements. For instance, strangling/entanglement in nets can be due to the hoisting of active fishing nets and not to litter. The revised decision has included this criterion (10DC4) in order to take this into account in the GES.

Types of impacts observed in deep diving

There is a direct link between the occurrence of entanglement in epibenthic invertebrates and the spatial distribution of fishing efforts. Typically, high levels of entanglement are reported for fishing grounds where the use of longlines and gillnets is more widespread (Pham *et al.*, 2014), whereas less entanglement is found in areas where bottom trawling is the predominant fishing gear. There is very little data for assessing any change in the level of entanglement of epibenthic invertebrates over time as most information is obtained opportunistically from video footage collected for other purposes.

The most common organisms found entangled are corals and sponges, probably due to their complex morphologies, but also because they form dense aggregations. As a result, larger and more complex species, such as gorgonians, black corals, scleractinians and habitat-forming sponges are more susceptible to being entangled in debris. Distribution of species and available data on epibenthic invertebrates, and in particular corals and sponges, are abundant and widespread in the MSFD region and OSPAR area (Claro *et al.,* 2018). Presently, surveys to explore the seabed are common in the framework of national explorations (for example, MEDSEACAN in France or EMEPC in Portugal), scientific endeavours or commercial exploitation of seabed resources (e.g. seabed mining or oil exploitation). These cruises, which operate in coastal areas or in the deep sea, collect hours of videos, which are generally available from oceanographic institutes upon request or directly from websites (for instance, see cartographie.aires-marines.fr).

In the Bay of Biscay, fishing-related items were found throughout the Bay and, unlike plastics, were not preferentially associated with corals or with any other complex habitat. This suggests that fishing-related items may be too heavy for displacement by currents. However, 15 to 20% of the marine litter found in corals (including cable and ropes) was related to fishing activities, highlighting this additional, potentially strong pressure on the health status of corals (Van der Belde *et al.,* 2017). It also appeared that 13.6% of the observed litter items in the Bay of Biscay were colonised, being this percentage considerably lesser than in the Mediterranean Sea. Such litter/species associations may increase with local diversity, as seen in Portuguese canyons.

Epibenthic invertebrates may be good indicators for entanglement, depending on the complexity of their morphologies. No biological constraints were identified for corals, whereas for sponges, their ability to grow on and entirely cover the marine debris over time may represent a bias. Underwater imaging technologies such as ROV and towed cameras are now widely used, with footage that may be georeferenced. The methods might be expensive, but ocean-going equipment and vehicles are present in quite a number of EU and RSC Member States. For monitoring purposes, a specific sampling scheme has to be defined (length of transects, distance above the seafloor for adequate image resolution, etc.). In particular, the locations should be chosen according to strict criteria, based on the level of the available knowledge for the area (understanding of fishing activities, etc.). An important constraint lies in determining how the occurrence of entanglement is linked to the number of litter items. Cold-water corals and sponges are mostly predominant in the deep ocean, restricting our monitoring to a certain type of litter items, mostly related to fishing gear.

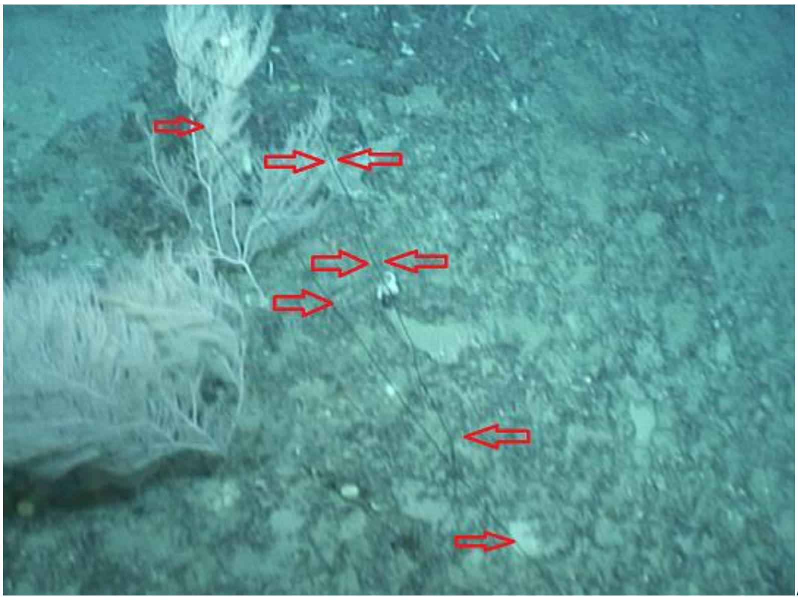
As stated in Claro *et al.* (2018), previous feasibility studies suggest that “marine debris entangling corals” could be proposed as a candidate indicator of impact of marine debris on marine biota, and that further work should be undertaken in order to collect and share data which would enable this indicator to be tested and developed (criteria, methods/ common approach, thresholds, common data collection and storage procedure etc.). Another proposal would be to consider more globally an indicator like “marine debris entangling (including smothering) epibenthic invertebrates”. In the first case, the metric could be the number of individuals entangled or damaged/injured (typology to be defined), while in the second case, the metric could consist of the number of individuals entangled/smothered by debris, or the surface of invertebrate communities covered by debris.

