MEDSEALITTER

Developing Mediterranean-specific protocols to protect biodiversity from litter impact at basin and local MPAs scales

Priority axis - Investment Priority-Specific Objective 3-2-1
Priority Axis 3: Protecting and promoting Mediterranean natural and cultural resources PI 6d
3.2: To maintain biodiversity and natural ecosystems through strengthening the management and networking of protected Areas

DELIVERABLE 3.2.1

EXISTING MONITORING PRACTICES REPORT

State of the art on existing monitoring methods of floating macro-litter and ingestion in biota

Work Package 3 – Studying
Activity 3.2: Review of the state of the art

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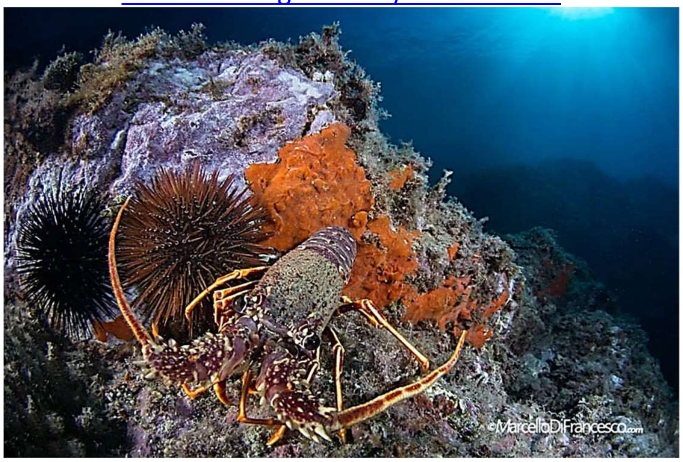

Summary

INTRODUCTION

Reduction of marine litter -any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment- is globally acknowledged as a major societal challenge of our times due to its significant environmental, economic, social, political and cultural implications (Cheshire et al. 2009; Galgani et al. 2010). Marine litter is one of the main causes for sea pollution and it is dominated by plastics (Barnes et al. 2009; Coe & Rogers 1997; UNEP 2015)
First measures to tackle marine pollution were taken by the OSPAR 72/74 convention and the International Convention for the Prevention of Pollution from Ships (MARPOL 73/78), becoming the main policy drivers of monitoring of coastal and offshore waters. More recently, new EU directives started to specifically target the reduction of waste and to monitor progress of these measures: the Waste Directive (2008/98/EC), the Packaging Directive (94/62/EC) and the plastic carrier bags Directive (2015/720/UE amending 94/62/EC) ask Member States to reduce the annual average production of waste and consumption of plastic bags. Other European directives introducing the ecosystem-based approach have been largely integrated in the existing measures and enforced into State legislation. These directives, such as the Water Framework Directive (WFD, EU 2000) and the UNEP/MAP Regional Plan for Marine litter Management in the Mediterranean (UNEP/MAP IG.21/9), highlight that policy drivers may change over time but maintain similar overall purposes.
In 2008, the European Commission adopted the Marine Strategy Framework Directive (European Commission, 2008/56/EC) whose objective is to achieve Good Environmental Status (GES) by 2020, considering 11 qualitative Descriptors. Marine litter is the Descriptor 10 of the Directive and GES is reached when the "*properties and quantities of marine litter do not cause harm to the coastal and marine environment*" (European Commission, 2008/56/EC; Galgani *et al*., 2010).
However, the lack of comparable data across all seas still presents a major obstacle for an European marine assessments. Effective measures to tackle marine litter are in fact seriously hampered by the insufficient scientific data (Ryan 2013). To monitor effectiveness of measures, the need for more accurate and coherent monitoring on marine litter is evident in order to set priorities for marine protection actions in a cost-effective way (Cheshire et al. 2009; Galgani et al. 2013a; Sheavly 2007; UNEP 2015).

