MEDSEALITTER

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

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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 3.3.2

Shared monitoring protocols
first draft of shared protocols for marine litter monitoring

Work Package 3 – Studying
Activity 3.3: Analysis and fine tuning of methods for monitoring Marine Litter

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Summary

INTRODUCTION

Reduction of marine litter 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), which became the main policy drivers of coastal and offshore waters monitoring. More recently, new EU directives specifically targeted the reduction of waste and asked monitoring programs to assess the 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 the Good Environmental Status (GES) by 2020, based on 11 qualitative Descriptors. Marine litter is the Descriptor 10 and, according to the Directive, 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 poses 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). 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 and to monitor the effectiveness of measures (Cheshire et al. 2009; MSFD Technical Subgroup on Marine Litter., 2013; Sheavly 2007; UNEP 2015).

The Mediterranean context

The Mediterranean Sea**,** considered one of the most affected seas worldwide for marine litter, but information 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, which prohibited the disposal of garbage at sea and leaded 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 centres (MAP/UNEP, 2001; UNEP 2015). Additionally, at-sea activities such as shipping and fishing can heavily contribute to the inputs of litter in specific contexts (Carić & Mackelworth 2014; Coe & Rogers 1997; Vlachogianni et al. 2016).
Floating macro litter is considered a pertinent indicator of the pressure of marine litter in the marine ecosystem: it is completely included in the marine compartment, is a "timeliness" (JRC 2008) indicator as include the first fraction of litter entering the sea (which only successively submerges and sinks to the sea bottom, is washed ashore, or is fragmented in micro particle), and can give indications on the main sources and sinks and pathway, and the effects of waste prevention measures (Thiel et al., 2013; Veiga et al., 2016). Since it is responsible for direct harm to marine species, monitoring macro litter can also help identify risky areas and seasons to design appropriate mitigation measures (e.g. Arcangeli et al., 2015; Di-Méglio and Campana, 2017). At Mediterranean level both the up to date documents of the MSFD and the

Barcelona Convention UNEP-MAP highlight the primary need for the assessment of litter pressure even in the surface layer compartment (Tab.1).

Tab. 1 MSFD and UNEP-MAP requirement on floating litter

COMMISSION DIRECTIVE (EU) 2017/845 of 17 May 2017 amending Directive 2008/56/EC of the European Parliament and of the Council as regards the indicative lists of elements to be taken into account for the preparation of marine strategies	**Theme: Substances, litter and energy** **Assessments of pressures** (Intensity and spatial and temporal variation of pressures on the marine environment and, if pertinent, at the source): Input of litter (solid waste matter, including micro-sized litter)
Marine Strategy Framework Directive (MSFD) Common Implementation Strategy 17th meeting of the Working Group on Good Environmental Status (WG GES) 10 March 2017	**Primary Criteria** Pressure: **D10C1** and D10C2 relate to the level of the pressure (litter and micro-litter) in the marine environment (coastline, **surface layer of the water column**, sea-floor and sea-floor sediment, as appropriate).
Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria UN Environment/MAP Athens, Greece (2017).	UN Environment/MAP will develop a specific Monitoring of floating litter protocol, on a regional basis. Common indicator: (17). Floating litter (items/km2) Min value = 0; Mx value = 195; mean value 3,9; Baseline 3-5.

Regarding the impact on biota, in the North European Seas the Northern Fulmar (*Fulmarus glacialis*, Linnaeus, 1761) has been used as a target indicator for many years, while in the Mediterranean Sea there has been no candidate species to be used as bio-indicator for litter ingestion until 2011. In 2011, DG ENV asked for a further development of the indicator and the adaptation of the methods implemented in the North Sea 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) was 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

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 (Galgani et al. 2013a; Zampoukas et al. 2014). Therefore, monitoring programmes should be consistent, coherent and comparable within marine regions. The choice of the most effective methodologies (cost-benefit approach, most appropriate indicator), as well as their 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).

Scope of the document

This document intends to describe methodologies and techniques already set for monitoring floating macro litter and litter ingested in biota, analysing in detail the parameters and covariates that can bias the results. The potential influence of the covariates and parameters involved in the monitoring methods are analysed and different experimental designs are proposed to be tested in the following WP4. Preliminary results of the pre-testing phase performed in WP3 are also reported as well as the requirements for the implementation activities that will be performed in WP4.
Considering that marine litter is widespread within the Mediterranean and that it affects both the offshore bodies of water and the coastal fringe, the proposed protocols are set considering the most effective

methodologies for these two spatial scales: large offshore bodies of water and the local coastal fringe. Moreover, the extreme variation in shape and size of marine litter also demands a multiscale approach so that the protocols are developed focusing both on the impact of macro and micro litter.

Giving the similarity of techniques involved, the document is organized in two sections dedicated to methods for floating macro litter monitoring (**Floating Macro Litter at large and local MPAs scales**) and for the analysis of litter ingested by indicators animal species (**macro and microlitter Ingested at large and local MPAs scales**). The floating macro litter methods are then explored considering first the general common settings of monitoring plans, and then the specific methodologies to be implemented for each platform type and/or technique.

1. MONITORING FLOATING MACRO-LITTER (FML) AT LARGE AND LOCAL MPAS SCALES

Scope of FML monitoring (for local and large geographical scale)

Following the legislative requirement, the information to be collected during monitoring programmes includes: 1) amount, distribution and composition of litter; 2) rates at which litter enters the environment (sources); 3) spatial and temporal variations; 4) impacts of litter. The protocols used need to be adequate to the information required, which depends on the goal of monitoring. Floating macro litter monitoring is indeed functional in order to:

- Evaluate trends
- Identify hotspots (seasonal and regional)
- Identify of pathways and geographical source areas
- Assess changes due to mitigation measures (long-term)
- Provide useful information to focus research and mitigation actions on specific areas, identify most sensitive areas for marine biodiversity, evaluate risks.

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 logistically more challenging (Ryan et al. 2009). The proposed methodologies take into account cost effectiveness, efficiency and sustainability of methods.

Variables and covariates influencing detectability of litter items

To evaluate amount, distribution and composition of litter the variables to be collected are number of items, size class, composition and geographical position. Many parameters (Covariates) influence the detectability and the identification of items other than the environmental parameters, such as prevailing winds, local and offshore currents, proximity to land based sources, etc.. All these elements have to be taken into consideration when comparing results at more detailed level (Tab. 1).

Tab. 1. Variables and covariates influencing detectability and identification of items

Variables	Covariates (observation parameters that could influence the sighting probability)
Number of items	a. Sampling design and period
	b. Type of platforms (height and speed)
Size class	c. Experience of the observers
Composition	d. Meteo and visibility conditions (Beaufort, wind direction, visibility, sun glare, etc.)
Geographical position	
	e. Strip width
	f. Size of items: lower size limit, classes
	g. Type and colour of items

Observation parameters (covariates) that could influence sighting probability

a. 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 a great temporal and spatial variability in litter loads in the sea compartments. Such variability requires a well-defined sampling design with sufficiently large replication in space and time to intercept these changes.

- **Site selection**. According to international guidelines, monitoring programmes should be consistent, coherent and comparable within marine regions and surveys. Giving the high heterogeneity of litter distribution, the criteria for the selection of site for survey could have crucial effect on results (e.g. special site selection on the basis of litter pollution levels, or a randomised selection of sites, UNEP-MAP 2016). Sampling should be stratified in relation to sources (urban, riverine outputs, offshore activities) to provide representative data in each location (Cheshire et al. 2009; Zampoukas et al. 2014) or it should cross expected low/high density areas to cover wide range of conditions (guidance completed by the MSFD technical subgroup on marine litter, Galgani et al., 2013).
- **Frequency of sampling**. 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. Seasonality can play a key role in the amount and distribution of litter, which is linked to seasonal variation in oceanographic and anthropogenic factors (Arcangeli et al., 2017). Pilot studies can be used to estimate variability in sample data, accompanied by successive power analysis to assess the most effective sample size necessary to detect a change (Ryan et al. 2009). Based on an exploratory data analysis on three years monitoring data from ferry using at least 5 replicates for season were used for collecting minimum samples data (Arcangeli et al., 2017)
- **Sample unit.** Surveys are usually based on transects, considered as sampling unit. The minimal length of the transect must be set in order to avoid bias due to small sample size.

b. Platforms of observation

As height and speed of the platforms affect visibility and the detection probability of litter (especially with regard to the minimum size of litter that can be detected and the effective strip width in which litter can be observed), a series of experiments are designed to define the appropriate combination of conditions (lower size/width of the strip) for each platform type. Different platforms of observation can be used:

- Ship based surveys:
    - Inflatable;
    - Sailing boat;
    - Ferries.
- Aerial surveys:
    - Drone;
    - Aircraft.

Floating marine litter can be monitored using different observation techniques:

- **Visual observation** of floating items is the most common methodology used and relies on competent, dedicated observers. 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 recording systems specifically set to acquire images from ships, aircrafts or drones, travelling along defined routes. In this situation, the bias is linked to observation conditions and the post-processing recognition of images.

Ship-based surveys. 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 from 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 sized 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; Arcangeli et al. 2017; Sà et al. 2016). In general, small boats can cover coastal waters, usually travelling at slow speed and detecting all items with at least one dimension bigger than 2.5 cm by naked eye (e.g. Day & Shaw 1987; Di-Méglio & Campana 2017; Gerigny et al. 2012; Thiel et al. 2003). The increase of observation height and vessel speed corresponds to a loss of ability to detect small size items. Large vessels, on the other hand can survey large open sea areas and provide data on larger size classes (>20 cm), considered adequate indicators for describing spatial patterns over larger scale (e.g. Sà et al. 2016; Arcangeli et al. 2017). 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).

Automated photographs can be obtained through cameras that, applied on platforms of opportunity as commercial vessels or cruises, acquire sea surface images in front of the ship bow during daylight (e.g. *SeaLitterCAM,* Hanke & Piha 2011; Galgani et al. 2013b).

Aerial surveys. *Aircraft*. To estimate the amounts of litter at sea, large scale monitoring programmes have been developed through aircraft surveys, even to locate areas of higher aggregations of litter (Lecke-Mitchell & Mullin 1992; Pichel et al. 2007; Unger et al. 2014). Aircraft surveys cover large areas but can detect only larger classes of items (i.e. the smallest size limit for aerial detection is *ca*. 30–40cm). Aerial surveys are considered valuable for detecting spatial differences in abundance, but the high costs of these surveys prevent from a large replication for monitoring changes over time (Galgani et al. 2013a; Ryan et al. 2009). Surveys are designed based on a line transect distance sampling technique (Thomas et al. 2010), but also strip transect are used especially when a multi-thematic monitoring is performed (e.g. French SAMM monitoring program, Laran et al., 2016). From aircrafts both visual observation and automatic detection techniques can be applied.

Aerial surveys. *Drone*. 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; the images are recorded permanently allowing subsequent statistic analysis (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. Adame et al. 2017; Hodgson et al. 2013; Koski et al. 2009). Two main categories of UAV can be used for marine monitoring, fixed-wing drones and multicopteres.

Apart from the 'traditional' RGB cameras, the use of thermic cameras and multi-spectral cameras is also being experimented for automated marine monitoring (Bryson & Williams 2015). The recognition analysis is performed afterwards, on the video/images acquired and 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).

c. Experience of observers

Giving the number of items to be recorded and the vast category types, only dedicated, experienced and well dedicated observers must be used during the monitoring. Experience of observers can in fact influence item detection and identification, leading to incoherent results.

d. Meteo/visibility conditions

Considering the need for a correct identification of items, a limit of Beaufort condition equal or lower than 2 is set generally for all the platforms. To avoid sun glare the monitoring is performed during central hours of daylight.

e. Strip width

Two methods can be applied:

- fixed width transects assume that all debris is detected within a pre-defined distance from the observer, considering a conservative strip width 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).
- Distance sampling methods assume that the perpendicular distance to each item has to be estimated to compensate for decreasing detection rate with increasing 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 the accurate definition of the monitored strip width or of the distance between the objects and the observers, measures that can be obtained with simple tools, as an inclinometer or range finder (Ryan 2013). Using the strip transect, however, the complexity of measuring is limited only to maximum two distances (the inner and outer edge of the strip) which stays fixed for all the survey.

f. Size of litter (lower size limit; classes)

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 on 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). The identification of size classes is applied in data collection.

- ***Lower size limit***: the minimum size of detectable litter depends on the type of platform used and in particular on the height of the observation point and the speed of the platform. The lower size limit will be defined for each platform type during the testing phase.
- ***Classes***: following MSFD guidelines, during monitoring, macro-litter will be divided into 7 classes:
  - (A: <2.5)
  - B: 2.5-5 cm;
  - C: 5-10 cm;
  - D: 10-20 cm;
  - E: 20-30 cm;
  - F: 30-50 cm;
  - G: 50-100 cm;
  - H: >100 cm.

g. Type and colour of objects

Field guides and litter identification tools are important to keep sampling consistency. 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). We suggest to specifically include to the list: 1) the fish aggregating device (FAD), as its origin is defined (in Sicily and in the Balearic Islands), the identification of this category can help tracing potential drifting routes; 2) the “natural debris” category (e.g. terrestrial and seaweed/marine plants), to compare seasonal/spatial differences between the litter and the organic fractions. In addition being most of the times the source of natural debris clear (terrestrial or marine) in can help tracing the drifting routes.

Master list and other parameters to be recorded

Following the above considerations and the pre-testing activities the following sheet is proposed as basic data collection sheet. The modifications with respect to the MSFD-TSG on Marine Litter report “Guidance on Monitoring of Marine Litter in European Sea” masterlist and proposed parameters are indicated, as well as the corresponding codes of JRC and UNEP (MSFD Technical Subgroup on Marine Litter, 2013; UNEP, 2009).

Data sheet		**Modification respect to JRC Masterlist on floating**	**JRC Code**	**UNEP-MAP Code**
M: from sea T: from land I: indet.				
P=fishing; F=other Food; S=sanitary; C=cosmetics; M=maritime; A=other; I=indeterm.		++		
‘+’ positive; ‘0’ neutral; ‘-’ negative		++		
Artificial polymer materials	Sheets		G67	PL16
	Bags		G2	PL07
	Polystyrene boxes	not all are fishing related	G58	PL17
	Plastic boxes	not all are fishing related	G57	PL17
	Bottles		G6	PL02
	Buoys		G63	PL14
	Buckets	+	G65	PL03
	Gloves	Aggregated	G39, G40, G41	PL09, RB03
	Beach-coastal amenities	++		
	Crates containers/baskets		G18	PL13
	Ropes		G48	-
	Foam/polyurethane		G74	-
	Jerry cans	+	G16	PL03
	Nets and lines	Aggregated	G51, G52, G53, G54, G55, G56, G59	PL20, PL18
	Mussel nets/ Oyster nets	+	G45	PL15
	Covers/packaging		G38	-
	Tableware	+	G34	PL04
	Tablecloth		G94	-
	Six-pack rings/strings	+	G1	PL05
	Sanitary towels strip	+	G96	OT02
	Other		G124	PL24
Glass	Bottles	+	G200	GC02
	Other	+	G210	GC08
Pr. Wood	Boards/Beams	Aggregated	G168, G159	-
	Pallets/Crates	Aggregated	G160, G162	WD04, WD04

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	Other		G173	WD06
Metal	Spray cans		G174	-
	Drums/barrels	Aggregated	G187, G192	ME05
	Cans food and drinks	Aggregated	G175, G176	ME03, ME04
	Wire/mesh		G191	ME09
	Alluminium foil/container	++ container	G177	ME06
	Other		G197	-
Texile	Clothing		G135	CL01
	Sail/Canvas		G143	CL03
	Carpet & Furnishing		G141	CL05
	Sanitary towel/tampons		G144	OT02
	Other		G145	CL06
Paper	Paper packaging/Bags		G149	PC03
	Cardboard		G148	PC02
	Carton/Tetrapack	+	G150, G151	PC03
	Newspapers/magazin e		G154	PC01
	Other		G158	PC05
Rubber	Tyres and belts		G128	RB04
	Balls		G126	RB01
	Balloons		G125	RB01
	Boots		G127	-
	Other		G134	RB08
Natural Organic	Seaweed/marine plant	++		
	Logs/plants parts	++		
	Other	++		
A <2,5; B=2,5-5; C=5-10; D=10-20; E=20-30; F=30-50; G>50; H>100; X>20			2,5-5; 5-10; 10-20; 20-30; 30-50	
W=white; T=trasparent; C=colored (specify)		++		

+ = inserted in floating

++ = added to the masterlist

SURVEY METHODS PER OBSERVATION PLATFORMS/TECHNIQUES

I. FERRY – LARGE VESSELS

Position of observer. Sampling is performed by a dedicated, experienced observer positioned on one side of the vessel in the vicinity of the bow (for example on the bridge, in the case of large ships), to have the best visibility of the strip of the sea avoiding the turbulence generated by the bow itself. The side to be sampled is the one with greater visibility, for example with fewer reflections on the water and the sun behind.

Meteo conditions. Monitoring is carried out with sea state ≤ 2 on the Beaufort scale.

Speed of the vessel. The speed of the vessel should not exceed 27 knots for an observation height about 15/25 m. It is important, however, also to take into consideration the frequency of occurrence within the strip of the recorded objects. Speed, in fact, does not affect the survey if the there is time to identify and take record of the items crossed by.

Fixed observational strip width. At the beginning of each transect, the observer defines *a priori* the width of the strip to be monitored from the ship up to a maximum of 100 m. This distance is defined a priori but could be smaller in relation to the type of craft in which the observer is located (height and speed), the force of the sea and the visibility: the basic assumption of the method is to define a strip width such that the observer can detect, to the naked eye in all the fixed strip surface, items equal or larger than 20 cm passing through. The width of the strip must be scored on the board. The upper and lower limit of the fixed observational strip are calculated using a measuring stick or a range finder and are continuously controlled during the survey to assure that only items spotted within the fixed strip are recorded. The measurement can be initially calibrated with a known distance in the port or from a known point of the ship itself. The strip can start from the very edge of the ship, if it is visible, or from the first point detectable by the observer. The distance of the inner edge and the outer edge of the strip to the route is indicated on the data collection sheet. Though with a graduated stick or just with scotch tape on the glass of the command deck this distance range can be easily have under control.

Equipment. The necessary equipment consists of: binoculars, dedicated GPS, measuring stick/range finder/inclinometer, digital camera and recording data sheet/tablet or computer with the dedicated app. The observation is made mainly to the naked eye and binoculars are used to confirm the sighting of litter larger than 20 cm.

The GPS is used to record the track of the monitored transect, to mark the opening and closing of the transect and the waypoints that indicate the position of the sighted objects.

The observer uses the data collection sheet or the dedicated app to note the characteristics of the debris observed, such as:

- composition: the main materials listed (or first level) are plastic (polymer artificial), glass, wood, metal, rubber, paper and textile (in line with OSPAR, UNEP and TSG_ML). For each type of material, the category (general name or second level) is then identified in more detail. Sightings that do not fall into the categories are scored as OTHER and described by the observer. For the plastic material, there is also a third level classification for Bags, Polystyrene and bottles. If a FAD is detected, its floating components (plastic) shall be noted in the main board, while its description in the back of the data sheet. The presence of natural organic material on the surface, such as logs (from land) or seaweed (from sea), should also be noted, as it can provide information on currents and combinations of materials in the study area.

- source: the observer notes, when possible, the probable origin of the litter observed, indicating whether the source is "from land", "from sea" or "indeterminate", and to what industry is linked. For a plastic bottle, for example, origin is unspecified, since it could have become waste on the ground (abandoned on the beach or from a river) or by the sea (thrown from a boat). On the contrary. a box of Styrofoam can be traced, presumably, to the fishing industry.
- buoyancy: defined as positive when the debris emerges whole or in part from the surface; as negative, when it is completely submerged; neutral when it is aligned to the surface of the sea.
- dimensions: according to the main assumption of the protocol (every item that goes inside the fixed strip has to be seen) the minimum size of items is 20 cm (length of one of the three sides of the object). The size classes used are those suggested by MSFD-TSG on Marine Litter report "Guidance on Monitoring of Marine Litter in European Sea". The class of an object can be known in advance (for example a bottle for beverages 50ml is classified in the class E, 20 - 30 cm), or if there are uncertainties between the limits of classes, widened classes as X> 20 cm can be used.
- The presence within the strip of cetaceans, turtles and other marine organisms larger than 20 cm (or in aggregations larger than 20 cm; e.g. jellyfish-gelatinous plankton) should also be recorded.
- The collected data are stored on dedicated excel sheets, useful for the analysis of the results, while data recorded by GPS will be processed with georeferencing programs.