The RAMOGE cruise, organized in September 2018, with the aim of exploring deep Mediterranean areas (canyons and seamounts), was an opportunity to develop a protocol for observing both marine litter abundance and strangulation/entanglement. Indeed, as indicated below, since the distribution of seafloor litter, including fishing gear, and strangulation/entanglement are closely linked, it was difficult to separate the observation protocol for the two indicators. This cruise, performed aboard R/V Atalante, made 7 dives in ultra-deep conditions (2,200 - 500 m) collecting observations data from the Victor 6 000/Ifremer ROV.

Before initiating the cruise, an observation protocol had been drafted (Gérigny and Claro, 2018) and proposed for testing. For monitoring purposes, a sampling scheme was defined (length of transects, distance above the seafloor for adequate observation/image resolution etc.) and locations were chosen to enable and record observations. For this protocol, two definitions of indicator 10DC4 observations were proposed as examples:

- Entanglement

The entanglement impact is defined here as tangling action. During deep diving, species affected by entanglement are mainly the benthic macro-fauna such as gorgonians, sponges and corals, coralligenous formations, etc. These interactions can correspond to plastic or textile litter that partially or fully covers an organism; a fishing net or line tangled around an organism.


A


B

Figure 2 : Examples of impact due to entanglement on benthic invertebrate fauna. A: Entanglement, B: Coverage.
Photos by © Ifremer

- Strangling or/and adaptive behaviour

These impacts are defined as: (*i*) Strangling: accidental strangulation of an organ or part of the body performing a function that negatively impact the life of the organism or (*ii*) adaptive behaviour: when litter items are used by organisms in their carrying behaviour or as mobile shelter instead of other natural items (i.e. sponges, corals, shell, etc.). Some species, which are pelagic or benthic, can be strangled or adapt their behavior even at great depths. Many pelagic species can be affected by this type of impact / interaction such as fish but also benthic species, such as crustaceans, gorgonians, etc.

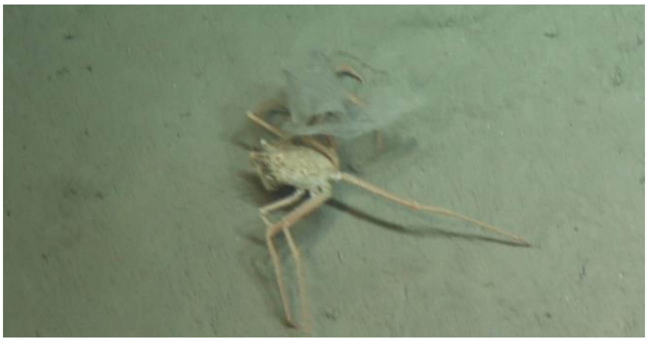


Figure 3 : Example of adaptive behavior of the crab *Paromola cuvieri* which was observed carry plastic on its exoskeleton. Photo by © Ifremer