The Mediterranean context

Information on marine litter in the Mediterranean Sea**,** considered as one of the most affected seas by marine litter worldwide, is still limited, inconsistent and fragmented (Barnes et al. 2009; Jambeck et al. 2015). The Mediterranean Sea was designated as a Special Area under MARPOL Annex V, that prohibited the disposal of garbage at sea and lead to the establishment of adequate port reception facilities for garbage: nevertheless, the efficiency of the shoreside management of waste often remains in doubt. A pilot survey organised in 1988 by UNEP/MAP and successive assessments showed that the main sources of coastal litter in the basin are river runoff, tourist activities and coastal urban centers (MAP/UNEP, 2001; UNEP 2015). Additionally, at-sea activities such as shipping and fishing grounds can heavily contribute to the inputs of litter in specific contexts (Carić & Mackelworth 2014; Coe & Rogers 1997; Vlachogianni et al. 2016).
Regarding impact on biota, until 2011 there were not Mediterranean candidate species to be used as bio-indicator for litter ingestion, while the Northern Fulmar (*Fulmarus glacialis*, Linnaeus, 1761), has been used as a target indicator in the Northern part of the European Sea, for many years.
In 2011, DG ENV asked for further development of the indicator and the methods implemented in the North Sea, and adaptation to other regions. This involved the identification of additional marine species to be used as indicators in Mediterranean EU countries and the Loggerhead (*Caretta caretta* Linnaeus, 1758) has been chosen as possible indicator (Matiddi et al., 2011; Galgani et al., 2013a)

As for the global scale, more and better data are needed to develop a marine protection framework in the Mediterranean Sea that addresses marine litter effectively, thus ensuring the sustainable management and use of the marine and costal environment at a basin-scale (Cheshire et al. 2009; Galgani et al. 2013a; UNEP 2015).

MONITORING

The monitoring is intended to detect changes over time and should provide data representative of the location and time of sampling. Long-term monitoring programmes provide valuable data sets which are highly relevant to present-day policy drivers, in particular in response to MSFD requirements which focus on trends in pressures and impacts, and to assess the effectiveness of legislative measures (Galgani et al. 2013a; Zampoukas et al. 2014). Therefore, monitoring programmes should be consistent, coherent and comparable within marine regions and the choice of the most effective methodologies (cost-benefit approach, most appropriate indicator), as well as the implementation /adaptation with different ongoing projects, are important elements in monitoring planning. The application of well-documented procedures, experienced analysts, as well as intercalibration of methodologies, will assure the production of high quality and consistent data (Zampoukas et al. 2014).

Variables
Many parameters (Covariates) influence the detectability of items or the depositional environment (prevailing winds, local and offshore currents, proximity to land based sources, etc.).

- The variables to be collected are: 1) Amount, distribution and composition of litter; 2) Rates at which litter enters the environment (sources); 3) Impacts of litter. The protocols used need to be adequate to the information required, which depends on the goal of the monitoring:
- Size of litter, broadly divided into macro-litter (x>2,5 cm), meso-litter (5mm<x<2,5cm) and micro-litter (<5mm), has also great influence on the sampling methodologies that can be applied, as well as for the results. Therefore, each study should clearly indicate the smallest size of items recorded. For macro-litter, this information might depend on the characteristics of the observation platform (height, speed) and the identification of size classes is applied in data collection.
- Even the categorisation of objects has to be clearly defined. Recent studies should now refer to the MSFD litter category list (Galgani et al. 2013a), which reviewed the original OSPAR and UNEP categories (Cheshire et al. 2009); field guides and litter identification tools are important elements in the maintenance of sampling consistency.

All these elements have to be taken into consideration when comparing results at more detailed level.

Sampling design and period
To monitor marine litter, we need to understand the dynamic linkages between sources and sinks. The combination of multiple diffuse and point-source inputs and the variable transportation of debris by winds and currents results in great temporal and spatial variability in litter loads in the different sea compartments. Such variability requires a well-defined sampling design with sufficiently large replication in space and time to intercept these changes.

- According to international guidelines, monitoring programmes should be consistent, coherent and comparable within marine regions. Therefore, sampling should be stratified in relation to sources (urban, riverine outputs, offshore activities) in order to provide representative data in each location (Cheshire et al. 2009; Zampoukas et al. 2014).

- A minimum sampling frequency of one year is required, although seasonal replication is recommended (Cheshire et al. 2009; Galgani et al. 2013a). At least 20 sampling units should be randomly allocated within each site, but given the heterogeneity in the amounts of marine litter, the adequate number of samples might be adjusted: for example, pilot studies can be used to estimate variability in sample data, with successive power analysis to assess the most effective sample size necessary to detect a change (Ryan et al. 2009).