Visual surveys data sheet MEDSEALITTER

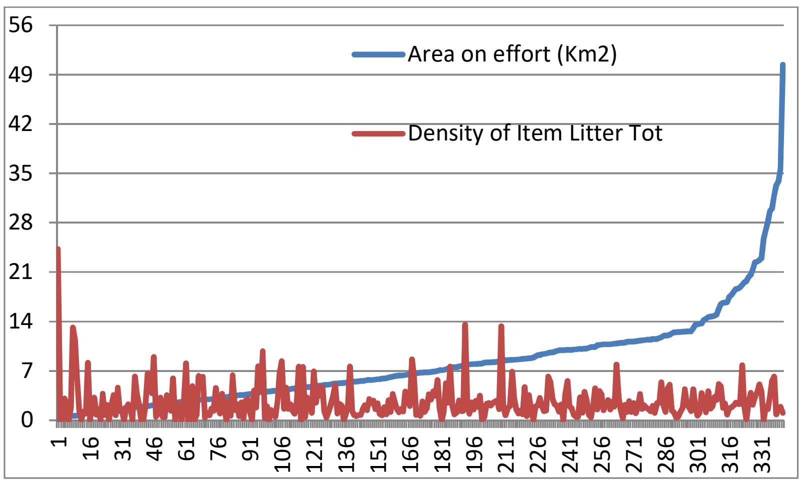
Date		COD Effort		Ship		Observer		Sea state		Speed	
Strip widht	mt= 25; 50; 75; 100	BEG (Hour + lat-long):				END (Hour + lat-long):					

Cod GPS/TIME GPS	Source	Industry	Buoyancy	COMPOSITION																																																		SIZE	Colors	State
				Artificial polymer materials																					Glass		Pr. Wood					Metal					Texile				Paper					Rubber					Organic.					
	M: from sea T: from land I: indef.	P=fishing; F=other Food; S=sanitary; C=cosmetics; M=maritime; A=other; I=indeterm.	+ positive; ' 0 neutral; '- negative	Sheets	Bags	Polystyrene boxes	Plastic boxes	Bottles	Buoys	Buckets	Gloves	Beach-coastal amenities	Crates containers/baskets	Ropes	Foam/polyurethane	Jerry cans	Nets and lines	Mussel nets/ Oyster nets	Covers/packaging	Tableware	Tablecloth	Six-pack rings/strings	Sanitary towels	Other	Bottles	Other	Beams/Dunnage	Wood boards	Pallets	Crates	Other	Spray cans	Drums/barrels	Cans food and drinks	Wire/mesh/foil	Other	Clothing	Sail/Canvas	Carpet & Furnishing	Other	Paper packaging/Bags	Cardboard	Carton/Tetrapack	Newspapers/magazine	Other	Tyres and belts	Balls	Balloons	Boots	Other	Seaweed/marine plant	Logs/plants parts	Other	A <2,5; B=2,5-5; C=5-10; D=10-20; E=20-30; F=3 50; G>50; H>100; X>20	W=white; T=trasparent; C=colored	E= Entire (def.); F=Fragments; FAD

Ferry - Preliminary results and indication for implementation of monitoring guidelines

1. Sampling design (ref. covariates: a. Sampling design)

Based on three years of monitoring data from ferries along different fixed transects distributed in the all Mediterranean sea region (Arcangeli et al., 2017) an exploratory data analysis was performed to verify the minimum effort needed to avoid biases due to outlier values. Results confirm the high variability of samples expressed as density values (number of items / km^2 of effort), and are likely linked to the natural variability of the phenomenon. No correlation is found between the effort and the density values (r=-0.034) and no trend is shown (fig.1). The boxplot highlights outliers over the density value of 7 item/km^2 on effort so that the limit of 7 Km^2 of effort could be considered as the minimum effort needed per survey to avoid outliers values.



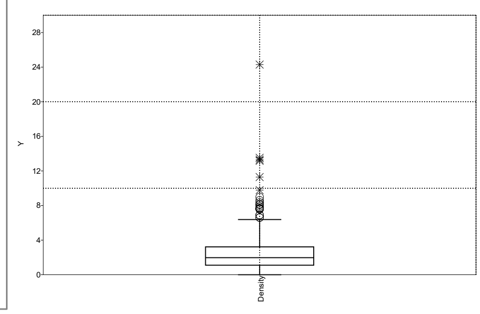


Fig 1 area surveyed on effort and density of recorded item (left) boxplot with outliers of density values (right)

Considering the effort needed to record the categories, a preliminary analysis using the macro categories was performed. The maximum number of material categories are 7 and results show no trend among km^2 of effort and the number of categories recorded (fig 2) so that the all 7 categories are recorded even in low effort (fig 2, table 1 and 2).

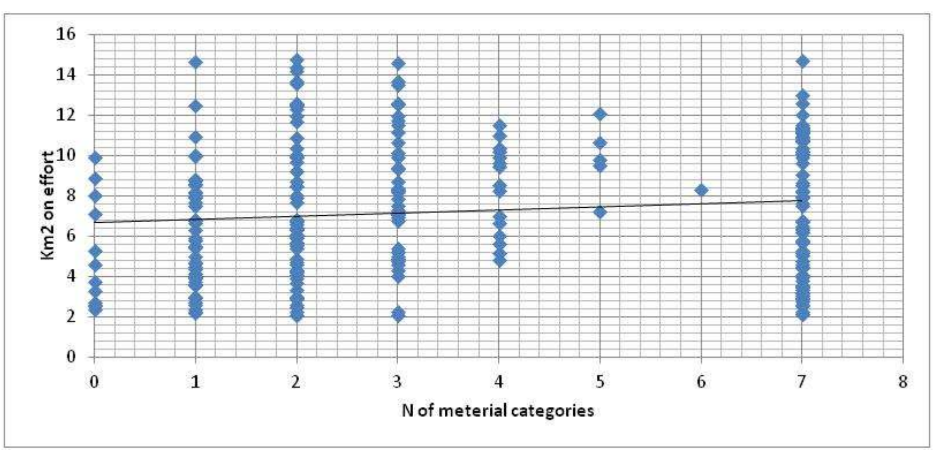


Table 1 N of material categories (n= 7) / Km2 on effort

	0	1	2	3	4	5	6	7
N	18	62	80	46	21	7	2	105
Min	0,102812	0,041148	0,607526	0,980176	4,814778	7,204486	8,324062	0,552999
Max	25,78305	33,26641	50,40526	33,80999	35,68714	20,304	31,85628	18,6406
Mean	**5,015139**	**6,212926**	**9,194115**	**9,904986**	**12,99623**	**12,33001**	**20,09017**	**6,268696**
Std. error	1,409117	0,665858	0,852516	0,975168	1,865201	1,729489	11,76611	0,398129

Table 2 mean N of material categories per > or < 7Km2 of effort

0	<7_Km2_effort	>=7_km2_effort
N	178	163
Min	0	0
Max	7	7
Mean	**3,511236**	**3,687117**
Std. error	0,200969	0,178467

Protocol tested on floating marine litter from ferry (Report of test on ferry, summer 2017- EcoOcean Institute and MPA Capo Carbonara - Villasimius).

The protocol developed by the FLT, based on the classification and size classes of the MSFD-TSG on Marine Litter masterlist (see Annex macro-litter sheet), has been used. The method of strip transect was used. The macro-litter observations were collected in a strip of 100m maximum width when the wind force was less or equal to 2 Beaufort Scale.

The pre-testing phase is described below according to the previously covariates (e.g. strip width and size of litter/item).

Montpellier 19 September 2017, debriefing after the trip: observers have found several problems in estimating the size of litter and the strip width.

2. Strip width for floating marine litter (ref. covariates: e. Strip width)

EcoOcean. To determine the 100m strip width, an inclinometer was used. The angle for each ferry used was calculated based on the height of the eye of the observer (height command deck+height observer) (Table1). A mark was set on the glass of the command deck to maintain the same bandwidth between the different observers.

Tab 1: Parameters of the different ferries to determine the angle for a 100 m bandwidth.

	Height of the ferry+observer (m)	Angle (°) read in the inclinometer for a bandwidth of 100 m
Mega Express 1	28.2	15.7
Mega Express 2	23.2	13.1
Mega Express 3	21.55	12.2
Mega Express 4	21.2	12.0

Mega Express 5	23.2	13.1
Mega Smeralda	39.2	21.4
Mega Andreas	39.2	21.4

Problem. The strip of 100m is too wide and forces the observer to sweep with its head with the risk of losing visual contact with the whole strip, leading to missing litter at the speed of 22 to 26 knots. This results in a non exhaustive count of the waste passing in the strip.
Solution: we measured the natural angle or field of view of an observer, so that he has not to move the head, and can be sure to see everything in the strip. This angle is 60°. This angle can be measured with an inclinometer (30°).
Improvements proposed for the protocol:
The angle of view to observe will be constant, measured with an inclinometer (30°) and the width of the strip will be calculated afterward, depending on the height of observation (Tab. 2).

Tab. 2: Width of the strip depending on the height of ferries

	Height of observation (m)	Width of the strip (m) for an angle of view of 60° (30° measured with inclinometer)
Mega express 1	28.2	48.8
Mega express 2	23.2	40.2
Mega express 3	21.55	37.3
Mega express 4	21.2	36.7
Mega express 5	23.2	40.2
Mega Sméralda	39.2	67.9
Mega Andreas	39.2	67.9

- **Influence of the position of observer (on front or on side) on strip width :** in order to define the best strip width, the position of the observer on the bat was taken in consideration, as this could influence the size of the observational strip.

2.1. EcoOcean. Observation on the front or on the side for the strip width was carried out:
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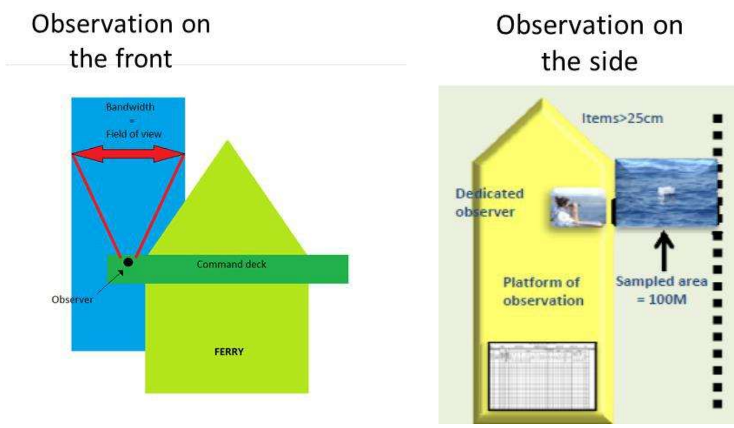


Figure 1.Scheme of observation on the front or on the side for the strip width

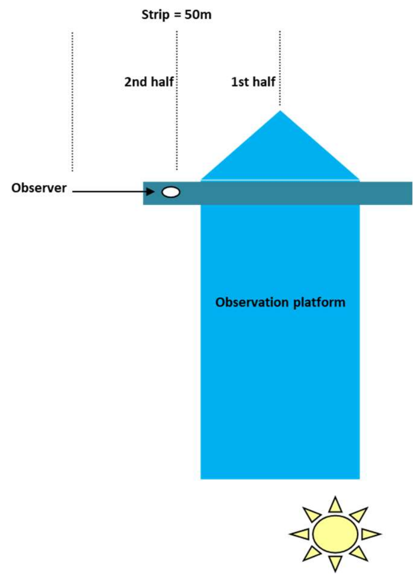
For an observation on the front of the ferry, two marks on the glass, one left and one right, will limit the strip. For example, for the Mega express 4 (height of observation 21.2 m), the 60° angle of view corresponds to a distance between both marks of 81 cm on the glass, for a distance eye/glass of 71 cm. The width of the strip at the surface of the sea reaches 24.19 m, calculated as:

$$\textbf{\textit{width of the strip}}\ (\boldsymbol{s}) =$$
$$\textbf{\textit{Height of the observation x size measured with the ruler / distance between eye and glass}} =$$
$$\mathbf{21.2}\ \boldsymbol{x}\ \mathbf{0.81 / 0.71} = \boldsymbol{24.19\ m}$$

In the case of an observation on the side of the ferry, the limit of the strip will be represented by a mark on the glass (for an observation inside the command deck).

2.2. MPA Capo Carbonara. Observation on the front of the ship was carried out on ship “BONARIA” of the Tirrenia Shipping Company

The strip was 50 metres wide and the zero was placed on the ship bow. During the monitoring the selected strip was divided in two parts: the 1st half, from the bow to outward, ranging between 0 and 25 metres, the 2nd ranging between 25 and 50 metres and the observer was positioned on side wing above the strip, like in the picture of Fig 1a. Paired T-Test and the Wilcoxon rank test, on all size categories pooled together, was performed together with One Way Anova and Mann-Whitney aiming to test the hypothesis of no differences on observed items between the two parts. Correlation of marine litter between first and second half of the strip was analyzed.



Density of marine litter:

D = n/(w x L)

n: number of items observed
w: width of the strip
L: length of the strip (Km)

Figure 1a.Scheme of observation on the front or on the side for the strip width

Dividing the width of 50 meters into two parts of 25 meters to compare the density we obtained:

ID-effort	Items 1st	Items 2nd	Area on effort	Density 1st	Density 2nd
CAPAL_2017_1	34	41	8,123881825	4,19	5,05
CAPAL_2017_2	11	6	3,994966525	2,75	1,50
CAPAL_2017_3	55	50	8,931015925	6,16	5,60
CAPAL_2017_4	20	21	4,41636395	4,53	4,76
CAPAL_2017_5	32	62	8,2691421	3,87	7,50
CAPAL_2017_6	14	8	3,583601225	3,91	2,23
CAPAL_2017_7	27	47	7,83828885	3,44	6,00
CAPAL_2017_8	6	18	4,0977469	1,46	4,39
CAPAL_2017_9	31	55	7,058403125	4,39	7,79
CAPAL_2017_10	11	24	4,137782225	2,66	5,80
CAPAL_2017_11	44	71	7,67085155	5,74	9,26
CAPAL_2017_12	18	25	7,234840375	2,49	3,46
tot	303	428	75,35688458	4,02	5,68

Tab. 3. Density of the items recorded on the 1st and the 2nd half of the strip.

While the paired comparison test showed a significant difference between the number of objects observed in the two parts the unpaired tests showed no significant difference

3. Size of items (ref. covariates: f. Size of litter)

EcoOcean. To determine the size of the litter, the "apparent size" is used, that is, the size obtained by holding a rule in front of the observer and measuring its apparent size.

$$\textbf{Real size } (s) = \textit{Height of the observation x size measured with the ruler / distance between eye and ruler}$$

With a distance eye-ruler of 30 cm, the following apparent sizes (measured) are shown in Tab. 4.

Tab. 4: Apparent size of the litter

Ferry	Height of the ferry+observer (m)	Size of the litter at the surface of the sea (real size) 20 cm	30 cm	50 cm	100 cm	
M.E. 1	28.2	0.21	0.32	0.53	1.07	Apparent size (measured with the ruler) (cm)
M.E. 2	23.2	0.26	0.39	0.65	1.30	
M.E. 3	21.55	0.28	0.42	0.70	1.40	
M.E. 4	21.2	0.28	0.43	0.71	1.42	
M.E. 5	23.2	0.26	0.39	0.65	1.30	
M.Smeralda	39.2	0.15	0.23	0.38	0.77	
M.Andreas	39.2	0.15	0.23	0.38	0.77	

Several parameters can influence the calculation: the distance of litter and the distance between the eye and the ruler. We tested the influence of each parameter on the resulting size of the litter:

- **Influence of the distance of the observation (height of the observer).** We took into account the vertical height of the command deck and the observer's viewing height for the Mega Express 2: 21.6m + 1.6m = 23.2m.
  - ***Influence of the observer height (eye).*** The height of the command deck is constant (21.6m). The eight of the observer can vary. We tested the difference for heights of observers (measured to their eyes) ranging between 1.50 and 1.80 m. Distance between eye and ruler is also constant in that case (30cm). There is very little variation in the apparent size of the waste depending on the observer height (Tab. 5).

Tab. 5: Variation of the apparent size of litter depending on the variation of the height of the observer

	Height of the observer's eyes	Size of the litter at the surface of the sea (real size) 20 cm	30 cm	50 cm	100 cm
Apparent size (cm)	1.5 m	0.2564	0.3846	0.6410	1.2821
	1.8 m	0.2597	0.3896	0.6494	1.2987
	standard deviation	0.0010	0.0015	0.0025	0.0050

The variation is less than a millimeter and is therefore not visible to the naked eye. The influence of the size of the observer is negligible.

  - ***Influence of the position of the litter within the strip.*** To test the influence of the position of the litter, the apparent size is calculated for positions between 0m (vertical to the ship) and 100m (end of the strip) with a pitch of 5m distance (Tab. 4). The height of the ship (21.6m), the observer's eye height (1.6m) and the distance between the eye and the ruler (30cm) are constant.

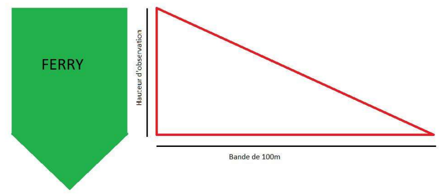


Figure 2: Scheme of the strip of observation (100m)

Tab. 6: Influence of the position of the litter within the strip, on its apparent size

Position within the strip (0 m= vertical 100 = 100 m away)	Distance observer-litter (cm)	Apparent size of the litter at the surface of the sea			
		20 cm	30 cm	50 cm	100 cm
0	2320	0.26	0.39	0.65	1.29
5	2373	0.25	0.38	0.63	1.26
10	2526	0.24	0.36	0.59	1.19
15	2763	0.22	0.33	0.54	1.09
20	3063	0.20	0.29	0.49	0.98
25	3411	0.18	0.26	0.44	0.88
30	3792	0.16	0.24	0.40	0.79
35	4199	0.14	0.21	0.36	0.71
40	4624	0.13	0.19	0.32	0.65
45	5063	0.12	0.18	0.30	0.59
50	5512	0.11	0.16	0.27	0.54
55	5969	0.10	0.15	0.25	0.50
60	6433	0.09	0.14	0.23	0.47
65	6902	0.09	0.13	0.22	0.43
70	7374	0.08	0.12	0.20	0.41
75	7851	0.08	0.11	0.19	0.38
80	8330	0.07	0.11	0.18	0.36
85	8811	0.07	0.10	0.17	0.34
90	9294	0.06	0.10	0.16	0.32
95	9779	0.06	0.09	0.15	0.31
100	10266	0.06	0.09	0.15	0.29

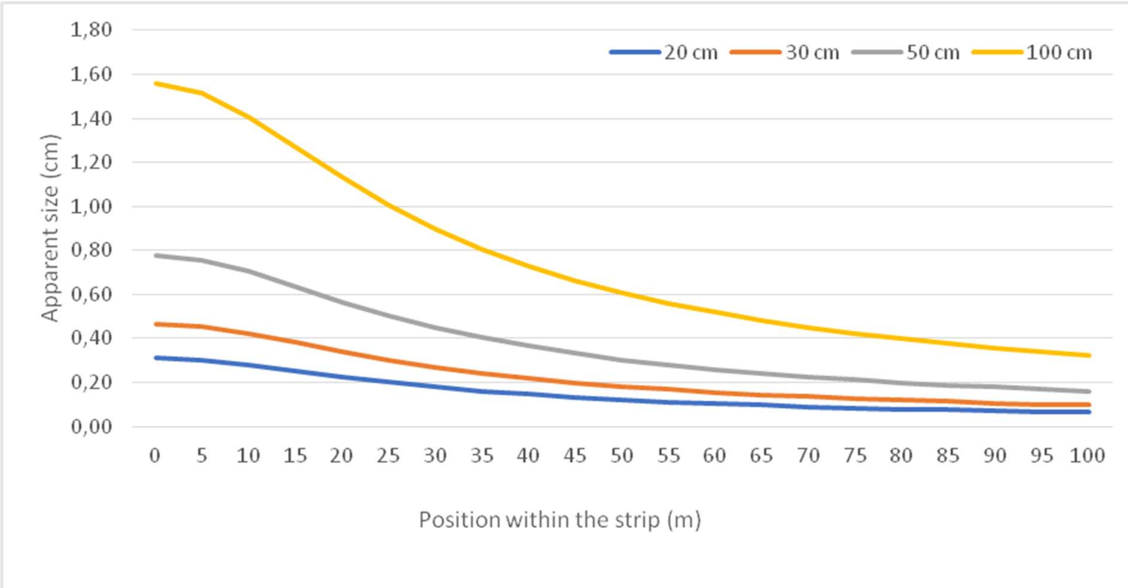


Figure 3: Influence of the position of the litter within the strip, on its apparent size, for 4 different real sizes (20, 30, 50 and 100 cm).