Following the survey and post-processing of the seafloor litter data and strangulation / entanglement / adaptive behavior events, recommendations were formulated and the protocol has been improved (Gérigny and Galgani, 2019) as shown in the protocol presented in the next section. The protocol focuses on observation techniques and methods concerning seafloor litter and strangling or entanglements of marine organisms by litter observed on deep dives. There are only descriptive data for this indicator at this stage, and the protocol can only be considered on an experimental basis. Although it needs to be further tested on several cruises and under several conditions to be validated, it constitutes the first step in the implementation of an indicator for such criteria. In addition, research and development of protocols to be used during the project partly provided a working basis and an element of input for the preparation of the updated D10 European guidance which was discussed during the TG ML workshop on harm on biota, held in Berlin in 2019 (Angiolillo et al. in prep.). This updated protocol provides the definitions and illustrations of 6 types of interactions between epibenthic organisms and litter.

III. A PROPOSED PROTOCOL FOR THE OBSERVATION OF D10C1 AND D10C4 INDICATORS DURING ROV DIVING OR USING TOWED CAMERAS

In this section, a simplified version of the protocol of observation of the seafloor litter and their impacts developed taking into account the feedback of the RAMOGE cruise is presented. All changes and recommendations can be found in Gérigny and Galgani, 2019. It should be kept in mind that this protocol is designed with the aim of being implemented on any campaign using an ROV or towed camera, not necessarily dedicated to monitor marine litter and their impacts, and it can be utilized by any user (including non-specialist of marine litter). Moreover, it has been revised to facilitate video post-processing of seafloor litter and data on litter/organisms interactions.

During a dive, various elements coming from the observation of litter and their impacts must be captured and/or noted in the comments in order to optimize the viewing of videos during post-processing. They are listed below:

- Save a **picture for each observation** of marine litter or interaction between biota and litter.
- In the dive comments (and/or on the observation sheet – Appendix 1) and if time allows this to be done directly (otherwise, to be completed during video playback after the survey):
    1. Indicate the **Litter/Debris** observation**, letter D** (Table 3)
    2. When zooming in on the same litter, indicate the **zoom by the letter Z;** for an observation of new litter, a few seconds after another one, mention **new observation NO=** New Observation (Table 3)
    3. For a **litter** observation, record the type of object (fishing line, fishing net, plastic bottle, glass bottle, etc. See table 5). If the litter type is too difficult to identify, the **category** of litter will be indicated, for example: **D/Plastic; D/Glass; D/wood**
    4. **Impact** observation will be noted as letter **I**. **Take a picture.** When possible, indicate the type: **covering**, **entanglement**, **strangling, etc.** Register as much information as you can, particularly:
        - The entangled **species** [if the name of the species is unknown, at least indicate a group (gorgonians, coral, sponge, etc.)].
        - The **apparent nature** of the litter as given in table 4 (plastic, glass, metal, etc.);
        - When possible, the source and/or the descriptive detail of the litter (fishing gear or net, plastic/plastic bottle, metal/anchor etc., examples are given in table 5);
        - The potential impact of the entanglement (broken branches, affected mobility, etc.);
        - The approximate percentage of individual covered by the litter/ part affected by the litter.

Table 3: Coding for comments on the observation of marine litter and their impacts during ROV/ towed camera dives

CODE	ACTION
D	Debris
Z	Zoom
NO	New Observation
I	Impact

Table 4: Simplified table of litter materials, when compared to the master list of TG ML (2020), in order to facilitate the observation of marine litter and interactions with biota for non-marine litter specialists in the context of opportunistic use of ROV/towed camera campaigns

N°	Category	N°	Category
1	Plastic	6	Metal
2	Processed wood	7	Paper / Cardboard
3	Rubber	8	Natural Product
4	Sanitary litter	9	Glass and Ceramic
5	Cloth / Natural fibers	10	Miscellaneous

Table 5: Examples of litter types

Plastic	Plastic bottle
	Rope
	Food packaging
	Sheets, tablecloths, tarpaulins
Cloth	Rags
	Clothing
Fishing litter	Fishing net
	Fishing line
	Fishing-related (float, pot, etc.)
Metal	Boat anchor
	Pot

A data management support for the implementation of this protocol can be brought from the database developed in CleanAtlantic project. Indeed, this PostgreSQL database has been sized and adapted to host data collected in MSFD monitoring programs on beach litter (10DC1), seafloor litter (10DC1), floating macro and microlitter (10DC1 & 10DC2) and litter ingested by Marine Organism (10DC3). It could also be used to store data on entanglement/covering caused by litter, collected during shallow or deep diving (10D4).