FLOATING MACRO-LITTER SURVEYS

Effective monitoring of floating litter at-sea requires huge sample sizes to overcome the very large spatial heterogeneity in litter distribution linked to wind strength and sea state. For this reason, these surveys are costlier and more challenging logistically, given the intensive sampling needed (Ryan et al. 2009).

Sampling platforms
Marine litter floating on the sea surface can be directly sampled from different observation platforms:

- Visual observation of floating items is the most common methodology used and relies on competent, motivated observers. Studies comparing detection ability show marked differences among observers (e.g. Ryan & Cooper 1989), which need to be evaluated when multiple observers are working, especially when volunteers are involved (Sheavly 2007). Direct observations need less resources, but are fraught with other potential biases linked to differences in litter detectability due to observation conditions and platform types.
- Automatic recording of floating litter was used in more recent applications and is provided by unmanned systems specifically set to acquire images from ships, aircrafts or drones, travelling along defined routes. In this situation, the bias is reduced to the post-processing recognition of images.

Sampling units
Surveys are usually based on transects, considered as sampling unit. The survey effort is carried out in standard conditions (constant speed, sea state, observers' height) to perform objects counts: within the MSFD the number of items is requested for suitable comparison on litter amounts (Galgani et al. 2013a) providing a crude index of abundance (number of items per distance unit) or an estimate of objects concentration (number of items per surveyed area). Two surveys can be performed:

- fixed width transects assume that all debris is detected within a pre-defined distance from the observer, considering a conservative strip based on preliminary measures; strip transect method is applied for density estimation (e.g. Hinojosa & Thiel 2009; Thiel et al. 2003; Topcu et al. 2010).
- line transects, where the perpendicular distance to each item has to be estimated to compensate for decreasing detection rate with distance from the observer and separate detection curves should be estimated for different sea states; distance sampling is applied for density estimation (Buckland et al. 1993)(e.g. Ryan 2013; Suaria & Aliani 2004).

The main constraints are in the accurate definition of the monitored strip width or the distance between the objects and the observers, measures that can be obtained with simple tools, as an inclinometer or range finder (Ryan 2013).

<u>Ship-based surveys</u>

Direct observations of macro-litter items from vessels have been conducted in several studies around the world since the 1980's. Observations are conducted only on the side of the ship with the best viewing conditions, because variable detection rates depend on sea state, light conditions and the characteristics of floating objects (Galgani et al. 2013a).
Different platforms have been used ranging from small-medium boats (Di-Méglio & Campana 2017; Dufault & Whitehead 1994; Shimoto & Kameda 2005; Thiel et al. 2003) to large ships (Aliani et al. 2003; Day & Shaw 1987; Matsumura & Nasu 1997; Ryan 2013; Suaria & Aliani 2004; Thiel et al. 2011; Topcu et al. 2010), including platforms of opportunity (ferries, cargo ships, ISPRA 2015; Sà et al. 2016). Different sampling designs are usually applied in small boats and large ships surveys, implying the different class sizes recorded, the geographical coverage and the sampling period. Both fixed width and line transect methods can be applied, accurately defining the strip width or distance of the objects from the ship.

- Small boats can cover coastal waters, usually travelling at slow speed and detecting items by naked eye (e.g. Day & Shaw 1987; Di-Méglio & Campana 2017; Gerigny et al. 2012; Thiel et al. 2003). The Legambiente campaign *Goletta Verde* (Italy, 2013-2015) performed visual sampling of macro-litter during its itinerary along Italian coasts, allowing large spatial coverage within the same season and interesting insights about the main local sources. Di-Méglio & Campana (2017) analysed a large dataset during survey campaigns along the French coast, investigating temporal trends in the same area.
- The increase of observation height and vessel speed corresponds to a loss of detection ability of small size items. Large vessels, on the other hand can survey open waters and provide data on larger size classes (>20 cm), considered adequate in describing spatial patterns over larger scale (e.g. ISPRA 2015; Sà et al. 2016). Moreover the use of platforms of opportunity can enhance the survey effort in a cost-effective way, supporting more regular observations (Cheshire et al. 2009). For example, in 2008 the HELMEPA association of maritime stakeholders (Greece) invited its member managing companies with ships travelling in or transiting the Mediterranean to implement the program of UNEP-MAP for the monitoring and recording of litter floating on the sea surface, obtaining data all around the basin in few months.