The apparent size of the litter varies greatly according to its position within the strip (Tab. 7 and Figure 3) and the real size of the litter too (Tab. 7).

Tab. 7 : Variation of the apparent size depending of the position of the litter within the strip

Position within the strip (0 m=vertical, 100 = 100 m away)	Height of observer +command deck	Size of the litter at the surface of the sea (real size)			
	(cm)	20	30	50	100
0	2320	0.26	0.39	0.65	1.29
100	10266	0.06	0.09	0.15	0.29
	Standard deviation	0.067	0.100	0.167	0.334

The position of the litter within the strip will lead to an underestimation of size and confusion in the size classes. The influence of the position is quite important and requires an improvement of the protocol.

We suggest setting a fixed area within the strip where we will measure the apparent size of the litter with the ruler. For example, for the Mega Express 4, with an observer on the front, we considered the nearest part of the strip for the measurement. We measured the angle of this sector with an inclinometer: this sector was at 48°. Each litter coming within the strip was measured at this position. This angle will be used to calculate the real size of the litter.

The fixed sector to measure the litter is easy to implement to observations from the front of the ferry. We suggest observing on each ferry only from the front.

- ***Influence of the distance between the eye and the ruler.*** To test the influence of the distance between the observer's eye and the ruler, the apparent size is calculated for distance between 10 cm and 80 cm with a pitch of 5 cm. The observation height (23.2m) is constant.

Tab. 8 : Influence of the distance between the eye and the ruler, on the apparent size of the litter

	Distance eye-ruler (cm)	Size of the litter at the surface of the sea (real size)			
		20 cm	30 cm	50 cm	100 cm
Apparent size(cm)	10	0.09	0.13	0.22	0.43
	80	0.69	1.03	1.72	3.45
	Standard deviation	0.1928	0.2891	0.4819	0.9638

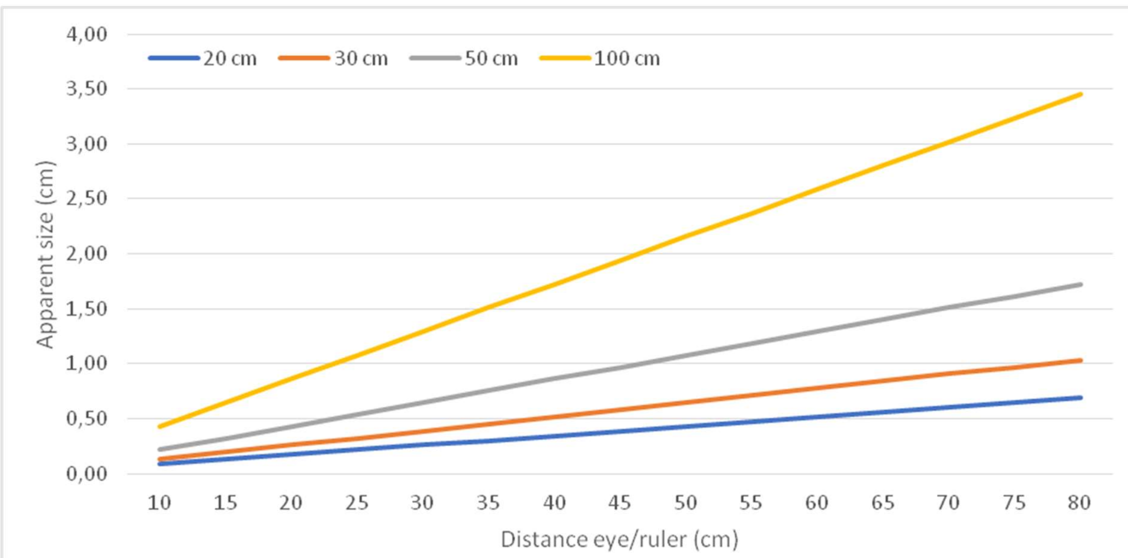


Figure 4 : Influence of the distance between the eye and the ruler on the apparent size of the litter, for 4 different real sizes (20, 30, 50 and 100 cm).

The apparent size of the litter varies greatly according to the eye / ruler distance (Tab. 8 and Figure 4). The greater the distance between the eye and the ruler, the greater the apparent size. The variation of the apparent size is higher as the distance between the eye and the ruler increases (Tab. 8).
The distance between the eye and the ruler will cause an over or under-estimation of the size of the litter and thus confusion in the size classes. The influence of the distance eye / ruler is important and requires an improvement of the protocol

To set a fixed distance between eye and ruler, whatever the observer, we suggest attaching the ruler to a cord that will be put around the neck.

4. Marine litter sheet (Categories g. type and colour of objects).

EcoOcean experience. The sheet appears to have too many categories and is not quickly "fillable", especially when the speed is over 20 knots, which poses problems when the density of macro-litter is high. We suggest simplifying the sheet as the example in annex. We suggest also trying with a tape recorder.

Annex: Data sheet

Nom du Bateau:		Date:		Fiche n°		Côté du ferry:	Droit / Gauche

Paramètre de difinition de la bande:

Largeur de bande au niveau de la fenêtre:		Distance entre l'œil et la fenêtre:		Secteur de lecture de la taille du déchet	

Heure	Nb	Taille lue sur la règle (cm)	Flottaison	Couleur	Etat	COMPOSITION Type de déchets: Plastique, polystyrene, verre, bois, métal, textile, papier, caoutchouc et organique					
			+ = positive 0 = neutral - = negative	B=Blanc; T=transparent; C=coloré	E= Entier ; F=Fragments; C=Concentration	Sac plastique	Emballage plastique	Bouteille plastique	Polystyrene	Autre	Description / Commentaire sur le nature du macrodéchet

MPA Capo Carbonara experience. It is not easy to fill the data on the sheet when the density of macro-litter is high; it is suggested to modify the sheet as the example in annex.

Date:		Cod effort:		Ship:	name of the ship and height of the bridge	Speed:		Side	
								Obs	
BEG (lat-long):				END (lat-long)					

Cod GPS/TIME GPS	Source	Industry	Buoyancy																																																				SIZE	Colors	State
	M: from sea T: from land I: indef.	P=fishing; F=other Food; S=sanitary; C=cosmetics; M=marittime; A=other; I=indeterm.	+ positive; ' 0 neutral; '- negative	CATEGORY								SUBCATEGORY																																										D=20-30; E=30-50; F>50; H>100; X>20	[illegible] T = transparent; C = colored	E= Entire (def.); F=Fragments; FAD
				Artificial polymer materials	Glass	Pr. Wood	Metal	Texile	Paper	Rubber	Organic	Bags	Balloons	Balls	Beach-coastal amenities	Beams/Dunnage	Boots	Bottles	Buckets	Buoys	Cans food and drinks	Cardboard	Carpet & Furnishing	Carton/Tetrapack	Clothing	Covers/packaging	Crates containers/baskets	Drums/barrels	Fish box (plastic)	Foam/polyurethane	Gloves	Jerry cans	Logs/plants parts	Mussel nets/ Oyester nets	Nets and lines	Newspapers/magazine	Pallets	Paper packaging/Bags	Polystyrene boxes	Polystyrene other	Rope, string and nets	Sail/Canvas	Sanitary towels	Seaweed/marine plant	Sheets	Six-pack rings/strings	Spray cans	Tablecloth	Tableware	Tyres and belts	Weight, sinkers, hooks (P)	Wire/mesh/foil	Wood boards	Other			

II. SAILING BOAT

The protocol to be used in sailing boat refers to the one used for ferry/large vessels except for the lower size limit of items which is set at 2.5 cm.

Basing on the pre-testing activities performed on sailing boats, consideration must be given to:

- Identification of lower size limit, especially by non-expert observers
- Setting of the strip width to a max of 10 m
- Use of a vertical graduate stick, indicating only the upper and intermediate limits of the strip

Sailing boat - preliminary results and indication for implementation of monitoring guidelines

1. ISPRA together with Legambiente set some experiments for floating litter protocol/data collection during the Goletta Verde campaign in July 2017. In particular, experiments were performed with regard to the definition of observation parameters from sailing boat. Characteristics of Goletta Verde: observer height above sea level: 2 m; mean speed: 6-7 kn.
2. EcoOcéan Institut set some experiments using a pole or an inclinometer to delimit the strip width.

3. Experience of observers (ref. covariates: c. Experience of the observers)

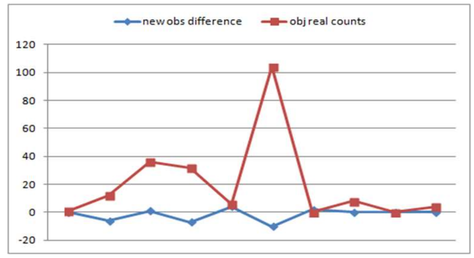
Experiments were set to evaluate how the detection probability is related to the experience of the observer and set minimal experience levels.

Fig. 1 Double observers experiment

Data collection was performed synoptically by two observers within the same monitoring conditions in order to compare detection probability between experienced and inexperienced observer. Tests were carried out in two separate days of sailing, using 4 inexperienced observers who were told to look for floating objects within a 10 m strip at the side of the boat. Each session lasted 15 minutes, to avoid fatigue, with the experienced observer conducting the monitoring behind the new observers to avoid any influence on their detection ability (Fig. 1).

A total of 10 replicas were carried out, in areas with different pollution conditions (from 0 to 104 floating items recorded). The inexperienced observers resulted to have good detection ability in these conditions (10 m strip, sea state 2), with only 1% less items detected then the experienced one. Even information about material was coherent between the two observers, but no further detail was asked to the new observers (this surely requires more training). The main difficulty for the new observers was setting the minimum size to record.

Difference in the number of objects detected by the two observers



NEXT: further experiments would be necessary using an experienced observer and a camera recording the same strip: the video would be analysed successively to verify the accuracy of the automatic recording compared to the direct observation.

4. Strip width for floating marine litter (ref. covariates: e. Strip width)

Esperiments will be set to evaluate the influence of strip width in the detection probability, to define the appropriate strip width for each platform type and/or to calculate the correction factors for the different platforms (height of the observation, speed).

Comparison of data from the first and second half of a fixed strip to obtain the correction factor.

We compared the number of objects seen within the 10 m strip and beyond, up to a maximum distance of 25 m. We used data from the 11 transects performed in the first period of monitoring from Goletta Verde. Of all floating objects seen, 11.3% were detected beyond the 10 m strip and among these, the most frequent were polystyrene boxes.

→ NEXT: further data are needed to better estimate the detection difference in the two sections of the fixed strip and to evaluate the correction factor.

- **Different tools to evaluate the strip width:**
    - *Measuring stick/rangefinder stick*. A small measuring stick was firstly used to define different distances within the 1000 m from the boat, based on the height on the sea level and the distance eye-hand of the observer (Fig. 2a). During the monitoring session, the strip distance can be easily checked frequently to maintain the same width of observation. A vertical graduate stick was also built, indicating only the upper and intermediate limits of the strip (10-5 m, Fig. 2b). → NEXT: further tests are needed to fix the stick to the boat and maintain a visible indication of the strip during the whole monitoring session.
    - *With the inclinometer*, knowing the height of the eye above the surface, the width of the strip can be measured at the beginning of the survey. Then a mark can be put on the shrouds, or on a stick tight on the side of the sailing vessel, so the observer know at what limit he should not take into account marine litter (out of the strip) (Fig. 2c).

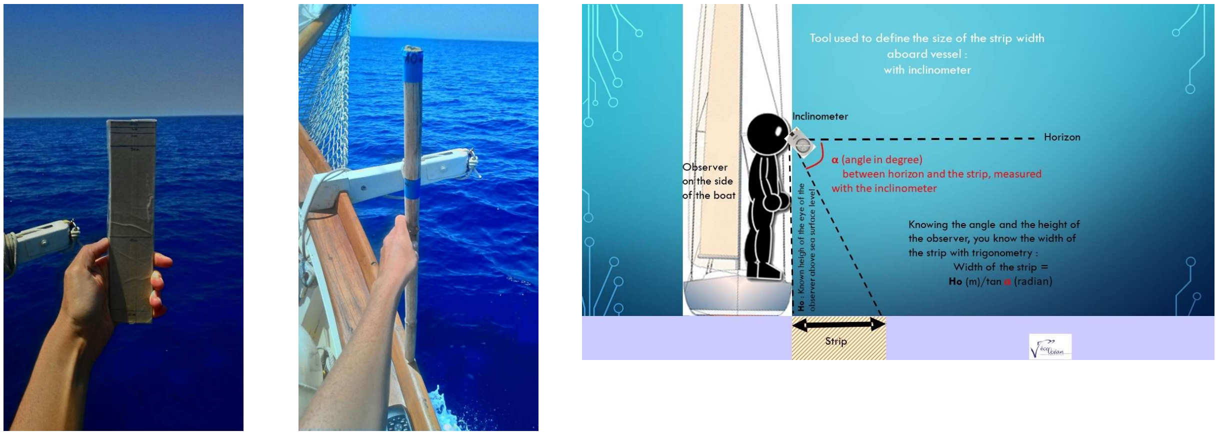


Fig. 2 Measuring tools: 2a measuring stick; 2b graduate stick; 2c inclinometer

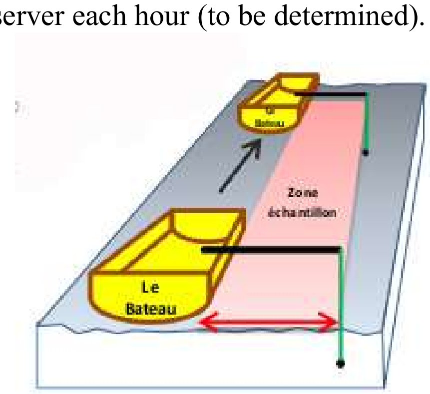
- *<u>Protocol for counting marine litter with a pole to delimit the strip width</u>* (EcoOcéan Institut)

Material:

- 1 thread weighted (rope, fishing line) / 1 rigid pole (fishing rod, spinnaker pole, ...)
- 1 GPS / 1 Log / observation sheet / 1 pencil

Implementation

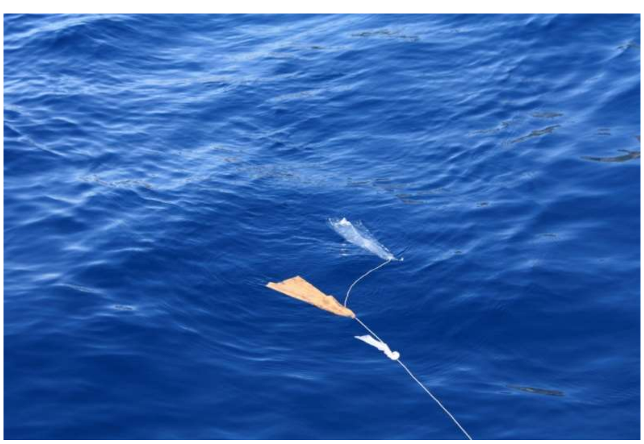
- Attach the weighted thread to the end of the pole
- Fasten the pole securely so that:
    - it extends widely on one side of the boat, perpendicularly
    - the ballast plunges into the water
- Calibrate the boat at a constant log speed of 5 to 6 knots
- Note the parameters relating to the observation conditions (wind strength, latitude, longitude, log, etc.)
- position yourself comfortably so as to see everything that passes between the hull of the boat and the weighted thread
- For the duration of the sample (30 minutes for example), note on the sheet all the waste that passes between the hull and the weighted thread.
- At the end of the observation, re-record the parameters (time, latitude, log etc.)
- If possible, do several counting sessions per day or do the counting continuously, with a rotation of observer each hour (to be determined).



- **Array with multiple items at different distance from the observation.** An array was built with 7 items representative of different materials (plastic, paper), colors (white, transparent, colored) and sizes (from 2.5 to 50 cm approx.) using objects commonly found floating at sea. The array was deployed at sea with the boat stopped, at 5 and 10 m distance from the boat; the same experiment was repeated in the port pier, with the array put 20 m away from the boat to verify the maximum distance of detection of the different objects. Results are reported in the following table.

ITEM	COLOR	SIZE CLASS	MATERIAL	5 m	10 m	20 m
Plastic sheet	transparent	F	artificial polymer	ok	vis,no dist	NO
Paper bag	brown	F	paper	ok	ok	Ok
Bottle 1.5 l	transparent	F	artificial polymer	ok	ok	Ok
Bar tissues	white	D	paper	ok	ok	vis,no dist
Plastic cutlery	white	D	artificial polymer (bio)	ok	ok	vis,no dist
Drinking glass	transparent	C	artificial polymer(bio)	ok	vis,no dist	NO
Bottle cover	white	B	artificial polymer	ok	ok	vis,no dist

The experiment confirmed the maximum detection distance from this kind of vessel to be 10 m, where all size of items are visible. Transparent items resulted visible but not easily distinguished, and in these few cases the use of binoculars can help with identification. At 20 m distance only the larger floating items are clearly visible, while the others can be detected only if white or twinkling, but the identification is harder; in fact, the monitoring within this strip would need a further confirmation with binoculars for many objects, making the sampling very challenging.
N.B. We observed a faster submersion of the items of biodegradable material compared to the common plastic objects (drinking glass or cutlery). On this basis we can assume that the buoyant floating items that are detected by visual observation are made of artificial polymers.



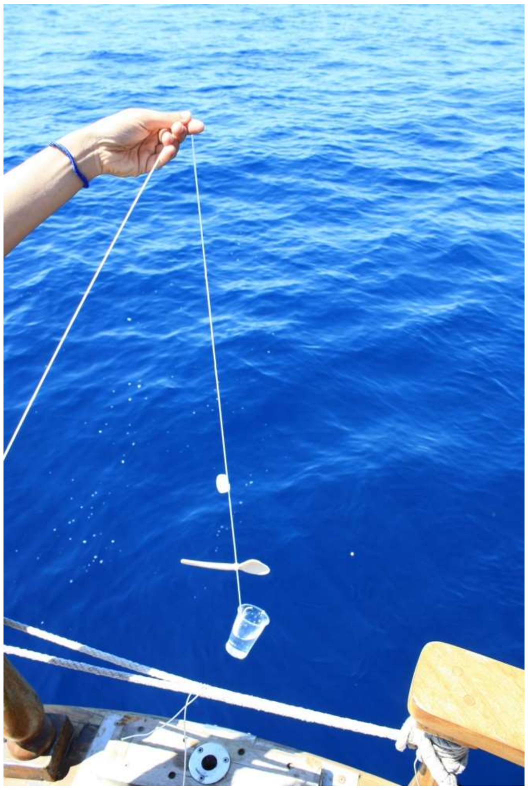


Fig.3 Array with multiple items at different distance from the observation.

5. Size of litter (ref. covariates: f. Size of litter)

To help defining the size classes, and the lower size detectable, the size classes used are those proposed by JRC: 2.5-5 (B); 5-10 (C); 10-20 (D); 20-30 (E); 30-50 (F); 50-100; >100cm.

A list of the size of different litter items (entire object) using the top 20 categories identified by literature and by previous data from ISPRA and Legambiente monitoring was completed, indicating the correct size class and material category as a reference.

ITEM	SIZE (cm)	SIZE CLASS	MATERIAL
Plastic bag standard	30-50	F	artificial polymer
Crates standard	50	F	processed wood
Polystyrene box	45-50	F	artificial polymer
Paper bag	30-40	F	paper
Maritime buoy medium	32-45	F	artificial polymer
Bottle 1.5 l	33	F	artificial polymer
Cover, bucket top	30	E	artificial polymer
Bucket standard	29	E	artificial polymer
Jerry can	27	E	artificial polymer
Sanitary towel	24	E	artificial polymer
Six-pack rings	22	E	artificial polymer

Plastic tableware	22-23	E	artificial polymer
Bottles 0.5 l	22	E	artificial polymer
Plastic cutlery	20	D	artificial polymer
Bar tissues	15-17	D	paper
Drink cans	11	D	metal
Drinking glass	9	C	artificial pol/paper
Cigarette box pack	9	C	paper
Tetrapack small brick	8,5	C	paper
Cotton bud stick	8	C	artificial polymer
Small coffee cup	5	B	artificial pol/paper
Bottle cover	3	B	artificial polymer
Cigarette butt/filter	2,5	B	artificial polymer

→NEXT: Given the frequency of some items commonly seen during the first monitoring sessions, it is suggested to add new categories from the MSFD Masterlist in the data collection sheet, such as cigarette butt, cotton bud, paper tissue, and to separate "covers and packaging" and "tableware" indicating if dish, glass, cutlery or straw.

I. METHODS FOR DETECTING FLOATING MACRO LITTER THROUGH AUTOMATIC PHOTOGRAPHY FROM UAV AND MANNED AIRCRAFTS

1. Introduction

Pollution from Marine litter has been increasing dramatically in recent years, raising concern on its potential impact on marine ecosystems and wildlife, as well as on its economic detriment to coastal communities (Eriksen et al. 2014, Gall and Thompson 2015). Various European and international conventions highlighted the importance of monitoring litter presence to effectively implement adequate protection measures (MSFD, 2008/56/EC; UNEP 2015).