Relevant advantages of using this PostgreSQL database are listed below

- Data have a structuration harmonized with the use of common referencial,
- Data have a security storage in a sustainable way
- Tools are developed to generate exchange formats for interoperability with other European systems, e.g. OSPAR, ICES, EMODnet, …
- It facilitates indicators calculation for assessments

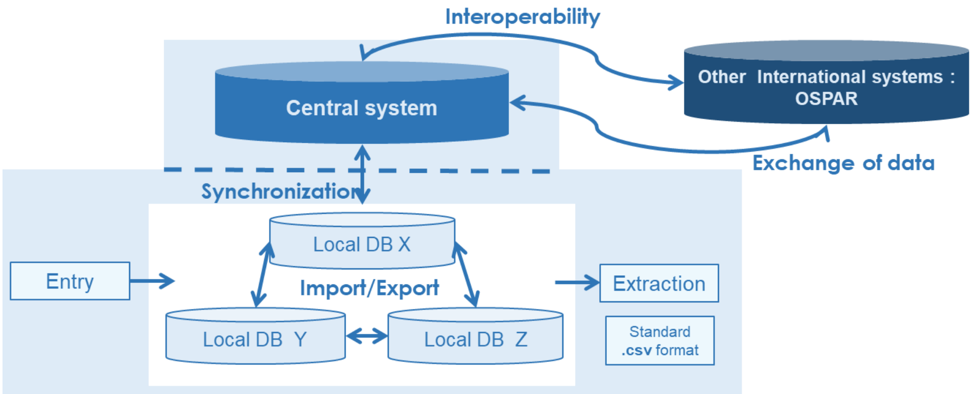


Figure 4 : Storage and handling System representation

IV CONCLUSION

Numerous studies on impact of marine litter have recommended potential indicator species for marine litter entanglement and ingestion monitoring. There are, however, very few standardized protocols. As a next step, the available data on entanglement and ingestion for the different species could be evaluated using risk assessment approaches such as cross mapping the distributions of both litter and target species.

Ingestion indicators are already monitored on regular basis in the North Atlantic, within MSFD and OSPAR areas. In addition, benthic invertebrates, such as mussels, may support regular assessment of harm, as sessile organisms are easy to monitor, and they filter higher amounts of microparticles. While samples of fish from fish markets could be considered for impact monitoring purposes, the use of demersal fish stock assessment cruises may also support the evaluation of microparticles ingestion by nectobenthic fishes, taking advantage of regular sampling (IBTS cruises) and stomach content analysis already performed by some institutions.

For entanglement, the rationalization of existing stranding, observation and rescue networks to better assess harm on biota together with the feedback from the RAMOGE cruise and the post-processing of data helped to set up a protocol dedicated to epibenthic fauna. However, results are still preliminary, and there is a need to validate them on a larger scale. For that purpose, it would be interesting in a second phase to have the protocol tested by a wider community on other surveys in the Atlantic, to get the necessary feedback that will enable to definitively validate it. In parallel, many groups of experts in different fields, in particular within TG ML, are working in this direction what will soon provide recommendations for the data collection procedure in the perspective of developing D10C4 indicators, notably for epibenthic fauna, as part of the new "monitoring guidance" scheduled for 2021 (Angiolillo *et al*. in prep). This guidance offers both a protocol for observation data (with inputs from the CleanAtlantic project), and indications on the metrics and calculation of indicators. Finally, data management will be another important step to support the implementation of monitoring. CleanAtlantic has addressed this issue, developing a marine litter database that also include data on entanglement.

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APPENDIX 1 – OBSERVATION SHEET

Observation Sheet on Entanglement/Strangling/Covering of the species by Marine Litter during ROV dives

Survey name / Vessel / Gear	
Diving Number	
Diving Area	
Diving duration	

Observation N°	Dive Time	Latitude	**Longitude**	**Entangled species**	**Recovery %**	**Litter Material**[1]	Litter Types[2]	Comments/Impacts

[1] See Table 4
[2] SeeTable 5