Thus, both approaches can be relevant for estimating trends in the amount of total litter present in the marine environment and can be complementary (Vlachogianni et al. 2016).

Automatic observations

Automated photographs can be obtained through cameras applied on platforms of opportunity as commercial vessels or cruises. The JRC set a preliminary project of *SeaLitterCAM,* Image recognition technology for the large scale monitoring of floating litter (Hanke & Piha 2011). It consists in the acquisition of sea surface images in front of the ship bow during daylight, along weekly itinerary covered by Costa Crociere ships in the Western Mediterranean Sea. Galgani et al. (2013b) presented a first application of a camera on a Wave Glider to monitor floating marine litter. Images are successively processed for recognition analysis and the output is a list with georeferenced objects and their size, to be computed as abundance index (number of items per unit distance).

Aerial surveys

To estimate the amounts of litter at sea, large scale monitoring programmes have been developed through aerial surveys, even to locate areas of higher aggregations of litter (Lecke-Mitchell & Mullin 1992; Pichel et al. 2007; Unger et al. 2014). Aerial surveys cover large areas and only detect very large litter items (i.e. the lowest limit for aerial detection are objects of *ca*. 30–40cm), so they are less prone to changes in litter detectability linked to wind strength and sea state. Aerial surveys are considered more valuable for detecting spatial differences in abundance than for monitoring changes over time, even because the high costs of these surveys prevent from a large replication (Galgani et al. 2013a; Ryan et al. 2009). Line transect methodology is usually applied from aircrafts, through a rigorous sampling design (Buckland et al. 1993). Set-ups for aerial surveys are usually prepared from surveys designed for the evaluation of the abundance of marine fauna, including marine mammals (e.g. Gómez de Segura et al. 2007; Panigada et al. 2011), sea turtles (e.g. Gómez de Segura et al. 2006; Lauriano et al. 2011) and fishes (e.g. Bauer et al. 2015).

Surveys should be designed accordingly to a line transect distance sampling technique, in which a high representation of the study area is homogenously covered (Thomas et al. 2010). The recommended aircraft is a two-engine high-wing with flat or bubble-windows flying at constant speed and altitude. Beside of the pilot, two experienced observers and a dedicated data logger should form the crew. Environmental and weather conditions should be recorded at the start and end of every transect, as well as any time when these change. For each sighting, positional data will be recorded using a GPS and observers should determine the strip width in which debris is observed. Note that in studies where marine fauna abundance is estimated, observers use a clinometer to measure the declination angle to each sighting, which is used along with the plane altitude to estimate the perpendicular distance of each sighting to the track line (Gilles et al. 2009; Gómez de Segura et al. 2011; Panigada et al. 2011). However, it has been suggested that aerial litter surveys need an adaptation to this method (Piha et al. 2011), as the collection of distance data on all marine debris could interfere by taking effort away from target (i.e. cetaceans, sea turtles) species (Scheidat and Feindt-Herr 2012). In any case, data for both marine fauna and marine debris has been successfully recorded simultaneously (e.g. Darmon et al. 2016; Panigada et al. 2017).

Considering that the lowest limit for aerial detection are objects of *ca*. 30-40 cm, there are limitations on the categorization of floating litter observed from aerial surveys according to the Master List of items proposed by Galgani et al. (2013a). Therefore, observers should identify the following characteristics of marine litter: (1) material and litter item, (2) size (3) level of aggregation of items and (4) possible source (see Galgani et al. 2013a and Darmon et al. 2016). Despite the possible limitation and litter categorization of a long-distance observation of marine debris, aerial surveys are a useful monitoring technique in which large areas can be surveyed and large litter accumulations spotted.