Traditional methodologies for monitoring floating macroscopic litter have been mostly based on visual observation by naked eye from different kinds of platforms such as boats and airplanes (Ribic et al. 1992, Veenstra and Churnside 2011); but the same platforms can be used to obtain photographs and implement automatic detection techniques for marine litter monitoring.

Automated recording of floating objects can be done through a variety of recording systems applied on Unmanned Aerial Vehicles (UAVs) or other devices that can be used to monitor the presence of marine litter at different special scales in the sea. Automatic detection techniques present some advantages when compared to traditional visual techniques: human error of visual surveys is reduced, as well as human risk (for pilots and/or observers); survey effort can be increased; the images are recorded permanently, allowing subsequent statistic (re-)analyses and to answer future questions of biological interest. Moreover, automatic detection is a reliable technique, in which the geo-referencing of observations is accurate and precise; it is constantly improving (e.g. through improvements in image resolution), and, when applied using UAV, it can allow to reach inaccessible areas and repeatedly sample the same sites with minor costs than aircrafts (Bryson and Williams 2015).

The use of UAVs 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 present some disadvantages related to the operations of take-off and landing, especially at sea. They are also less stable, sometimes 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.

- Multi-copters: they are multi-rotor drones, generally with less endurance than fixed-wing drones, but with a much more stable structure, 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 can be performed using other kinds of remotely controlled systems, such as aerial balloons (Kako et al. 2012), but automated surveys can also be carried out through manned vehicles, such as small aircrafts. Aerial surveys involve traditionally the implication of dedicated observers, looking from bubble windows located on the two sides of small planes. Large-scale monitoring programmes through aerial surveys have been performed to locate areas of higher aggregations of litter and estimate its amount (Lecke-Mitchell and Mullin 1992, Pichel et al. 2007, Unger et al. 2014), covering large areas and allowing the detection of spatial differences in abundance. According to local legislations, these surveys normally occur at an average height of 250 m (750 ft approximately) over the sea level, thus allowing the observer to detect only large litter items (bigger than 30–40 cm). The application of automatic detection techniques on this kind of surveys could lower substantially this limit, if cameras with adequate resolution are used.

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 task of recognition analysis is performed afterwards, on the video/images acquired. Various algorithms for automated image analysis and object detection are being developed and proposed, based on the characterization of pixels and the analysis of colour and shape of objects (e.g. Maire et al. 2013): these techniques are under constant improvement and their applicability on marine litter surveys is under evaluation.

2. Aim

The aim of this document is to provide a guideline for monitoring floating macro litter through the use of automatic detection techniques, applied on UAVs and small aircrafts. This draft protocol on field techniques and image processing is based on the preliminary operational experiments conducted by the University of Barcelona and CSIC during the WP Studying of MedSeaLitter project.

3. Methodology

3.1 Field experiments

3.1.1. UAVs

To evaluate the performance of the different types of drones and cameras that could be used for monitoring, experiments were set on the field, aiming also to determine the most effective conditions to detect the presence of floating marine litter (flight height, camera resolution, etc).

Two arrays were built, reproducing the structure of a long-line, and including, hanging from the main line, items representative of the materials (e.g. plastic, aluminum, wood, rubber), colors (e.g. white, black, transparent, colored) and sizes (from a few cm to 1 m approx.) of objects commonly found floating at sea. A similar array was also built including a series of polystyrene models representing profiles of dolphins and turtles of different sizes (small, medium, large) (see figure 1 - 2 and annex 1 for the detailed list).

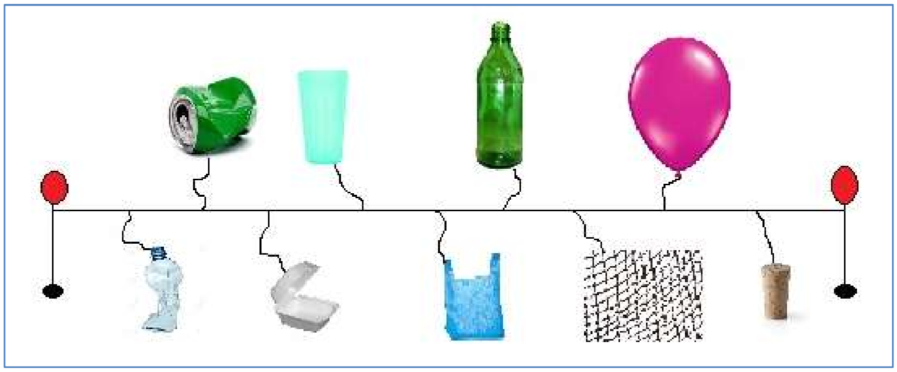


Fig.1


Fig.2

Two field experiments were carried out deploying these arrays at sea and recording images and videos through aerial photography: the first using multi-rotor drones, and the second using fixed-wing drones, equipped with similar sets of cameras.

Both experiments were performed in the area of Blanes (approx. 60 km N of Barcelona), in a marine coastal area located N of the mouth of the small river Tordera (see figure 3). Drones, provided by the professional company Hemav, were flown by the personnel of Hemav from the beach, while the arrays were deployed at sea approx. 100-200 m from the coastline, from a small motor boat (4.5 m Solar Congo), rented from the local company Boat Rental Blanes.

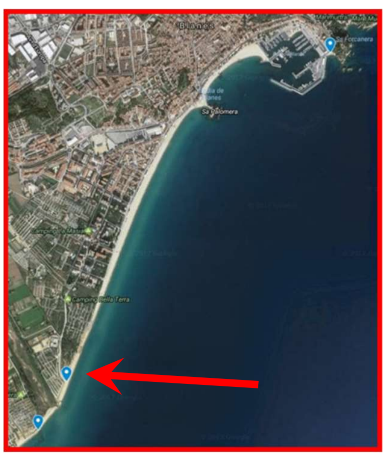


Fig.3

The first field experiment was performed on May 16th 2017. Three different drones were flown over the area where the arrays were deployed:

- Multi-rotor 'topografía', equipped with a visible RGB camera Sony Alpha 7R (figure 4).

Fig.4

- Multi-rotor 'agricultura', equipped with a multi-spectral camera Micasense Red-Edge (figure 5).

Fig.5

- Multi-rotor 'inspecciones', equipped with a visual + thermic system, composed by a thermal imaging sensor (FLIR TAU-2 640) and a visual sensor (Sony cx240) (figure 6).

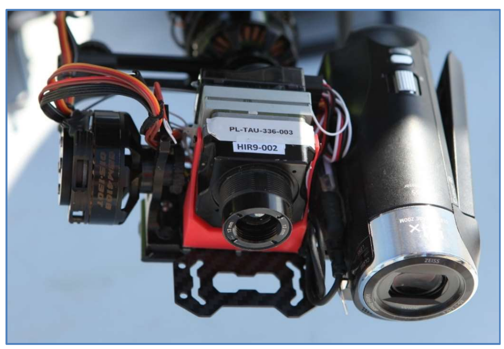


Fig.6

Flights were performed starting at 8 am, when the sun was lower on the sea, in the following order:

- 1st flight: RGB camera taking photos from heights of 20; 50; 100 and 120 m. approx., perpendicularly over the area where arrays were deployed.
- 2nd flight: RGB camera taking photos from a height of 100 m. approx., at various angles from the arrays and the sun.
- 3rd flight: RGB camera tilted 30º, taking photos from heights of 50; 100 and 120 m. approx.
- 4th flight: multi-spectral camera taking photos from a height of 100 m approx., perpendicularly on the arrays.

- 5th flight: visual + thermic system, taking videos from a height of 100 m approx., perpendicularly on the arrays. For this flight, a person was also recorded while swimming at sea, to check for thermic variations in the videos.
- 6th flight: same as 1st flight, but repeated at 10 am, when sun was higher on the sea, thus increasing the amount of sun glare on the sea.

The second experiment was performed on July 3rd 2017, starting at 6:00 am, before the sunrise, to perform the first flight with no sun glare.

For this experiment only one drone was used: the Fixed-wing drone HP1, flown from the beach using a ramp-system (figure 7), and recovered on the beach using a small parachute.

Fig.7

This drone was not transmitting live recordings to the operator of the remote controller, for this reason flights had to be previously programmed, along transect centered on the approximate position of the boat and the arrays (figure 8). Due to the local safety regulations, the flight height was established at 100 m, and the max distance from the coast (the controller) was 500 m.

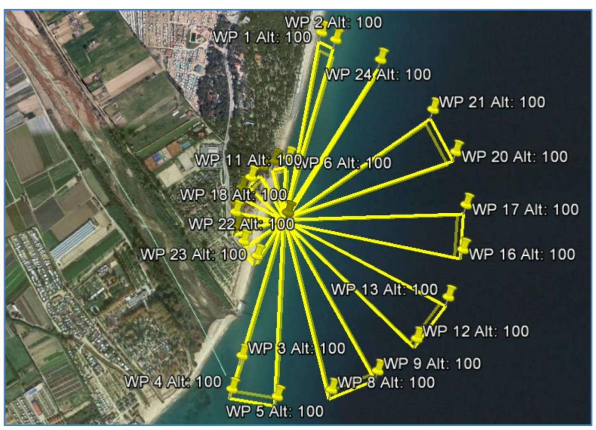


Fig.8

Between flights sensors were changed, to use a set of sensors similar to the one used for the first experiment, following the order:

- Visual RGB camera: SONY QX1 with wide-angle lenses
- Multi-spectral camera: Micasense Red-Edge
- Thermic camera: FLIR TAU-2 640.

Flights were performed over the designated area, and photos and videos were taken perpendicularly to the water, with different angles from the arrays and the sun. The last flight was performed at 10 am approx.

3.1.2 Aerial images from aircrafts

Ideally, an experiment would be set with arrays similar to those used for taking images with UAVs, during which a small plane equipped with an automated detection system and two observers onboard would fly over the area where arrays are deployed. Due to logistic difficulties, such an experiment has still to be set.

The University of Valencia was able to perform a series of aerial surveys of marine litter with observers (see the relative protocol, and figure 9) onboard of an aircraft provided with a camera Canon EOS Rebel SL1, and obtained some pictures from the transect that we could include in our preliminary analysis.

Fig.9 Aerial surveys of marine litter with observers.

3.2 Image processing

Once images are downloaded, they must be processed to estimate the detectability of litter and evaluate the best parameters for monitoring (e.g. flight height, image resolution, effect of glare, minimum size of detectable litter).

Processing of images involves 3 steps:

1. Statistical analysis of detectability

As we are still in the initial attempts of marine litter detection from aerial imagery, it is important to keep a formal monitoring of the actual accuracy. On this regard, it is necessary to:

1.1. Select a sub-set of test images in which litter is present.

1.2. Interactively delineate training polygons of the different types of categories according to what the photo-interpreter can distinguish (at least “litter” and “water”) (see figure 10).

In case polygons are drawn with QGIS (which does not have the option of setting an arbitrary Euclidean coordinate system), and to ensure ulterior bulk-processing, it is necessary to:

- set the photo to a coordinate reference system (CRS) with a rectangular geographic projection (e.g. ETRS89 UTM31N, epsg 25831);
- make sure to create the vector file in the same projection system.

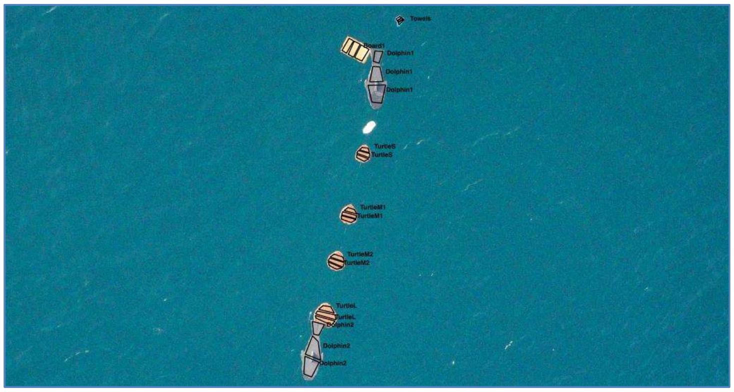


Fig.10 training polygons of the different types of categories

1.3. Extract RGB values for the polygons, run a Linear Discriminant Analysis (LDA) to visualize discrimination in LD space and classify using cross-validation to produce a confusion matrix and calculate global, user's and producer's accuracy, along with rates of True Litter (TL), True Water (TW), False Litter (FL) and False Water (FW) cases.

2. Sun-glare Mask

Having found sun-glare as a major cause of miss-detection, it is important to run an automatic process that detects, in a conservative way, the areas of sun-glare and creates a mask. This mask will define the area not to be used for detection (see figure 11 for an example, in which sun glare affects the top left corner of the image).

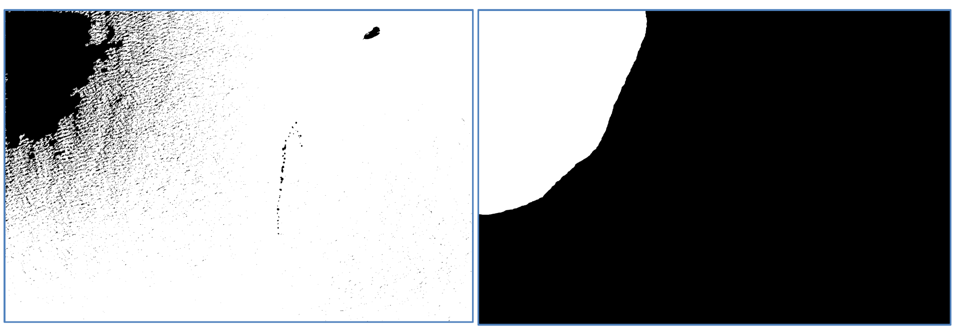


Fig.11 example of sun glare affects

As we are still in the initial attempts of marine litter detection from aerial imagery, it is important to evaluate the percentage of masked area for each photo and save this data in a table along with camera orientation, date and time, in order to further study the relationship between sun-glare on one hand and photo and sun angles on the other.

3. Candidate Objects

Classifying every pixel of the image would be too demanding in terms of computing power, hence it is necessary an automatic process that detects patches in the image that could be objects.

4. Classification

Using results of the LDA, all candidate objects are then classified as "Litter" (eventually, different types of litter depending on the results obtained from the previous LDA) or "water" (see figure 12 for an example: red dots represent TL, pink dots FL, dark blue dots TW and light blue dots FW).

Classifiers other than LDA will be considered in the future.

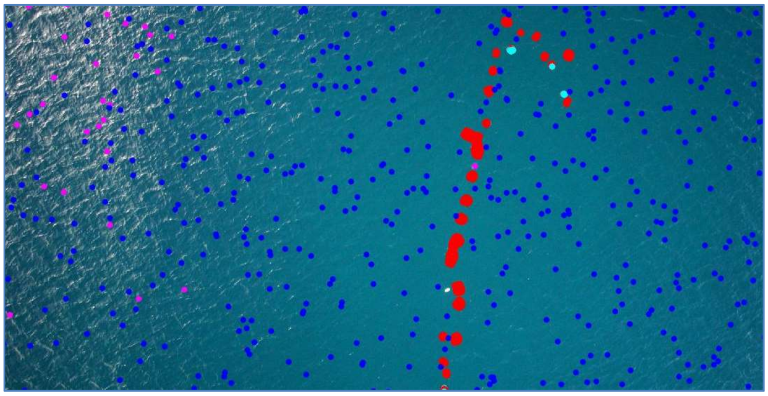


Fig.12 red dots represent TL, pink dots FL, dark blue dots TW and light blue dots FW

4. Preliminary results and indication for implementation of monitoring guidelines

To run a preliminary analysis of images, a subset of RGB images was selected, including photos taken during the two field experiments, from different heights, both perpendicularly to the horizon and tilted, with different percentage of sun glare.

Images were first processed following the point 1.2 of image processing, to create vectors of polygons representing the various litter items, classified by colour and material (see Appendix 1). As shown in figure 13, not all items were detected in the images by the photo-interpreter, especially if items were floating in an area covered by sun glare. Overall, the number of litter items that could be detected by the photo-interpreter was found to decrease as height from which the photo was taken increased. This amount was further reduced when sun glare was present.

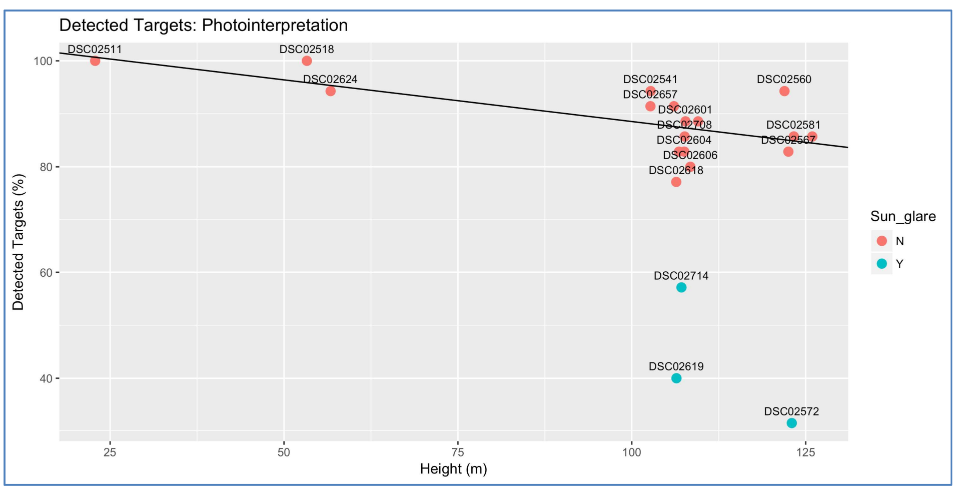


Fig.13

Once RGB values were extracted from a preliminary subset of photos, a series of LDAs were run, to see discrimination between water and litter and discrimination among litter items of different colours and water (see figure 14 and 15 for an example of these preliminary results). In general, water discriminated well from litter, and some of the colours were also grouping quite well in LD space (e.g. red, white, brown).

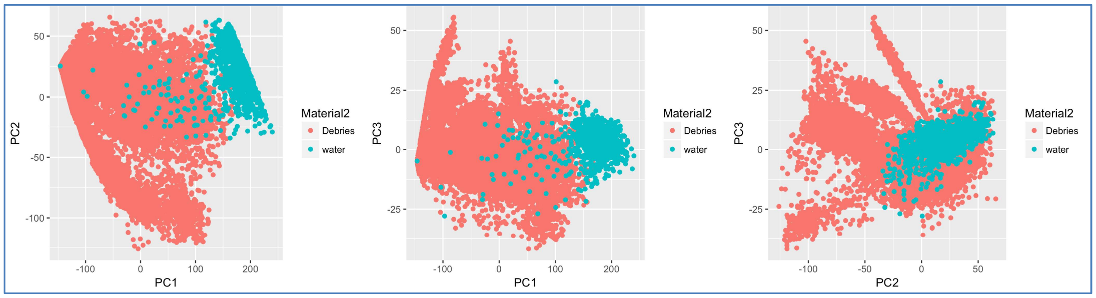


Fig.14

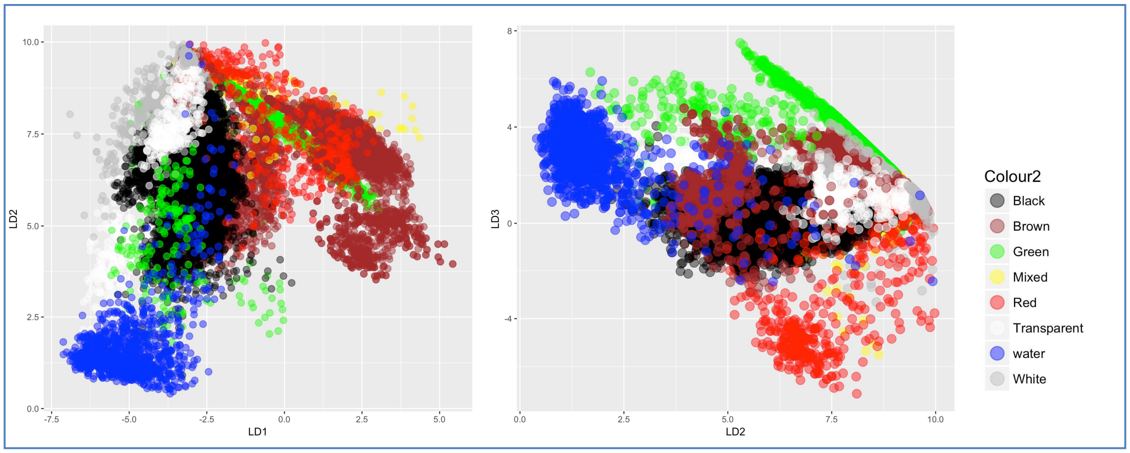


Fig.15

Following the point 1.3 of image processing, cross-validation was used to produce a confusion matrix and calculate rates of TL (points correctly classified as litter), TW (points correctly classified as water), FL (points representing water that were misclassified as litter) and FW (points representing litter that were misclassified as water). The results of this validation are represented in LD space in figure 16, grouped by flight height (20, 50, 100 and 120 m., respectively). Also in this case, results indicated that the rate of discrimination between litter and water was generally high.