Automatic observations

Automated recording of floating objects can be obtained through a variety of recording systems applied on Unmanned Aerial Vehicles or other remote controlled devices that can be used to monitor the presence of marine litter at different special scales in the sea. The use of these devices presents some advantages when compared to traditional visual techniques: human error of visual surveys is reduced; human risk (for pilots and observers) is reduced, while at the same time survey effort can be increased; it is a reliable technique, in which the geo-referencing of observations is accurate and precise; the images are recorded permanently, allowing subsequent statistic (re-)analyses and making possible to answer future questions of biological interest; it is a technique that allows to reach inaccessible areas and repeatedly sample the same sites with minor costs than aircrafts; and finally it is a constantly improving technique (e.g. improvements in image resolution) (Bryson and Williams 2015). The use of Unmanned Aerial Vehicles for marine monitoring has seen a rapid development in recent years, especially with regard to marine mammal and other marine fauna monitoring (e.g. Koski et al. 2009; Hodgson et al. 2013; Adame et al. 2017). These systems have an equal interest for their application for surveying human activities at sea and documenting possible illegal activities, as well as for identifying debris presence and its localization in the oceans. Two main categories of UAV can be used for marine monitoring:

- Fixed-wing drones: they have longer endurance with regard to flight distance and duration, but they also present some disadvantages related to the operations of take-off and landing, especially at sea. They also are less stable, thus limiting the quality of images recorded. Their use is recommended for the inspection of medium-scale marine areas and the identification of areas of high concentration of marine debris.
- Multicopteres: they are multi-rotor drones, generally with less endurance than fixed-wing drones, but their structure is much more stable, allowing easy take-off and landing, and stable flights. The quality of images taken using these drones can be extremely high, allowing an accurate characterization of objects at sea. These drones are recommended for small-scale investigations, when a more accurate classification of sightings is needed.

Pilot remote-sensing surveys of marine litter have also been performed using other kind of remotely controlled systems, such as aerial balloons (Kako et al. 2012). More recently, Merlino et al. (2017) used pictures taken from drones to follow the dispersion and accumulation of drifters delivered at Arno's mouth to investigate the dispersal patterns of debris transported through the river.
Apart from the 'traditional' RGB cameras, the use of thermic cameras and multi-spectral cameras is also being experimented for automated marine monitoring (Bryson and Williams 2015).
Independently from the platform and the instruments used for image recording, in this kind of surveys the recognition analysis is performed afterwards, on the video/images acquired. Various algorithms for automated image analysis and object detection have been developed and proposed, based on the characterization of pixels and the analysis of colour and shape of objects (e.g. Maire et al. 2013), but these techniques are still under constant improvement and their applicability on marine litter surveys has still to be evaluated.

Fixed stations

A first estimate on marine litter abundance in the Mediterranean Sea was obtained by Morris (1980) from a fixed station in the Sicilian Channel, followed by the study of McCoy (1988) in the same area: the two obtained very different densities indicating the gaps deriving from unrepresentative samplings.
Floating debris counts performed from fixed stations (vessel or other platform) consist in repeated scans of 1 minute covering the sampling area over a defined radius (10 m – Morris 1980 - up to 200 m– McCoy 1988; Aliani et al. 2003) depending on the observers height. These measures give a semi-quantitative index of litter abundance from the number of litter items observed per scan over the searching radius. In some studies this sampling has been used in combination with transects (Aliani et al. 2003).

Land-based monitoring

Most of litter originates from land and it is carried by water via rivers and storm-water (Jambeck et al. 2015), increasing after rain events. Therefore, surveys of litter before it reaches the sea can also provide useful information on debris abundance and sources. Moreover, monitoring the amounts of litter entering in the marine environment and their changes is necessary to assess the efficacy of policy measures (Sheavly 2007; Zampoukas et al., 2014).
To date, most studies of litter in urban runoff have focused on macro-litter (e.g. Marais et al. 2004). The first European-scale quantification of loads of floating litter to the European seas was developed by JRC in 2015 (González-Fernández & Hanke 2017). The *RIMMEL* project, RIverine and Marine floating macro-litter Monitoring and Modelling of Environmental Loading, intended to quantify floating macro-litter loads through rivers to marine waters, by collecting existing data and developing an European observation network focused on the rivers. The project developed the RiverLitterCam

methodology for continuous recording of floating macro-litter in rivers, providing a new tool for observation and assessment of litter amounts in freshwater/estuarine environments.

IGESTION OF MARINE LITTER BY BIOTA

Marine litter may harm marine animals in different ways: many species could be entangled by macro litter, while other species can ingest smaller items accidentally (Laist, 1987; Schuyler et al., 2014a;b). Ingestion occurs when animals are unable to discriminate items, in other cases they confuse marine litter with prey (plastic bags/jellyfish), in certain cases, items work as support for attractive organisms (Barnes et al., 2009; Derraik, 2002; Mrosovsky, 1981; Schuyler et al., 2014b). The recent review of Kühn et al. (2015; 2016), show more than 500 animals threatened by litter, including planktonic organisms (de Lucia et al., 2014; Fossi et al., 2012), sea bird species (Spear et al., 1995; Van Franeker et al., 2011), fishes (Boerger et al., 2010; Lusher et al., 2013), marine mammals (de Stephanis et al., 2013; Baulch and Perry, 2014) and all species of sea turtles listed as globally vulnerable or endangered (IUCN 2013, Schuyler et al., 2014a).

Sea turtles

The loggerhead sea turtle (*Caretta caretta*, Linnaeus, 1758) is adopted worldwide as a bio-indicator of environmental conditions such as pollution (Foti *et al*., 2009; Keller *et al*., 2006) and it is the most abundant chelonian in the Mediterranean Sea (Casale and Margaritoulis, 2010; Margaritoulis *et al*., 2003). Presence of ingested anthropogenic debris in loggerhead, has been documented in Mediterranean Sea since many years (Casale *et al*., 2008; Lazar and Gracan., 2011; Tomàs *et al*., 2002). These studies analysed stomach contents of turtles showing, for the first time, presence of artificial materials in turtles diet, with a frequency of occurrence of 48,1%; 35,2% and 75,9% respectively. Nevertheless until 2011 none proposed the use of sea turtles as bio-indicator for marine litter ingestion.

In the last years different studies correlated the marine litter ingestion rate in sea turtles with level of sea pollution, confirming loggerhead as a target species for monitoring marine litter ingested by marine organisms in the Mediterranean (Camedda et al., 2014; Campani et al., 2013; Galganì et al., 2013a ; Matiddi et al., 2011;2017).

Comparison of data collected is difficult if different methods, different spatial and temporal scales, different size scales of litter items and different lists or categories of litter items recorded are used within the Regional Seas Convention and the EU as a whole.

For this reason a first harmonized protocol has been produced by the Technical Subgroup on Marine Litter (TSG-ML), an international group of experts established in 2010, by the Directorate-General for the Environment (DG ENV) to address gaps and further develop Descriptor 10 (Galganì et al., 2013a). The stomach contents of stranded Loggerhead sea turtles *Caretta caretta* (Linnaeus, 1758) are used to measure trends and regional differences in marine litter. The dissection procedure, measurement, and litter analysis are reported in the document, and they originated from a pilot study conducted during 2012 by ISPRA, CNR-IAMC Oristano, Stazione Zoologica Napoli; University of Siena and Arpa Toscana. The final results are presented in a scientific paper produced by Matiddi et al., 2017.

Methods consist in a dissection of death animals, empting the gastro intestinal tract on a 1 mm sieve. Marine litter categorization follow the Fulmar protocol (Galgani et al 2013a), and the items are subdivided into 4 main categories (IND-Industrial plastic, USE-User plastic, RUB-Non plastic rubbish, POL-Pollutants), including different subcategories, plus food remains (*Foo*) and natural non food remain (*Nfo*), (Table 1).

Table 1: Categories for classification of items ingested by Fulmar, in Galganì et al., 2013a.