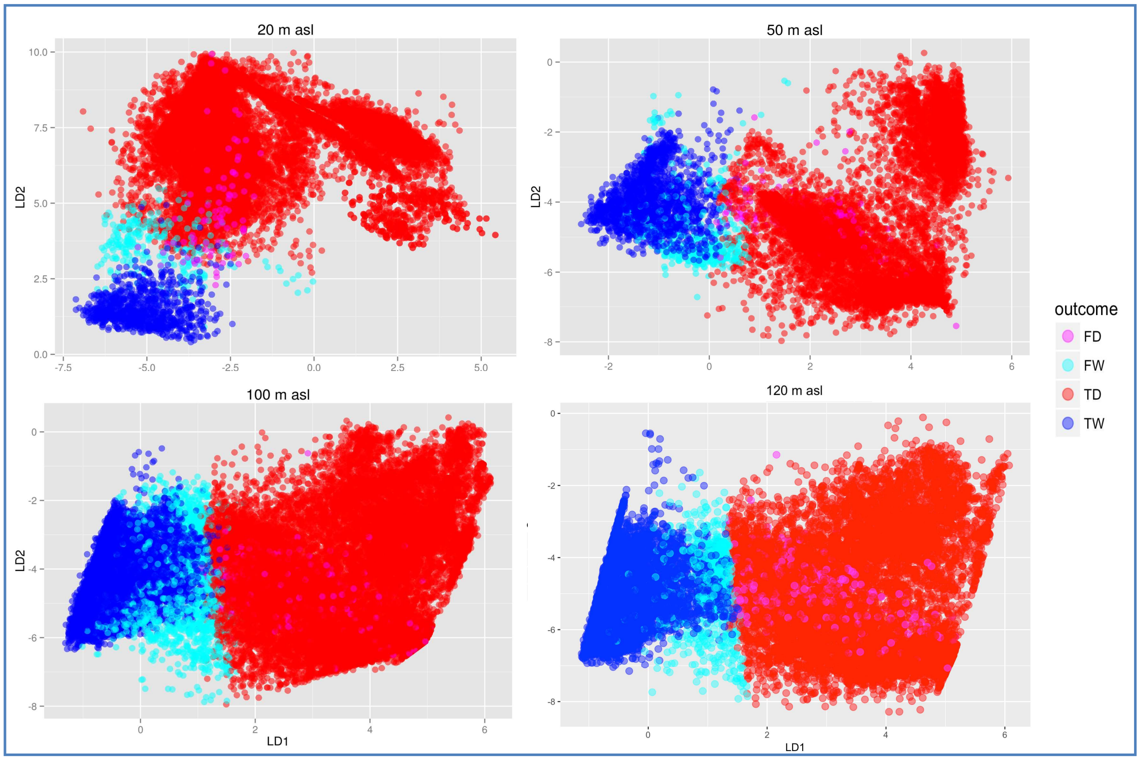


Fig.16

Results of this validation allowed to calculate average classification accuracy for each flight height. Table 1 summarizes these results, distinguishing photos that were taken perpendicularly from those that were taken with the camera tilted 30º. The average area covered by pictures taken at different heights was also calculated, basing on the resolution of the camera and the lenses used.

Table 1 average classification accuracy for each flight height

Flight height (m)	**Inclination**	**TD**	**FD**	**TW**	**FW**	**Accuracy**	**Transect area (m^2)**
20	0	34168	96	901	396	0.9867	1836
50	0	7556	89	910	99	0.9767	9617
	30º	5196	44	956	317	0.943	
100	0	2684	45	955	357	0.97	33067
	30º	2164	47	952	185	0.93	
120	0	2057	74	924	85	0.9467	48753
	30º	1176	28	971.3	119	0.9367	

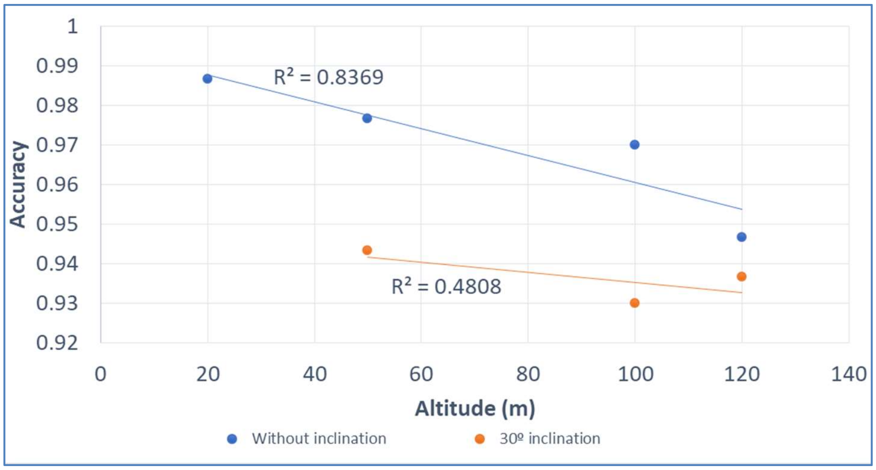


Fig.17 average accuracy of detection against flight height

As showed from table 1 and figure 17, representing average accuracy of detection against flight height, the discriminability of litter items from water was generally high, with a limited number of water misclassified as litter, and a relatively higher number of misclassification of litter as water, but overall a high amount of correct classifications. The average accuracy was decreasing slightly with the height from which photos were taken, especially in photos taken not perpendicularly to the sea surface, due to the distortion of these images and their consequent decrease of resolution.

A final analysis was performed to relate the resolution of images (size of pixels) and the height of flight, using the characteristics of the RGB cameras and lenses mounted on the drone (15 mm lenses) and the Partenavia aircraft (40 mm lenses) and, as a reference, an image of dolphins taken from the observer on board the Partenavia aircraft using a camera with 79 mm lenses.

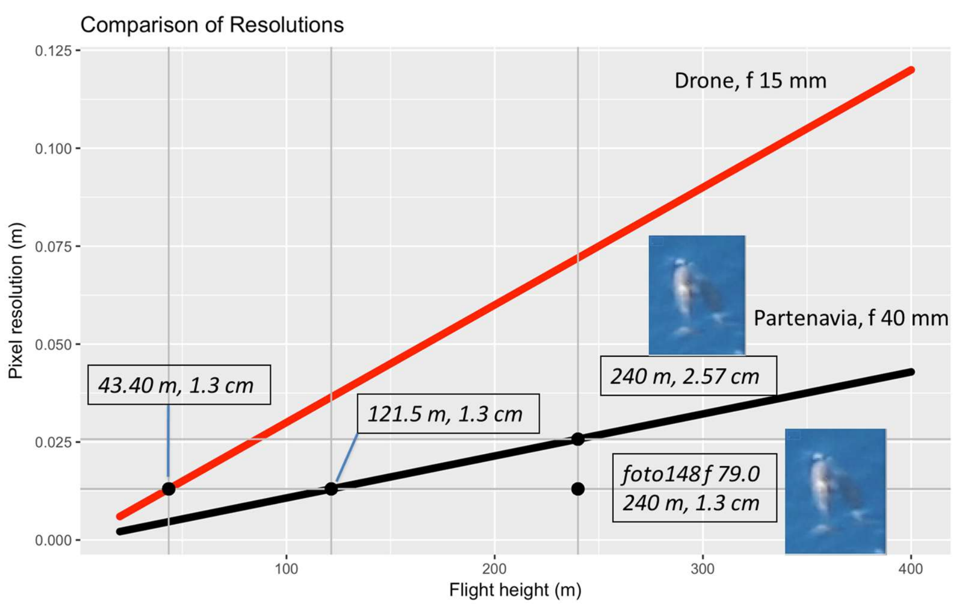


Fig.18 relation between flight height and pixel resolution for the images taken from the drone

Figure 18 shows the relation between flight height and pixel resolution for the images taken from the drone (15 mm lenses, red line) and those taken from the camera mounted on the Partenavia (40 mm lenses, black line). The isolated black dot represents the pixel size of the dolphin image taken using 79 mm lenses at a height of 240 m. As it can be seen from the graph, to get a similar resolution (that is, a pixel size of 1.3 cm), with smaller lenses, the flight height must be reduced, in the case of a 40 mm lens, to 121.5 m, and in the case of a 15 mm lens, to 43.4 m. The area covered by photographs taken at similar heights would be reduced noticeably (see table 1), thus increasing dramatically the number of images required to cover a designated transect – and thus the post-processing effort. Nevertheless, the dolphin image re-sized as if it was taken with a 40 mm lens from an height of 240 m, shows a possible compromise, being still of an acceptable resolution (pixel size of 2.57 cm).

These calculations suggest that aerial photographs taken from a small aircraft using an average lens of 40 mm could easily detect medium-sized object of approximately 30-40 cm, covering relatively big areas when flying at the legally required height of 750 ft (240 m approximately).

4.2 Guidelines for monitoring marine litter through automatic aerial photography

Results obtained from field experiments and the preliminary analyses of photographs taken, allow to draw some preliminary guidelines for monitoring marine litter through automatic aerial photography.

Sampling scale & platform: The first thing to consider when planning marine litter monitoring through aerial photography is spatial scale. The platform from which the photos are taken must in fact be selected according to the size of the area to be surveyed.

When monitoring large areas (such as for basin scale surveys, or regional surveys), the use of a small aircraft is suggested, providing that sensors are selected with a resolution compatible with the height limits set by local legislation (i.e. increasing resolution with increasing height).

If monitoring is to be carried out over smaller areas, such as small MPAs or limited segments of the coastline, the use of UAVs is recommended. Also in this case, it is necessary to consider local regulations related with the maximum distance allowed from the remote controller, and the height limits. In general, the use of fixed wing drones, due to their higher endurance, is recommended for larger areas, but considering the difficult operations of take-off and landing, the use of these UAVs is not recommended when conducting surveys from boats or from rocky coasts. The use of multi-rotor drones, which are easier to manoeuvre, and whose recording can be transmitted directly to the control station, is instead recommended when operations are performed from boats or other less-stable platforms, or when high resolution photos of specific areas are required.

Sampling design: When monitoring marine areas through automatic photography, the width of transects is directly dependent on the camera resolution and lenses used, and/or the height from which the photos are taken. Therefore, according to the needs of each monitoring program, flight height can be reduced to obtain more detailed pictures but covering smaller areas, or increased to cover bigger areas but with lower quality images.

It is important that once width is set, transects are designed to cover homogeneously the study area, and that along each transect photos are taken at a pace that allows a certain level of overlapping between subsequent images. Timing, height and geographic positions must be recorded automatically from the sensor for each photo, to allow the subsequent geo-referencing and precise mapping of the areas of litter concentration.

When planning large scale monitoring through aircrafts, transects should be oriented approximately perpendicularly to the coast, to follow a depth gradient and a systematic saw-tooth pattern. Despite this, it is also fundamental to consider the angle of the sun, and modify the orientation of transects accordingly.

Environmental conditions & sun glare effect: The effect of sun glare reduce dramatically the probability of detecting marine litter, both when images are checked by human eye and when the detection is run automatically. Therefore it is important to plan monitoring when the sun glare is limited, preferring the early morning or the late afternoon hours, when the sun is lower on the horizon. It is also important to consider the position of the sun at each time of the day, to plan transects accordingly and avoid transects oriented against sun.

Sea state surface (i.e. Beaufort scale) is also a factor to be considered, as the presence of white caps in the sea, like it happens with visual monitoring, could bias the probability of observation of marine litter. Thus, monitoring should take place only with Beaufort < 3. Strong winds conditions must be avoided also because they limit the possibility to fly, both through UAVs and manned aircrafts.

Finally, visibility and a cloud covering must also be considered, as a reduced visibility (e.g. because of fog) or a spotted cloud covering could also decrease the probability to detect floating object through automatic detection.

Type of marine litter: The accuracy of marine litter identification is dependent on the quality of the images taken (which in turn is dependent mainly on the flying altitude and type of sensor used). Type and composition of marine litter observed must be based on the Master List for floating objects proposed by the technical group on marine litter within the Marine Strategy Framework Directive (Galgani et al. 2013), despite many of the item listed in the list are of difficult identification. Larger categories of floating marine litter, distinguishing at least litter composition, could then be considered for classification. The use of multi-spectral cameras could help identifying the composition of litter, as each material presents different spectral characteristics, but the results of this preliminary analysis have still to be confirmed.

Size of marine litter: Size of marine litter can be easily determined when considering the resolution of each image. If the size of a pixel is known, the size of floating objects can be calculated using image analysis software. The lower size limit, as explained above, is dependent on the curve of height/resolution (figure 18), and we calculated that with a pixel size of approximately 2.5 Cm it would be possible to distinguish objects of approximately 30 Cm. When pixel size is reduced, the probability to detect smaller objects increase: in photos taken from drones at 20 m height also smaller objects (such as cans, see Appendix 1 for sizes) could be detected.

Image processing: Guidelines for image processing and on how to carry out preliminary analyses of images are already given in paragraph 3.2.

5. References for monitoring FLM through automatic photography

Bryson, M., Williams, S., 2015. Review of Unmanned Aerial Systems (UAS) for Marine Surveys. Australian center for Field Robotics, University of Sidney.

Eriksen M, Lebreton LCM, Carson HS, Thiel M, Moore CJ, Borerro JC, Galgani F, Ryan PG, Reisser J, 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLOS ONE, 9:e111913. DOI: 10.1371/journal.pone.0111913.

Galgani F, Hanke G, Werner S, Oosterbaan L, Nilsson P, Fleet D, Kinsey S, Thompson RC, Van Franeker J, Vlachogianni T, Scoullos M, Mira Veiga J, Palatinus A, Matiddi M, Maes T, Korpinen S, Budziak A, Leslie H, Gago J, Liebezeit G, 2013. Guidance on Monitoring of Marine Litter in European Seas. Scientific and Technical Research series, Report EUR 26113 EN.

Gall SC, Thompson RC, 2015. The impact of debris on marine life. Marine Pollution Bulletin, 92:170-9. DOI: 10.1016/j.marpolbul.2014.12.041.

Hodgson, A., Kelly, N., Peel, D., 2013. Unmanned Aerial Vehicles (UAVs) for surveying marine fauna: A dugong case study. PLoS ONE 8(11): e79556.

Koski, W. R., Allen, T., Ireland, D., Buck, G., Smith, P. R., Macrander, A. M., Halick, M. A., Rushing, C., Sliwa, D. J., McDonald T. L., 2009. Evaluation of an Unmanned Airborne System for monitoring marine mammals. Aquatic Mammals 35(3), 347-357.

Lecke-Mitchell KM, Mullin K, 1992. Distribution and abundance of large floating plastic in the north-central Gulf of Mexico. Marine Pollution Bulletin, 24:598-601. DOI: 10.1016/0025-326X(92)90279-F.

Pichel W, Churnside J, Veenstra T, Foley D, Friedman K, Brainard R, Nicoll J, Zheng Q, Clemente-Colón P, 2007. Marine debris collects within the North Pacific Subtropical Convergence Zone. Marine Pollution Bulletin, 54:1207-1211. DOI: 10.1016/j.marpolbul.2007.04.010.

Ribic CA, Dixon TR, Vining I, 1992. Marine debris survey manual. NOAA Technical Report NMFS 108, NOAA National Marine Fisheries Service, Seattle, WA. 92pp.

UNEP, 2015. Marine Litter Assessment in the Mediterranean. UNEP/MAP Athens, 45 pp.

Unger B, Herr H, Gilles A, Siebert U, 2014. Evaluation of spatio-temporal distribution patterns of marine debris in the SCI Sylt Outer Reef. 28th Conference of the European Cetacean Society, Liège (Belgium).

Veenstra TS, Churnside JH, 2011. Airborne sensors for detecting large marine debris at sea. Marine Pollution Bulletin, 65:63-68. DOI: 10.1016/j.marpolbul.2010.11.018

Appendix 1. Items included in the arrays deployed during field experiments.

LINE	Item	Material	Size (Cm)	Colour
	Buoy	Plastic	30x12	White
1	Six-pack ring (x2)	Plastic	20x10	Transparent
1	Bottle	Glass	35x7 (diameter)	Green
1	Food tray	Polystyrene	22x13	Black
1	Milk Brick	Tetrapack	20x9	White-blue
1	Can	Aluminium	12x7 (diameter)	Green
1	Bag	Plastic	32x32	Green
1	Bag (x2)	Plastic	40x24	Transparent
1	Food tray (eggs)	Plastic	22x21	Transparent
1	Can	Aluminium	22x6 (diameter)	White-red-blue
1	Jar	Plastic	19x10 (diameter)	Mixed
1	Toy baloon	Rubber	36x62	Green
1	Sanitary towels	Tissue	22x15	White
1	Board	Cork	62x38	Brown
0	Dolphin	Polystyrene	132x50	Black
0	Small turtle	Polystyrene	37x31	Brown
0	medium turtle	Polystyrene	42x37	Brown
0	medium turtle	Polystyrene	42x37	Brown
0	big turtle	Polystyrene	54x50	Brown
0	Dolphin	Polystyrene	134x46	Black
2	Crate	Wood	30x19	Brown
2	Bottle	Plastic	31x8 (diameter)	Green
2	Bottle	Plastic	30x11	White
2	Bottle	Plastic	30x13	Transparent
2	Can	Aluminium	26x6 (diameter)	Brown
2	Sack	Tissue	94x53	White
2	Can	Aluminium	12x7 (diameter)	Red
2	Bottle	Glass	30x6 (diameter)	Black
2	Milk Brick	Tetrapack	24x7	White-green
2	Net	Plastic	21x24	Red
2	Ball	Rubber	18 (diameter)	White
2	Bag	Plastic	43x40	White
2	Bag	Plastic	39x52	Black
2	Board	Polystyrene	55x35	White
	TURTLE	Keratin	45x40	brown

II. METHODS FOR FLOATING MACRO LITTER FROM AIRCRAFT IMPLEMENTED FROM THE UNEP/MAP AND MSFD PROTOCOLS

Summary

Summary
Among the available methods for monitoring floating macro litter in the ocean, aerial surveys are useful to assess large areas, detect and identify aggregations of litter and estimate its abundance. This protocol aims to synthesize methodologies used for monitoring abundance and distribution of marine fauna applied to the study of marine floating macro debris. Surveys should be designed accordingly to a line transect distance sampling technique, in which a high representation of the study area is homogenously covered. 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 all transects and 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. Although data for both marine fauna and marine debris has been successfully recorded simultaneously, a correction on the data obtained from marine litter is needed, as the collection of distance data on all marine debris could interfere by taking effort away from target (i.e. cetaceans, sea turtles) species. Considering that the lowest limit size for aerial detection is over objects of ca. 30-40 cm, a limitation on the categorization of floating litter observed from aerial surveys is imposed. Thus, observers will identify the following characteristics of the marine litter observed: (1) material and litter item, (2) size (3) sighting angle regarding the transect line.

1. Introduction
Recent concern has been raised regarding the quantity of ocean litter in the ocean due to its detrimental effects on human health and, the impact on marine ecosystems and marine wildlife as well as the economic adverse on coastal communities (Eriksen et al. 2014, Gall and Thompson 2015). Marine strategies have been developed worldwide with the aim of achieving a good environmental status for the oceans (UNEP 2016). These strategies rely on data obtained from different marine litter monitoring programs and provide guidelines to reduce ocean litter.
Methodologies for monitoring floating macroscopic litter are mostly by observation. Surveys can be done by naked eye or by using images from different kind of platforms such as land-based, boat-based and airplanes (Ribic et al. 1992, Veenstra and Churnside 2011). Large-scale monitoring programmes have been developed through aerial surveys to locate areas of higher aggregations of litter and to estimate its amount (Lecke-Mitchell and 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. 30–40 cm), 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 (Lecke-Mitchell and Mullin 1992, Galgani et al. 2013, Ryan et al. 2009).
The following protocol is based on a line-transect methodology (Buckland et al. 1993) and has been modified from previous methodologies implemented for assessing distribution and abundance of marine fauna (see Gómez de Segura et al. 2007, Panigada et al. 2011 for marine mammals; Gómez de Segura et al. 2006a, Lauriano et al. 2011 for sea turtles and Bauer et al. 2015 for fishes) to identify and quantify floating marine litter by aerial craft observers.