BIOTA categories for contents of digestive tract *(oesophagus, stomach(s), intestine)*			
PLA	**PLASTIC**	**acronym**	all plastic or synthetic items: note number of particles and dry mass for each category
IND	**pellets**	**ind**	industrial plastic granules (usually cylindrical but also oval spherical or cubical shapes exist
	probab ind?	**pind**	suspected industrial, used for the tiny spheres (glassy, milky, ...) occasionally encountered
USE	**sheet**	**she**	remains of sheet, eg from bag, cling-foil, agricultural sheets, rubbish bags etc
	thread	**thr**	threadlike materials, eg pieces of nylon wire, net-fragments, woven clothing; includes 'balls' of compacted such material
	foam	**foam**	all foamed plastics so polystyrene foam, foamed soft rubber (as in matras filling), PUR used in construction etc
	fragments	**frag**	fragments, broken pieces of thicker type plastics, can be bit flexible, but not like sheetlike materials
	other	**Poth**	any other, incl elastics, dense rubber, sigarette-filters, balloon-pieces, softairgun bullets; objects etc. DESCRIBE!!
RUB	**OTHER**	**acronym**	any other non synthetic consumer wastess: note number of particles and (in principle) dry mass for each category
RUB	**paper**	**pap**	newspaper, packaging, cardboard, includes multilayerd material (eg Tetrapack pieces) and aluminium foil
	kitchenfood	**kit**	human food remains (galley wastes) like oinion, beans, chickenbones, bacon, seeds of tomatoes,grapes, peppers, melon etc
	other user	**rva**	other consumber waste, like processed wood, pieces of metal, metal air-gun bulletes; leadshot, painchips. DESCRIBE
	FISHHOOK	**hoo**	fishing hook remains (NOT FOR HOOKS ON WHICH LONGLINE VICTIMS WERE CAUGHT - THOSE UNDER NOTES)
POL	**POLLUTANTS (INDUS/CHEM WASTE)**	**acronym**	other non synthetic indusrial or shipping wastes (number of items and mass per category (wet for paraffin)
POL	**slag/coal**	**sla**	industrial oven slags ('looks like non-natural pumice) or coal remains
	oil/tar	**tar**	lumps of oil or tar (also not n=1 and g=0.0001g if other particles smeared with tar but cannot be sampled separately)
	paraf/chem	**che**	lumps or mash of unclear paraffin, wax like substances (NOT stomach oil!) if needed subsample and estimate mass
	featherlump	**rva**	lump of feathers from excessive preening of fouled feathers (n=1 with drymass) (NOT for few normal own feathers)
FOO	**NATURAL FOOD**	**foo**	various categories, depends on the species studied, and aims of study
NFO	**NATURAL NON FOOD**	**nfo**	anything natural, but which can not be considered as normal nutritious FOOD for the individual

The Fulmar protocol is the results of different studies made on *Fulmarus glacialis* (Linnaeus, 1761) in the North European Country (van Franeker, 2004; van Franeker *et al*., 2011), where it has been used as a target indicator for litter ingestion by biota for the OSPAR Sea Convention.
The system of Ecological Quality Objectives (EcoQO) has been formulated as: "There should be less than 10% of Northern Fulmars having 0.1g or more plastic in the stomach in samples of 50-100 beached fulmars from each of 5 different regions of the North Sea over a period of at least 5 years".

Even if the turtle potocol follow the fulmar one, actually there is not an approved EcoQO or GES for turtle, due to the lack of data on this specie in the Mediterranean Sea.
During the last year two proposals on a GES definition for litter ingested by loggerhead have been made by Matiddi et al. (2017). The first is formulated following the fulmar one : "There should be less than X% of Caretta caretta having Y g or more plastic in the stomach in samples of 50-100 stranded loggerhead from each sub-region" in which Y is the average value of plastic ingested by the collected turtles and considering for X% the number of turtles with more plastic grams than Y. The secondo proposal is formulated as: *There should be less than X% of Caretta caretta having more plastic grams than food remain (Foo) in the stomach in samples of 50-100 stranded loggerhead from each sub-region"* in which there is not a fixed value for plastic but considering if the sampled individuals were in good or bad health condition, due to litter ingestion, before strand.

Fish

Even if there is a growing body of publications on ingestion by fish during the last decade and many species are documented to ingest plastic, not only in laboratory experiments but also on field, actually there is not already a common protocol of analysis. The ones proposed in Galgani et al., (2013), did not considered all the variables, different biases and use of solvents. Difficulties due to the microplastic detection and contamination, the different fish trophic level (benthic, demersal, pelagic), the feeding behaviors and the size of the sampled fish are the main variance reported in the papers with a lack of harmonization. Moreover fish analysis showed a high variability of results, respect to geographical location of catches. Even if the direct visual inspection is the main reported method of microplastic detection, it is also the less accurate and highly subject to secondary contamination. In general main methods for micro detection in fish consider , dissection of fish and isolation of the gastrointestinal tract, degradation of the organic material and tissue whit acid or alkaline solvent, filtration of the solution under vacuum pump and observation of the membrane under stereomicroscope. Nowadays no one specie has been chosen, neither the lab method of analysis and detection have been harmonized at national or international level. The purpose of MEDSEALITTER project is to work on this direction in order to detect the better fish species able to show higher level of ingested plastic and establish an harmonized method for lab analysis.

Polychaeta

Marine litter is a global threat for living marine organisms, affecting biodiversity in different ways. Marine plastics have become an emerging issue because it is well documented that the ingestion of plastic fragments results in the entanglement and suffocation of hundreds of marine species. Microplastic (particles smaller than 1 to 5 mm) ingestion has been demonstrated in a wide array of marine organisms (Wright et al., 2013; Van Cauwenberghe et al., 2015a). Over 260 species, including invertebrates, turtles, fishes, seabirds and mammals, have been reported to ingest or become entangled in plastic debris, resulting in impaired movement and feeding, reduced reproductive output, lacerations, ulcers, and death (Derraik 2002; GEF, 2012; de Lucia et al. 2014). Many species ingest debris such as plastic, monofilament line, rubber and aluminium foil (Derraik 2002). Regularly, fishes (Boerger et al., 2010), birds (van Franeker et al., 2011), cetaceans (de Stephanis et al. 2013) and marine turtles (Campani et al. 2013; Camedda et al. 2014; Lazar and Gracan 2011) accidentally swallow micro/macro plastic often found in their digestive tracts.

Experimental studies reveal that microplastics were ingested by invertebrates occupying different marine habitats as amphipods, lugworms and barnacles, despite their differences in feeding method, detritivore, deposit feeder and filter feeder, respectively (Thompson et al., 2004). Uptake of microplastics can take place via normal ventilation processes (Watts et al., 2014) , or they can be directly ingested when mistaken as food (Thompson et al., 2004) and can further be transported within different marine food webs (Setälä et al., 2014). A large set of data regarding microplastic ingestion by marine invertebrates resulting by exposure in controlled laboratory conditions (usually at unrealistically high concentrations). It has been shown that invertebrates, such as polychaete worms, barnacles, bivalves, crustacean amphipods and decapods and sea cucumbers, can ingest microscopic plastics particles during laboratory trials (Graham and Thompson, 2009; Thompson et al., 2004). Microplastic uptake under field conditions in lugworm, mussels and oysters was demonstrated by Van Cauwenberghe et al. (2014, 2015b) and De Witte et al. (2014) . How to apply results from laboratory experiments to natural habitats is challenging, because organisms and their habitat interact with each other, as well as different organisms do with each other. Plastic ingestion has been reported primarily in the vertebrates. Investigations examining the impacts of plastics on benthic invertebrates are virtually nonexistent. Yet, if plastic fragments are common in the benthos, organisms foraging in sediment are prone to ingesting them, particularly organisms exercising deposit feeding and non-selective methods of particle capture. Moreover, plastic ingested by benthic foragers, many of whom occupy a low trophic level, may be a means of reintroducing settled plastic debris to littoral, nektonic, and pelagic food webs.

Polychaeta are abundant in all over the marine environment and cover a wide size range and are the dominant macrofauna within fine sediments. The presence or absence of specific Polychaeta in such sediments provides one excellent indication of the condition or health of the benthic environment. They have a short life-cycle, can be transported easily, and laboratory cultures of certain species can be maintained. They are readily available, easy to sample and include different trophic level with sedentary, mobile, and tube-building species. Polychaeta are good monitors of the presence of anthropogenic compounds in marine and accumulate deleterious materials within their tissues in concentrations proportional to concentrations found in their environment. This ability makes them good indicators of the presence and bioaccumulation potential of marine microplastic. Nevertheless the study of interactions between sea worms and microplastic reveal some technical issues not easy to face as selection of the best species to check microplastic ingestion, sampling, laboratory dissection of body part potentially involved in microplastic ingestion, gastro intestinal tract, or pre ingestion, filtration tract as crown of feeding tentacle. During Medsealitter activities interaction between Polychaeta and marine microplastic will be tested following established an harmonized methods applied for other well studied marine organisms as fish.

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