2. Aim

The aim of this protocol is to provide a guideline for conducting aerial craft-based visual surveys of floating macro litter (>30 cm) by unifying different monitoring approaches, in order to ensure data comparability between surveys and across regions. There are three main objectives:

- To examine the spatial distribution and abundance of marine macro litter.
- To quantify marine litter at local and regional levels according to its use and other correlating parameters.
- To determine the type and concentration of marine litter present by making material categories..

3. Methodology

3.1 Protocol for monitoring floating macro litter assessed from aircrafts

This protocol has been modified from the one used by Gómez de Segura et al. (2006a,b) for studying the abundance and distribution of cetaceans and sea turtles in the Western Mediterranean. For the case of dedicated cetacean aerial surveys, it is common that not only target species are recorded. Further records of other marine mammals, birds and different type of vessels would provide valuable information on the distribution and abundance of these other non-target species and objects. It is important to note, however, that collecting data on marine debris can take effort away from the target species if the attention of the observers is diverted. Hence, effort for collection of marine debris data will not be the same throughout the survey and this must need to be taken into account during the analyses (see Scheidat and Feindt-Herr 2012).
Surveys must be conducted with good sea state (i.e., below 3 Beaufort state), as visibility will decrease with bad weather conditions. Aircraft must hold a constant speed and altitude. Under these conditions previous studies have shown that a minimum threshold of objects 30 cm (medium size of juveniles loggerhead sea turtles) or larger will be observed (Gómez de Segura et al. 2006a).

Sampling design (ref. covariates: a. Sampling design and period):

Line transects should be designed using the “Distance” software ver.6.1 Beta 1 (Thomas et al. 2010). The software allows creating a sampling methodology with homogeneous and highly representative coverage probability over the whole studying area, for example by using equidistant parallel lines or a systematic saw-tooth pattern (Fig 1). Each transect must be characterized by:
- Number and transect length.
- Date of survey and starting and finishing times.
- Geographic position at the starting and finishing points.
- Number of marine fauna sightings and the average distance between each two consecutive sightings (average distance = length between transects/number of sightings, Sá et al. 2016). This could also apply to marine litter.
- Oceanographic characteristics (i.e., depth, Beaufort state, cloudiness).

Platform for observation (ref. covariates: b. Type of platform):

Aerial surveys can be performed on a two-engine high-wing aircraft, like a ‘push-pull’ Cessna 337, with flat windows as described in Gómez de Segura et al. (2006a). Aircrafts with bubble windows are also recommended (Fig 2a). Transects are flown at a groundspeed of c.166 km/h (90 kn) and an altitude of 750 ft, which in both cases should be maintained constant. In some previous studies, the flying altitude was 500 ft; however, a 750 ft-altitude would guarantee identification of objects bigger than 30 cm while conforming to safety aerial procedures.

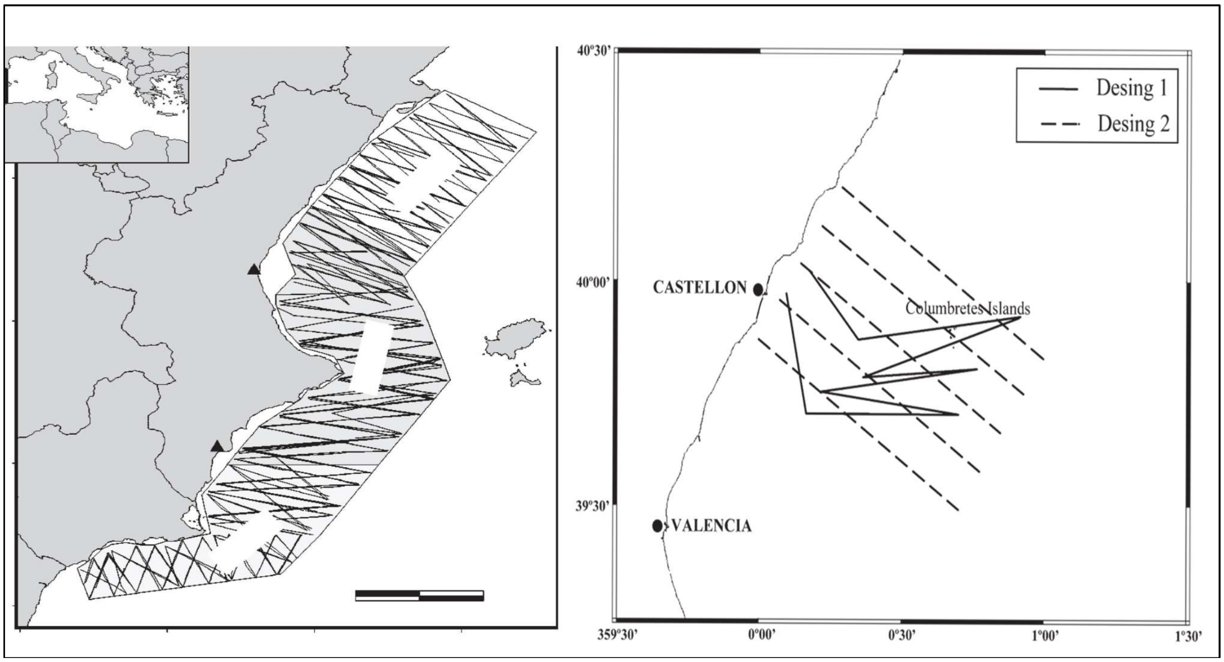


Figure 1. Two different line-transect designs for aerial surveys to study the abundance and distribution of cetaceans and sea turtles in the Spanish Mediterranean. In both cases, transects were oriented approximately perpendicular to the coast, following a depth gradient and a systematic saw-tooth pattern starting from a specific point (modified from Gómez de Segura et al. 2006a, b).

Crew (ref. covariates: c. Experience of observers):
A standard crew should include: pilot, recorder in the seat of the co-pilot and two experienced observers positioned behind them on each side of the plane, which will be preferably the same for all transects during the survey. An additional observer could be dedicated to photo recording; also this figure would be greatly beneficial to turn shifts with the main observers (Fig 2b).

Figure 2 (a) Aircraft for monitoring floating macro litter and (b) crew made by the pilot on the left hand of the plane, data recorder in the seat of the co-pilot, two observers positioned at each side of the plane and an additional observer dedicated to photo shooting.

Sampling at the beginning of each transect: The recorder should annotate the following items and all environmental conditions must be updated whenever any changes occur.

- Identification number and characteristics of each transect.
- Position of sun, intensity of glare (if any as low, medium or high) and angle of glare (from the right side = 0º to 180º; from the left side = 0º to -180º).
- Geographic locations at the beginning of each transect. A GPS will continuously record the position updated every few seconds.
- Position of observers (Left, Right).
- Environmental conditions (Beaufort sea state, cloud coverage, visibility, etc.).

Sampling within effort: 1) Duties of the recorder: The recorder will take note of all data in the "Visual Survey Data Sheet" (Appendix 2). Alternatively, data can be recorded on a laptop using any specific data recording software. A highly recommended software is the IFAW's Logger 2010 (Appendix 1), as it is suitable software to automatically record observations of marine litter and other marine fauna. Otherwise, recorder can use any other suitable method for data recording. Information on the location of each sighting, which will be also recorded in the GPS, the time and angle of sighting (see below), and changes in environmental conditions will be annotated.
2) Duties for observers: Each observer will record marine litter and will communicate to the recorder the following three aspects: 1) type of marine litter, 2) marine litter sighting angle (will be used to estimate the distance of the observed marine litter from the transect line), and 3) size of the object observed.

Type of marine litter (ref. covariates: g. Type and colour of items):

It is worth to mention the limitations posed over the accuracy of marine litter identification given the flying speed and altitude. Therefore, type and composition of marine litter objects observed will be based on a modified version of the Master List for floating objects proposed by the technical group on marine litter within the Marine Strategy Framework Directive (Galgani et al. 2013) (Table 1).

Table 1. Modified master list with the list of objects observable form an aerial survey.

Plastic, Polystyrene, Polyurethane	Bags
	Boxes
	Fish box
	Buoys(*)
	Buckets
	Fishing nets
Processed wood	Pallets
Vegetable	Seaweed/marine plant
	Logs/plants parts
Liquids	Oil slick
	Isolated foam
Glass	Bottles
Textile	Clothing
Rubber	Balloons
	Tyres

Animal	Animal carcases
Unidentified material	Ropes (plastic or textile) Pieces (non-organic material)

(*) Only adrift buoys will be considered.

Distance of the observed marine litter from the transect line (ref. covariates: g. Strip width):

This distance will be established accordingly to the angle of sighting within three fixed-width strips (Fig 4). These strips will be drawn on the window and the length of each strip will be estimated using a hand-held inclinometer and should be between 90º and 40º (observable area within 200 m from the transect line) (Fig 5). The data of the angle from each detected item, together with the flying altitude, will be used to calculate the perpendicular distance of the item from the line-transect; any other object observed above 40º are outside the 200m distance from the transect line and will not be recorded.

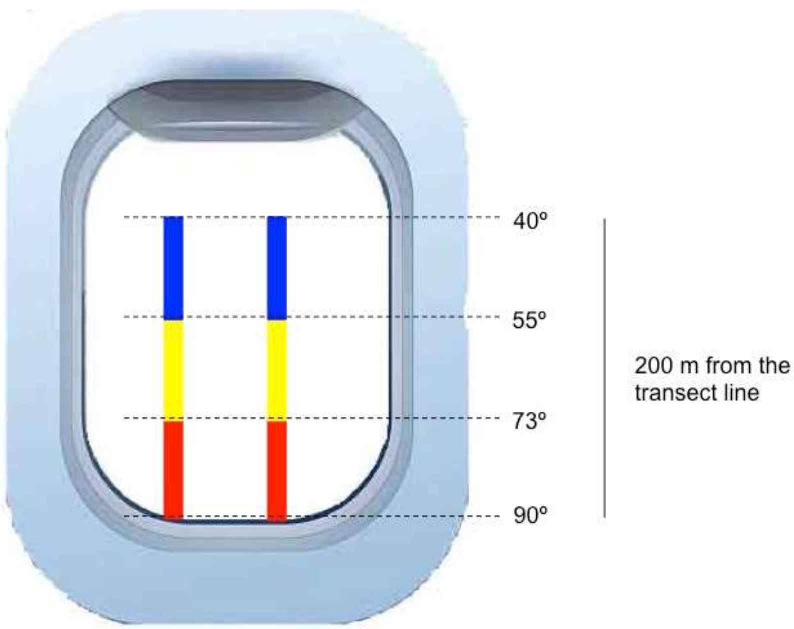


Figure 4. Observable angles to detect marine litter within 200m from the transect line.

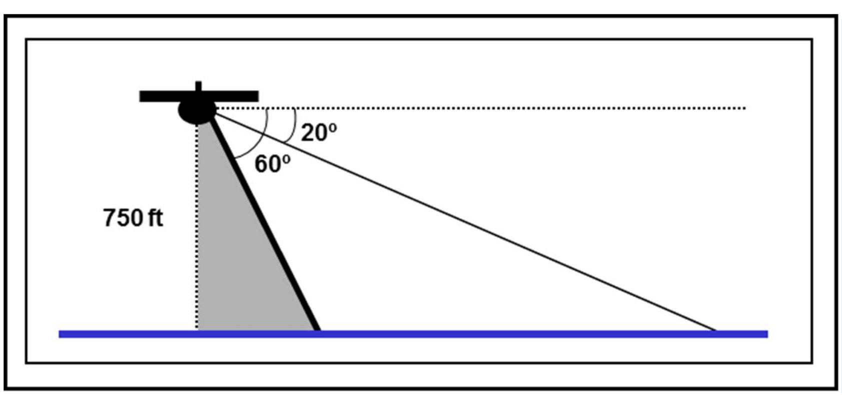


Figure 5. Schematic drawing of the visibility from the aircraft window with angles for distance estimation. Note that with a bubble window observers will be available to see from 0º to 90º. Marine litter

will be only recorded within the 40º distance from the line transect. The maximum angle of marine fauna sighting is 20º. The grey section of the scheme represents the 90º to 60º of non-observed area from a flat-window aircraft.

Size of the observed object (ref. covariates: f. Size of items):

A suitable method to standardize the size of the marine litter observed is to classify the object into three main categories: Small, Medium and Large. A small object will be the one measuring ca. 30–100 cm (as an estimate, the length of a juvenile loggerhead turtle is ca. 30 cm); a medium-size object would measure ca. 100–200 cm (body length of an adult striped dolphin is ca. 2 m); and a large object would be >200 cm.

3.2 Data analyses

Litter density and abundance will be analysed using standard distance sampling methods (Thomas et al. 2010), which fit a detection probability function to a distance–frequency histogram. Densities will be estimated for the overall data and from each type of litter according to their classification, using independent detection functions.

Kernel density maps for floating marine debris will be generated using the software Arc Map 10.1. These maps will show the geographic areas with a higher probability of marine litter occurrence. This approach will be used, as kernel density estimates represent a true probability density function to be used in statistical analyses. Spatial Analyst Tools in the software ArcGIS 10.1 will be used to select cell size and search radius, and probability contours will be used to show the probability of occurrence of litter (Sá et al. 2016).

*3.3 Strategy for classifying floating marine litter (*ref. covariates: g. Type and colour of items*)*

Different methodologies currently employed have been assessed for monitoring floating litter, and how to identify and classify the objects. Overall, marine litter can be classified in three different categories based on its characteristics: 1) source, 2) type of material and 3) the likely use of the item (Eriksen et al. 2014).

- Classification of marine litter by its source: Broad categories regarding the source of litter can be used to its identification and classification (Table 2). Whenever the source of an item cannot be specified, it will be established as "indeterminate", i.e., a plastic bag would be unspecified if observers cannot identify if it has become waste on the ground (abandoned on the beach or from a river) or by sea (thrown from a boat); further examples at: http://ec.europa.eu/environment/marine/good-environmental-status/descriptor-10/index_en.htm).

Table 2. Classification of marine litter by its source.

Source	Examples
Ocean-based sources of marine litter (OS)	- Merchant shipping, ferries and cruise liners - Fishing vessels - Military fleets and research vessels - Pleasure craft - Offshore oil and gas platforms - Fish farming installations
Land-based sources of marine litter (LS)	- Municipal landfills (waste dumps) located on the coast or inland - Riverine transport of waste from landfills or other sources along rivers and other inland waterways (canals) - Discharge of untreated municipal sewage, including storm water (including occasional overflows) - Industrial facilities: Solid waste from landfills, and untreated waste water

	- Tourism (recreational visitors to the coast; beach-goers)
Indeterminate source (IS)	The observers cannot specified the origin

- Classification of marine litter by its type of material and size: The most common materials that make up marine litter are cloth, glass, metal, paper, plastic, rubber, and wood (Table 3) (Suaria and Aliani 2014, Sá et al. 2016). Regarding size, aerial surveys allow the detection of large litter items, while allowing for broad area surveys. A conventional lower size limit for aerial surveys is ca. 30-40 cm. These monitoring techniques are therefore suitable for the detection of larger objects, such as abandoned fishing gears (i.e., floating nets) or large litter accumulation spots (Veenstra and Churnside 2011, Darmon et al. 2017).

Table 3. Classification of litter items in categories based on the type of material.

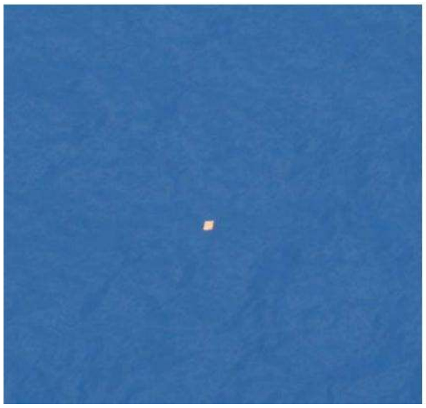
Material	Examples
Natural floating marine litter	- Driftwood, trunks, branches, canes
Anthropogenic floating marine litter	
Plastic items	- Fragments, plastic bags, bottles and containers
Foamed polystyrene	- Cooler or packaging material, coffee cups
Paper and cardboard	- Cartons, cups and bags
Rubber	- Tires, balloons and gloves
Processed wood	- Pallets, crates, particle boards
Clothing and textiles	- Cloths, hard hats
Glass	- Beverage bottles, bulbs
Metal	- Beverage cans, storage drums
Oil	- Oil slicks, Oil spill
Unidentified material	

- Classification of marine litter by its likely use: a third way to classify marine litter is by the type of activity that created the waste item and the associated behaviors that caused the waste to become marine litter (Table 4) (Galgani et al. 2013).

Table 4. Marine litter classified accordingly of the source activity.

Categories	Examples
Fishing	Buoy, floats, float flag, lines, rope, net, fish trays and other fishing gear
Packaging	Polystyrene and other foamed plastics, plastic bag, food wrapping or packing strips Bottles, tubs, tins, aerosols
Agricultural	Fruit box, fertilizing bottles, liquid drums
Non-identified items	Other plastic pieces (mostly fragments of items that could not be identified = plastic 'confetti', but some items too deep from sea surface that is not clear to see what it is can also can be placed into this category)

Examples of marine litter observed from aerial surveys in the Western Mediterranean.




SM1. Floating cardboard box

SM2. Floating plastic bag **S**


SM4. Patch of floating common reed grass

SM3. Oily waste from a ship bilge

4. References monitoring FML from aircrafts

Bauer R, Bonhommeau S, Brisset B, Fromentin J-M, 2015. Aerial surveys to monitor Bluefin tuna abundance and track efficiency of management measures. Marine Ecology Progress Series 534:221-234. DOI: 10.3354/meps11392

Buckland ST, Anderson DR, Burnham KP, Laake JL, 1993. Distance Sampling: Estimating Abundance of Biological Populations. Chapman & Hall, London.

Darmon G, Miaud C, Claro F, Doremus G, Galgani F, 2017. Risk assessment reveals high exposure of sea turtles to marine debris in French Mediterranean and metropolitan Atlantic waters. Deep-Sea Research II: Tropical Studies in Oceanography 141:319-328. DOI: 10.1016/j.dsr2.2016.07.005.

Eriksen M, Lebreton LCM, Carson HS, Thiel M, Moore CJ, Borerro JC, Galgani F, Ryan PG, Reisser J, 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLOS ONE, 9:e111913. DOI: 10.1371/journal.pone.0111913.

Galgani F, Hanke G, Werner S, Oosterbaan L, Nilsson P, Fleet D, Kinsey S, Thompson RC, Van Franeker J, Vlachogianni T, Scoullos M, Mira Veiga J, Palatinus A, Matiddi M, Maes T, Korpinen S, Budziak A, Leslie H, Gago J, Liebezeit G, 2013. Guidance on Monitoring of Marine Litter in European Seas. Scientific and Technical Research series, Report EUR 26113 EN.

Gall SC, Thompson RC, 2015. The impact of debris on marine life. Marine Pollution Bulletin, 92:170-9. DOI: 10.1016/j.marpolbul.2014.12.041.

Gómez de Segura A, Crespo EA, Pedraza SN, Hammond PS, Raga JA, 2006b. Abundance of small cetaceans in the waters of the central Spanish Mediterranean. Marine Biology, 150:149-160. DOI: 10.1007/s00227-006-0334-0.

Gómez de Segura A, Hammond PS, Cañadas A, Raga JA, 2007. Comparing cetacean abundance estimates derived from spatial models and design-based line transect methods. Marine Ecology Progress Series, 329: 289-299. DOI: 10.3354/meps329289.

Gómez de Segura A, Tomás J, Pedraza SN, Crespo EA, Raga JA, 2006a. Abundance and distribution of the endangered loggerhead turtle in Spanish Mediterranean waters and the conservation implications. Animal Conservation, 9:199-206. DOI: 10.1111/j.1469-1795.2005.00014.x.

Lauriano G, Panigada S, Casale P, Pierantonio N, Donovan GP, 2011. Aerial survey abundance estimates of the loggerhead sea turtle Caretta caretta in the Pelagos Sanctuary, northwestern Mediterranean Sea. Marine Ecology Progress Series, 437: 291-302. DOI: 10.3354/meps329289.

Lecke-Mitchell KM, Mullin K, 1992. Distribution and abundance of large floating plastic in the north-central Gulf of Mexico. Marine Pollution Bulletin, 24:598-601. DOI: 10.1016/0025-326X(92)90279-F.

Panigada S, Lauriano G, Burt L, Pierantonio N, Donovan G, 2011. Monitoring winter and summer abundances of cetaceans in Pelagos Sanctuary (Northwestern Mediterranean Sea) through aerial surveys. PLoS ONE 6(7): e22878. DOI: 10.1371/journal.pone.0022878.

Pichel W, Churnside J, Veenstra T, Foley D, Friedman K, Brainard R, Nicoll J, Zheng Q, Clemente-Colón P, 2007. Marine debris collects within the North Pacific Subtropical Convergence Zone. Marine Pollution Bulletin, 54:1207-1211. DOI: 10.1016/j.marpolbul.2007.04.010.

Ribic CA, Dixon TR, Vining I, 1992. Marine debris survey manual. NOAA Technical Report NMFS 108, NOAA National Marine Fisheries Service, Seattle, WA. 92pp.

Ryan PG, Moore CJ, van Franeker JA, Moloney CL, 2009. Monitoring the abundance of plastic debris in the marine environment. Philosophical Transactions of the Royal Society B: Biological Sciences, 364: 1999-2012. DOI: 10.1098/rstb.2008.0207.

Sá S, Bastos-Santos J, Araújo H, Ferreira M, Duro V, Alves F, Panta-Ferreira B, Nicolau L, Eira C, Vingada J, 2016. Spatial distribution of floating marine debris in offshore continental Portuguese waters. Marine Pollution Bulletin, 104:269-278. DOI: 10.1016/j.marpolbul.2016.01.011.

Scheidat M, Feindt-Herr H, 2012. Collecting data on marine debris during cetacean aerial surveys. International Whaling Commission, SC/64/E15.

Suaria G, Aliani S, 2014. Floating debris in the Mediterranean Sea. Marine Pollution Bulletin, 86:494-504. DOI: 10.1016/j.marpolbul.2014.06.025

Thomas L, Buckland ST, Rexstad EA, Laake JL, Strindberg S, Hedley SL, Bishop JRB, Marques TA, Burnham KP, 2010. Distance software: design and analysis of distance sampling surveys for estimating population size. Journal of Applied Ecology, 47:5-14. DOI: 10.1111/j.1365-2664.2009.01737.x

UNEP, 2016. Marine plastic debris and microplastics-Global lessons and research to inspire action and guide policy change. United Nations Environment Programme, Nairobi.

Unger B, Herr H, Gilles A, Siebert U, 2014. Evaluation of spatio-temporal distribution patterns of marine debris in the SCI Sylt Outer Reef. 28th Conference of the European Cetacean Society, Liège (Belgium).

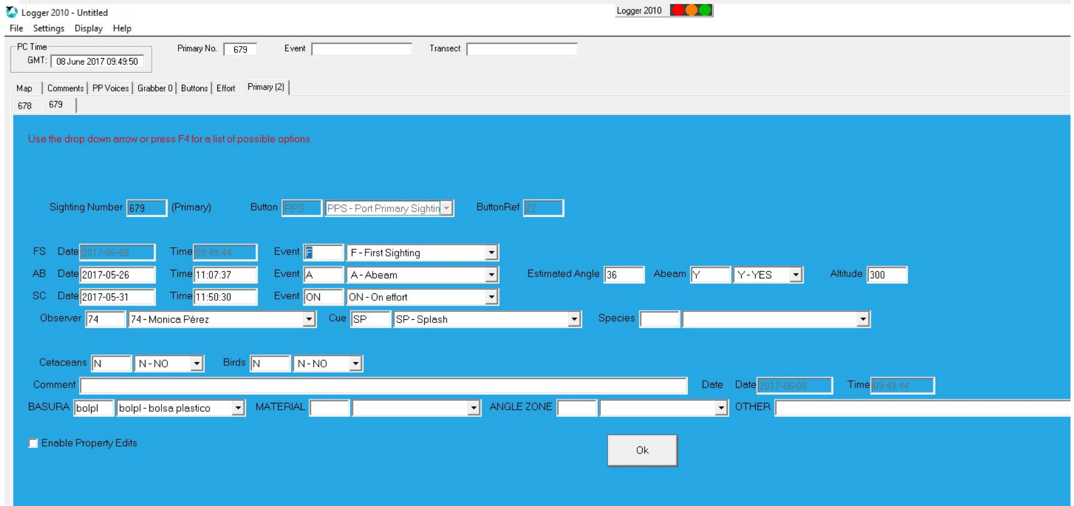
Veenstra TS, Churnside JH, 2011. Airborne sensors for detecting large marine debris at sea. Marine Pollution Bulletin, 65:63-68. DOI: 10.1016/j.marpolbul.2010.11.018.

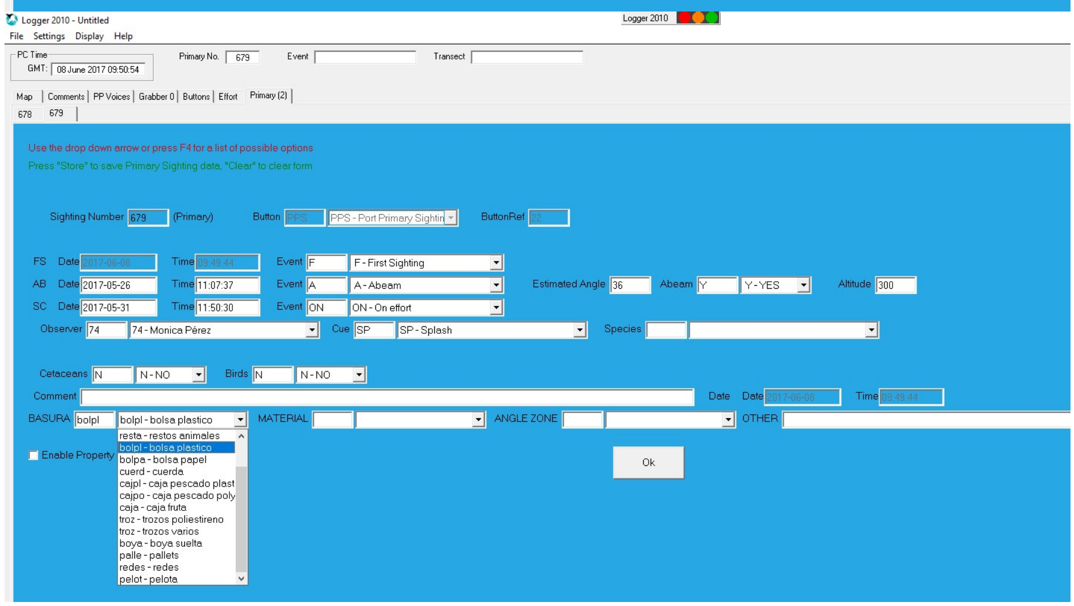
Thomas L, Buckland ST, Rexstad EA, Laake JL, Strindberg S, Hedley SL, Bishop JRB, Marques TA, Burnham KP. 2010. Distance software: design and analysis of distance sampling surveys for estimating population size. Journal of Applied Ecology, 47:5–14. Doi: 10.1111/j.1365-2664.2009.01737.x

UNEP. 2016. Marine plastic debris and microplastics – Global lessons and research to inspire action and guide policy change. United Nations Environment Programme, Nairobi.

Veenstra TS, Churnside JH. 2011. Airborne sensors for detecting large marine debris at sea. Marine Pollution Bulletin, 65:63–68. Doi: 10.1016/j.marpolbul.2010.11.018

Appendix 1. IFAW's Logger 2010 software screenshots





Appendix 2. Visual surveys data sheet

Date: ______ **Flying altitude:** ______ **Sea state:** ______

Cod GPS/ TIME GPS	Observer	Transect	Angle of sun glare	Angle strip	Plastic, Polystyrene, Polyurethane						Proc. wood	Vegetable		Liquids		Glass	Textile	Rubber		Animal	Unidentified material		Others/Pieces
	L (Left) R (Right)			R (Red) Y (Yellow) B (Blue)	Bags	Boxes	Fish box	Buoys	Buckets	Fishing nets	Pallets	Seaweed/marine plant	Logs/plants parts	Oil slick	Isolated foam	Bottles	Clothing	Balloons	Tyres	Animal carcases	Ropes (plastic or textile)	Pieces (non-organic material)	

Monitoring FML litter impact risk on biota through synoptic monitoring of key species of mega and macro-fauna

During systematic monitoring of marine litter, data on marine macro-fauna is collected each time it occurs within the assessed monitored strip. Potential macro-fauna data collected regards the species listed in table 1. From Aircraft, the list includes also cetacean species as listed below, while from other platform types it is recommended that marine mammal monitoring is performed by a dedicated Marine Mammal Observer, while at the same time litter monitoring is performed by another dedicated observer.

Table1 List of potential other species of marine mega fauna to be recorded.

##Other Species names##	**Eng name**
Caretta caretta	Loggerhead sea turtle
Dermochelys coriacea	Leatherback turtle
Mola Mola	Ocean Sunfish
Mobula mobular	Devil fish
Xiphias glaudius	Swordfish
Thunnus ssp	Tuna
Fam. Istiophoridae	Marlins
Shark	Shark
Jellyfish*	Meduse
Puffinus yelkouan	Yelkouan Sh (or Levantine shearwater)
Calonectris diomedea	Scopoli's Shearwater
Other	

Cetacean species to be recorded by a dedicated Marine Mammal Observer performing the cetacean monitoring at the same time as the litter monitoring:
Globicephala melas (Long-finned pilot whale)
Balaenoptera physalus (Fin whale)
Delphinus delphis (Short-beaked common dolphin)
Grampus griseus (Risso’s dolphin)
Ziphius cavirostris (Cuvier’s beaked whale)
Physeter macrocephalus (Sperm whale)
Stenella coeruleoalba (Striped dolphin)
Tursiops truncatus (Common bottlenose dolphin)
Megaptera novaeangliae (Humpback whale)

Other species data sheet

SPECIES	Cod GPS	TIME GPS	Number of individuals	Direction	Behaviour (1)	Size (2)	Note
Turtle							
JELLYFISH (3)			**Abundance (4)**	**Longitudinal strip/Patch (5)**	**Distance between individuals (6)**		
Other species (5)			**Number of individuals**				

(1) **R**) Resting; **T**) Travelling; **O)** Other
(2) Size perception: **I**) < 35 cm; **II**) tra 35 e 70 cm; **III**) > 70 cm
(3) name of the species.
(5) **L**) longitudinal strip (Langmuir current); P) patch
(6) **I**) single individual; **II**) individuals at a great distance from each other; **III**) within close distance; **IV**) swarm (compact individuals)

Monitor FML impact risk on biota - preliminary results

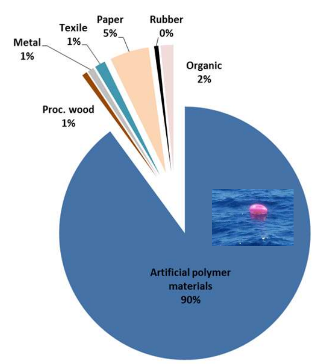
Survey from ferries on macro marine litter, Sea Turtles and Cetaceans were performed along representative fixed transects

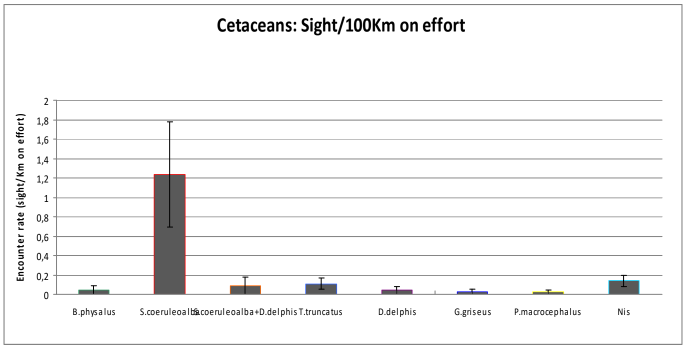
Summer 2017: from the 19th of July to the 7th of September
Trips on the ship "BONARIA" of the Tirrenia Shipping Company
Fast ship, around 20 knots speed
length: 214 meters
width 26 meters
height of the bridge: around 20 meters
Fixed transects: from Cagliari to Palermo roundtrip
4 researchers for each trip (1 for macro marine litter and 3 for Cetaceans and Sea Turtles)

Sampling:

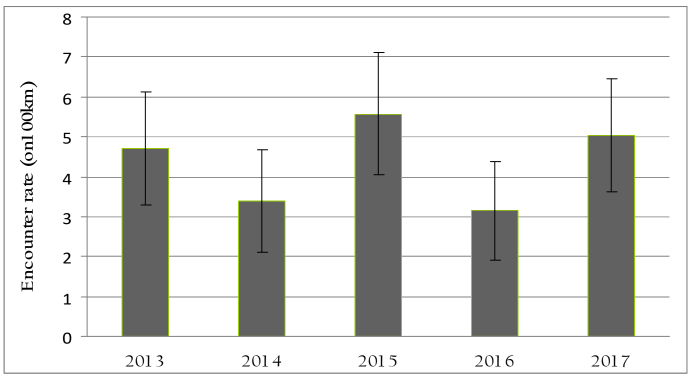
- an experienced observer positioned on one side (left or right) of the ferry in the vicinity of the bridge
- strip width: 50 meters divided into halves, first and second.
- equipment: binocular, GPS, digital camera and recording data sheet.
- monitoring is carried out with sea state ≤ 2 on the Beaufort scale.

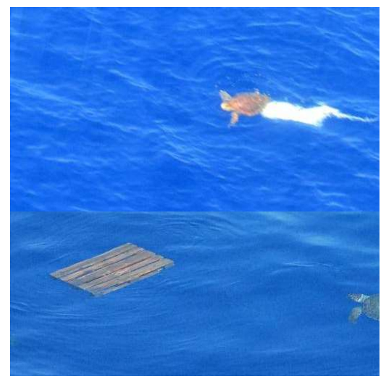
ITEMS	ABUNDANCE	DENSITY (on Km2)
Artificial polymer materials	657	4,359
Paper	37	0,245
Organic	12	0,080
Texile	10	0,066
Metal	6	0,040
Proc. wood	5	0,033
Rubber	4	0,027
Glass	0	0,000
TOTAL	**731**	**4,850**





Cetaceans - N = 49 (7 cetacean species)





Caretta caretta: 200 sightings and 223 individuals

Experiments to be implemented during the testing phase (WP4) to set conditions for monitoring Floating Macro Litter:

- **Analysis of litter size, type and classes**

Using the ecological boat of Cinque Terre MPA a direct removal and identification of items will be performed to compare the percentage of litter categories and size classes recorded from visual or automatic detection with the one recorded from direct litter removal and inspection.

A pre-testing activity was performed in Cinque Terre MPA during the summer 2017: 10 surveys in July and 15 in August (lasting about 4 hours/day) were performed by the Cinque Terre MPA to contribute to the identification of characteristics of floating macro litter items. Monitoring and direct removal and identification of items were performed by the "ecological boat" owned by the managing of Cinque Terre MPA, a special craft designed for recognition and direct removal of floating marine litter in the MPA. The study areas were selected in Cinque Terre MPA basing on predetermined navigation routes selected according to several parameters: main migration of floating material, profiles of surface currents, main winds and intensity, passenger maritime transport routes, etc. The selected route is a 10 miles circular route along thee coast line (about 100-200 meters from the coast, due to the summertime provisions for the protection of swimmers) (Fig.1). The detection was carried out by visual observation directly from the deck and command post by the MPA experienced staff.

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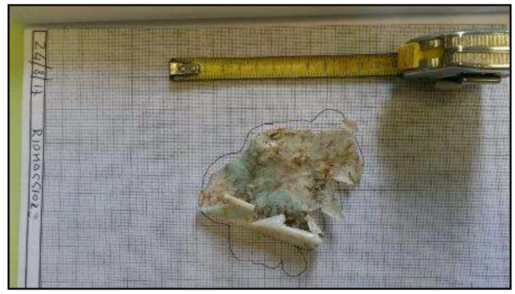
Fig 1 pre-selected route

Fig 2 The MPA ecological boat

Floating materials were recorded in the data collection sheet (considering the categories master list), and were identified and reported by colour, shape out of the water, buoyancy, etc. Results of the observation are reported in Fig 3:

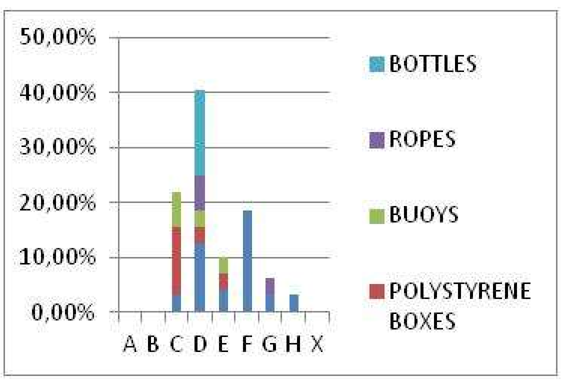
REMOVED SEA LITTER				
	hour	NM	Plastic	Wood
05.07.17	4	18	30	20
07.07.17	4,5	4	200	200
10.07.17	3	20	10	40
19.07.17	4	15	10	20
20.07.17	2	20		
31.07.17	4	20	5	10
01.08.17	4	10	10	20
02.08.17	4	11	160	
03.08.17	3	10	20	30
08.08.17	5	20	150	200
16.08.17	3	20	10	50
17.08.17	5	25	100	200
21.08.17	4	10	10	5
22.08.17	5	15		100
23.08.16	3	15	40	100
24.08.17	4	15	10	5
25.08.17	3	20	30	50
03.10.17	3,5	4	10	20



size litter	percent detection
D 20-30 cm	54,0%
E 30-50 cm	26,0%
F > 50 cm	17,0%
H > 100 cm	3,0%
X > 200 cm	0,0%

Fig. 3 items collected and percentage distribution of size classes of floating litter

During the testing were detected the presence of floating ropes, bags, polystyrene boxes (for the most part fragments), pieces of buoys, were detected in the following proportions (Fig3):



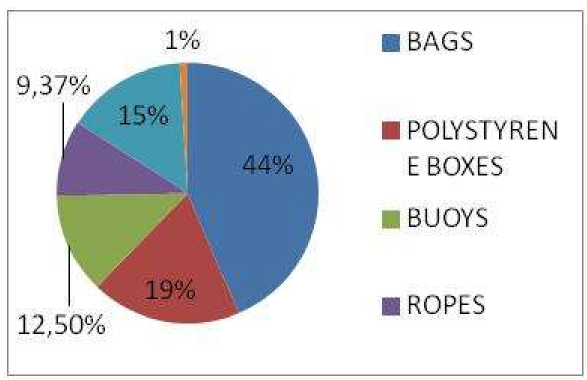


Fig 3 main categories of marine litter

First results highlight the accumulation of floating litter on the western MPA coast, likely due to the effect of predominant winds and currents (Fig. 5):

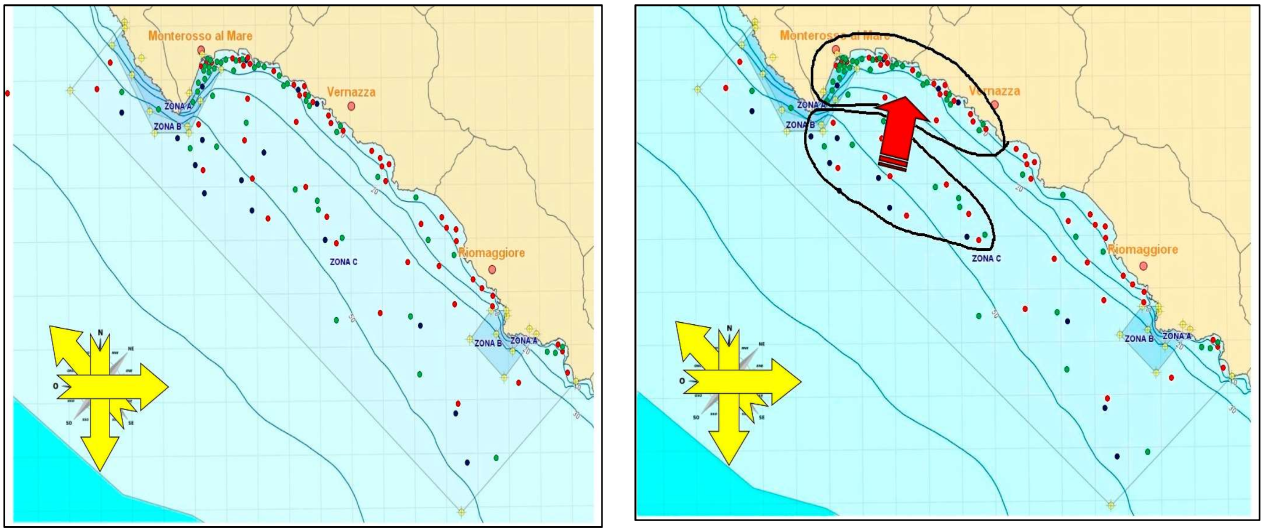


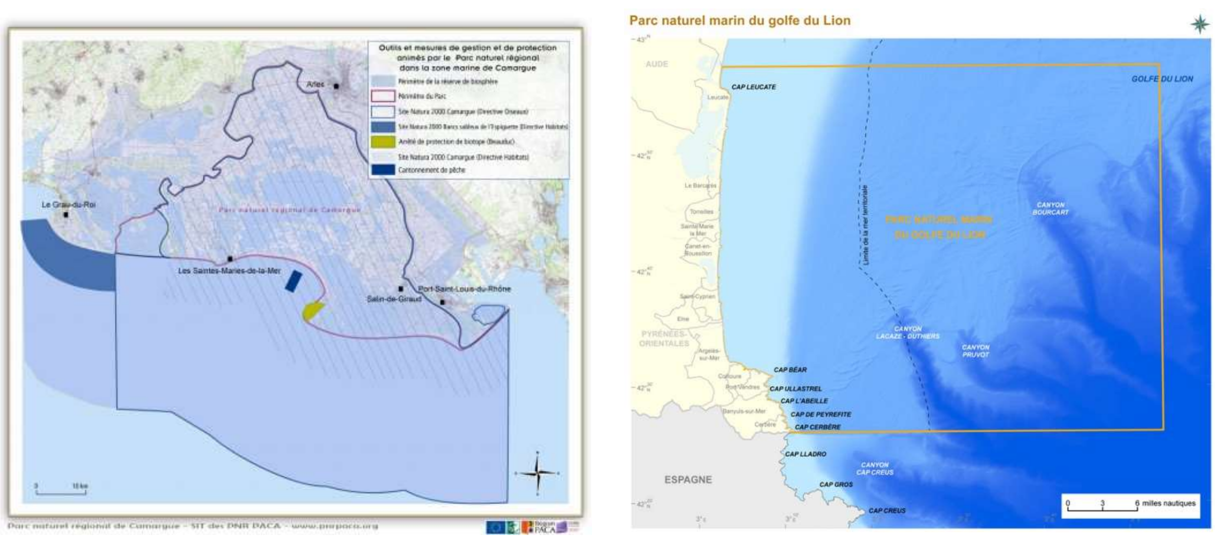
Fig 4 detection areas and migration of sea litter

- **Synoptic comparison of different observation platforms**

CEFE experiments to be implemented in WP4

1) **Study areas** (figure 1)

Study areas were selected according to several parameters (involvement of the MPA agents, availability of vessels, presence of fishermen willing to be involved in MedSeaLitter, degree of pollution by marine debris, presence of sea turtles, related studies carried out in these regions, etc.).
The two selected regions are: The Natura 2000 Camargue area and the *Parc naturel marin du golfe du Lion* (figure 1).



a b

Figure 1. Study areas (**a** : Natura 2000 Camargue ; **b**: Parc Naturel Marin du Golfe du Lion)

2) **Experiment 1**
   a) **The objective is to compare synoptically observations of litter and megafauna at the regional scale and by means available in MPAs** (experiment carried out by CEFE and EcoOcéan Institut) by (figure 2) :
      - Observers from vessels of different heights, using strip transect methodology (partnership made for inflatable, fishing and sailing boats accessibility),
      - Drone (partnership made with drone experts)
      - Trawling net (being constructed by artisanal fishermen involved in MedSeaLitter)
      - Pole camera (description of the device and experimentations in figure 3)

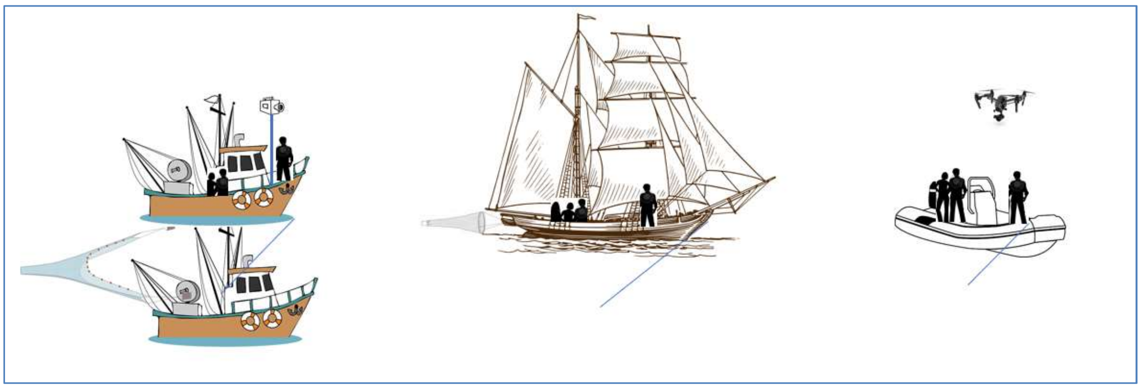


Figure 2. Experiment 1

Pole camera device (description and experimentations)
Tests are being carried out by CEFE to define the characteristics of the pole camera device: type of camera, angle of view, focal length, inclination of the device, height of the pole (figure a).

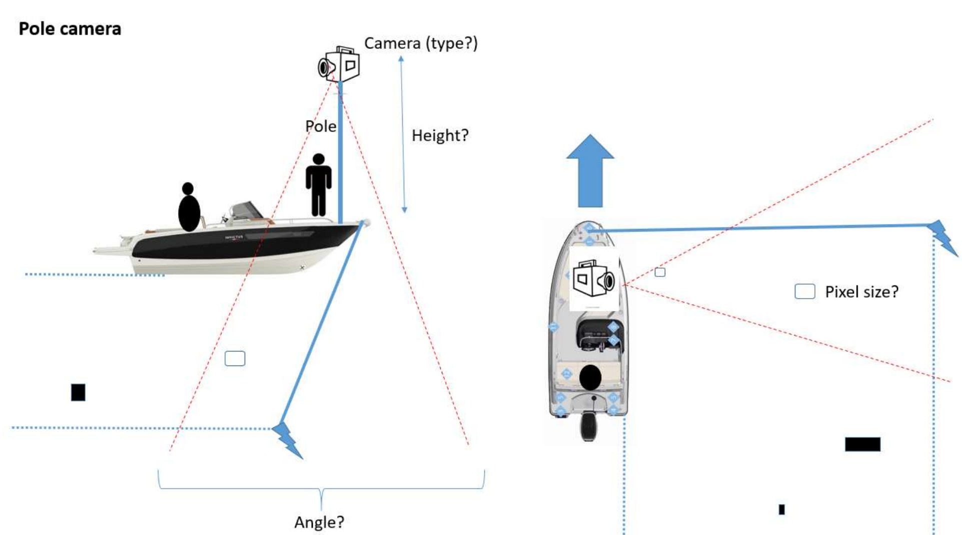


Figure a. Pole camera experimentation

Tests consist in comparing different types of cameras (reflex, GoPro, Smartphone) with different characteristics (focal length, angle of view) and to define: field of view, height of the camera, inclination of the camera, position of the pole on the boat, battery needs, memory card capacity, time lapse intervals, image distortion issues, image processing methodology (in collaboration with the University of Barcelona), costs and ergonomics, availability and robustness of the equipment, etc (figure b).

Figure b. Pole camera testings

According to the results of the first tests, the following equipment seems to be the most appropriate: Polarized *GoPro Hero Session* camera, inclined at 45°, MicroSDXC SanDisk ultra 200 Go memory card, combined with an *Anker PowerCore* 26800 external battery, protected in a homemade waterproof Plexiglas box (figure c). Further tests (at sea) are needed and will be carried out shortly.

Figure c. Polarized *GoPro Hero Session* camera combined with an *Anker PowerCore* 26800 external battery, protected in Homemade waterproof Plexiglas box.

b) **The Objective is to study the (potential) correlation between macro and micro litter** thanks to a micro-plastic net attached to the sailing boat and a trawling net attached to two artisanal fishing boats (experiment carried out by CEFE).

3) **Experiment 2:**

The objective is to compare synoptically litter observations by different means (available in MPAs) and using the “litter line” made by the University of Barcelona for detection by drone (experiment carried out by CEFE) (figure 4 & 5):

- Observers from vessels of different heights, following the band transect methodology
- Pole camera
- Drone

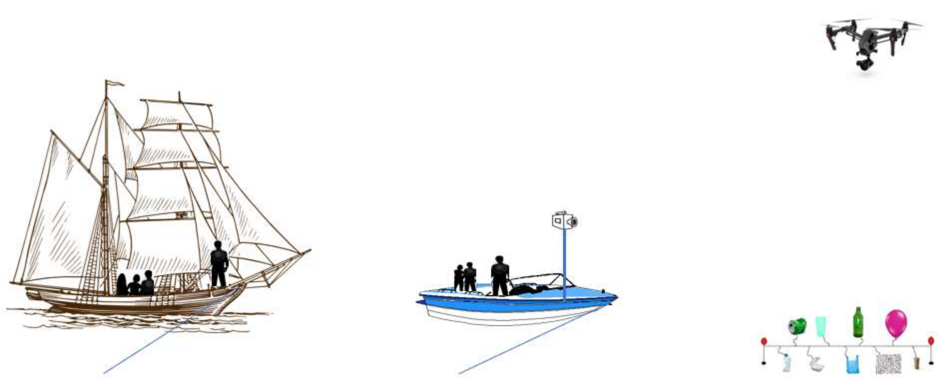


Figure 4. Experiment 2

Figure 5. Testing of experiment 2 at sea

4) **Experiment 3**

The objective is to estimate the probability of detection (visual observation with band transect) using “litter lines” thanks to transect replication to infer detection by: size/colour/material/distance to the vessel/meteorological conditions, etc.
(figure 6)

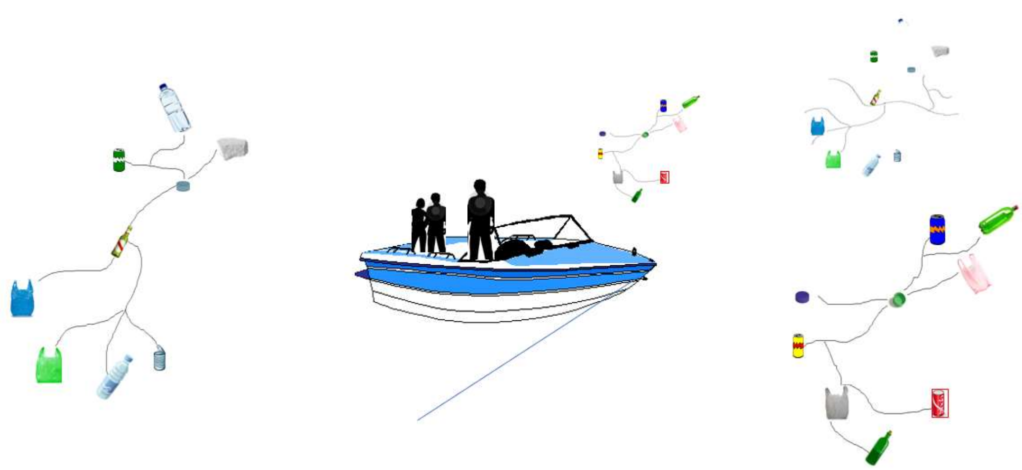


Figure 6. Experiment 3

- **Other experiments to be implemented during the testing phase (WP4)**

1. **Experience of observers:**
Experiments to evaluate how the detection probability is related to the experience of the observer in order to set minimal experience levels (double observers synoptically taking data within same conditions, plus camera as control):
    - From ferries (ISPRA, Capo Carbonara MPA)
    - From sailing boat (Legambiente and ISPRA,)
    - ISPRA will use the previous data collected from ferries (2013-2016) to analyze the influence of Beaufort state on sighting probability.
2. **Strip width**
To define the appropriate width of the strip for each platform type and/or calculate the correction factors experiments will be set for the different platforms. The main objective of the experiment to be carried out for the MEDSEALITTER project is to review the table 4 of JRC Guideline (JRC, 2013) in order to refine the appropriate strip in relation to height of the observation point and the speed of the platform. The proposed experiments are:
    a. Comparison of data from the first and second half of a fixed strip to obtain the correction factor:
        - From Ferries (ISPRA, Capo Carbonara MPA)
        - From sailing boat (Legambiente and ISPRA)
    b. Operation experiments to set up the strip width from:
        - Drone (University of Barcelona)
        - Aircraft (University of Valencia)
    c. Tools to evaluate the strip width (measuring stick/rangefinder stick/ graduate binocular/inclinometer) appropriately set for the different platform types:
        - From ferry (ISPRA, EcoOcéan Institut, Capo Carbonara MPA): tape on the glass to individuate the limit of the strip
        - From sailing/medium sized boat (EcoOcéan Institut, Legambiente): vertical graduate stick with strip limit
        - From inflatable boat (ISPRA, Capo Carbonara MPA): horizontal graduate stick with strip limit
        - Form aircraft (University of Valencia): inclinometer and angle ranges marked with color tape on the window.
3. **Size classes**
    a. A list of the size of different litter categories (entire object) using the top 20 categories identified by literature and by previous data from ISPRA monitoring starting from the list compiled by the University of Barcelona.

b. From all platforms observers will be trained with arrays and other experiments to identify the size class of the items from each specific platform.
c. From aircrafts, the University of Valencia proposes 3 size classes which can be comparable with the JRC size classes: <30 cm (ref. sea turtle size); 30-100 cm (ref. pallet); 100-200 cm (ref. bottlenose dolphin).
d. From ferries the ruler proposed by EcoOcéan Institut will be tested.
e. A category for "Aggregation" of items of small dimension or fragments is proposed.
f. Experiments are planned in order to identify the proportion of:
    - floating micro and macro litter (EcoOcéan Institut and CEFE from vessel equipped with net);
    - size classes and item categories (Cinque Terre National Park from the MPA boat for litter collection).

References FML

Adame, K., Pardo, M. A., Salvadeo, C., Beier, E., Elorriaga-Verplancken, F., 2017. Detectability and categorization of California sea lions using an unmanned aerial vehicle. Marine Mammal Science. DOI: 10.1111/mms.12403.

Aliani, S., Griffa, A., Molcard, A., 2003. Floating debris in the Ligurian Sea, north-western Mediterranean. Marine Pollution Bulletin 46, 1142–1149.

Arcangeli A., Campana I., Angeletti D., Atzori F., Azzolin M., Carosso L., Di Miccoli V., Giacoletti A., Gregorietti M., Luperini C., Paraboschi M., Pellegrino G., Ramazio M., Sarà G., Crosti R. (2017) Amount, composition, and spatial distribution of floating macro litter along fixed trans-border transects in the Mediterranean basin. Marine Pollution Bulletin. https://doi.org/10.1016/j.marpolbul.2017.10.028

Barnes, D. K. A., Galgani, F., Thompson, R. C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: Biological Sciences 364, 1985–1998.

Bauer, R., Bonhommeau, S., Brisset, B., Fromentin, J-M., 2015. Aerial surveys to monitor Bluefin tuna abundance and track efficiency of management measures. Marine Ecology Progress Series 534, 221–234.

Boerger, C., Lattin, G., Moore, S. & Moore, C. 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Marine Pollution Bulletin,60, 2275-2278.

Bryson, M., Williams, S., 2015. Review of Unmanned Aerial Systems (UAS) for Marine Surveys. Australian center for Field Robotics, University of Sidney.

Buckland, S.T., Anderson, D.R., Burnham, K.P., Laake, J.L., 1993. Distance Sampling: Estimating Abundance of Biological Populations. Chapman & Hall, London.

Camedda, A ., M arra, S., Matiddi, M., Massaro, G., Coppa, S., Perilli, A ., Ruiu, A., Briguglio, P., de Lucia, G.A ., 2014. Interaction between loggerhead sea turtles (*Caretta caretta*) and marine litter in Sardinia (Western M editerranean Sea). Mar. Environ. Res. 10 0, 25 e 32 .

Campani, T., Baini, M., Giannetti, M., Cancelli, F., Mancusi, C., Serena, F., Marsili, L., Casini, S., Fossi, M.C., 2013. Presence of plastic debris in loggerhead turtle stranded along the Tuscany coasts of the Pelagos sanctuary for Mediterranean marine mammals (Italy). Mar. Pollut. Bull. 74, 1330e13 34.

Caric, H., Mackelworth, P., 2014. Cruise tourism environmental impacts – The perspective from the Adriatic Sea. Ocean & Coastal Management 102, 350-363.

Cheshire, A.C., Adler, E., Barbière, J., Cohen, Y., Evans, S., Jarayabhand, S., Jeftic, L., Jung, R.T., Kinsey, S., Kusui, E.T., Lavine, I., Manyara, P., Oosterbaan, L., Pereira, M.A., Sheavly, S., Tkalin, A., Varadarajan, S., Wenneker, B., Westphalen, G., 2009. UNEP/IOC Guidelines on Survey and Monitoring of Marine Litter. UNEP Regional Seas Reports and Studies, No. 186; IOC Technical Series No. 83: xii + 120 pp.

Coe, J. M., Rogers, D. B., (eds) 1997. Marine debris: sources, impacts, and solutions. New York, NY: Springer-Verlag.

Coppola D., Cicero A.M., Panti C., Baini M., Guerranti C., Marsili L., Massaro G..,Fossi M.C. and

Darmon, G., Miaud, C., Claro, F., Doremus, G., Galgani, F., 2016. Risk assessment reveals high exposure of sea turtles to marine debris in French Mediterranean and metropolitan Atlantic waters. Deep Sea Research Part II. Doi: 10.1016/j.dsr2.2016.07.005

Day, R. H., Shaw, D. G., 1987. Patterns of abundance of pelagic plastic and tar in the North Pacific Ocean, 1976–1985. Marine Pollution Bulletin 18, 311–316.

De Lucia G.A., Caliani I., Marra S., Camedda A., Coppa S., Alcaro L ., Campani T., Giannetti M.,
de Stephanis, R., Gimenez, J., Carpinelli, E., Gutierrez-Exposito, C., Canadas, A ., 2013. As main meal for sperm whales: plastics debris. Mar. Pollut. Bull. 69: 206-214 .
De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., Robbens, J., 2014. Quality assessment of the blue mussel (*Mytilus edulis*): comparison between commercial and wild types. Mar. Pollut. Bull. 85 (1): 146 – 155 .
Derraik, J.G.B., 2002. The pollution of the marine environment by plastic debris: a review. Mar. Pollut. Bull. 4 4 (9), 842 – 852 .
Di-Méglio, N., Campana, I., 2017. Floating macro-litter along the Mediterranean French coast: Composition, density, distribution and overlap with cetacean range. Marine Pollution Bulletin 118, 155-166.
Dufault, S., Whitehead, H., 1994. Floating Marine Pollution in ' the Gully ' on the Continental Slope , Nova Scotia. Marine Pollution Bulletin 28, 489–493.
Galgani, F., Fleet, D., van Franeker, J., Katsanevakis, S., Maes, T., Mouat, J., Oosterbaan, L., Poitou, I., Hanke, G., Thompson, R., Amato, E., Birkun, A., Jansse, C., 2010. Marine Strategy Framework Directive Task Group 10 Report on Marine litter, European Union, IFREMER and ICES.
Galgani, F., Hervé, G., Carlon, R., 2013b. Wavegliding for marine litter. Rapport Commission International Mer Méditerranée 40, 306.
GEF 2012. Impacts of Marine Debris on Biodiversity: Current Status and Potential Solutions, Montreal, Technical Series No. 67, 61 pages.
Gerigny, O., Henry, M., Tomasino, C., Galgani, F., 2011. Dechets en mer et sur le fond. Un rapport de l'evalution initiale, Plan d'action pour le milieu marin - Mediterranee Occidentale, rapport PI Dechets en mer V2 MO, pp. 241-246.
Gilles, A., Scheidat, M., Siebert, U., 2009. Seasonal distribution of harbour porpoises and possible interference of offshore wind farms in the German North Sea. Marine Ecology Progress Series 383, 295–307.
Gómez de Segura, A., Hammond, P. S., Cañadas, A., Raga, J. A., 2007. Comparing cetacean abundance estimates derived from spatial models and design-based line transect methods. Marine Ecology Progress Series 329, 289–299.
Gómez de Segura, A., Tomás, J., Pedraza, S. N., Crespo, E. A., Raga, J. A., 2006. Abundance and distribution of the endangered loggerhead turtle in Spanish Mediterranean waters and the conservation implications. Animal Conservation 9, 199–206.
González-Fernández, D., Hanke, G., 2017. Toward a Harmonized Approach for Monitoring of Riverine Floating Macro Litter Inputs to the Marine Environment. Frontiers in Marine Science 4, 86.
Graham, E.R., Thompson, J.T., 2009. Deposit and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. J. Exp. Mar. Biol. Ecol. 36: 822-8 29 .
Hanke, G., Piha, H., 2011. Large scale monitoring of surface floating marine litter by high resolution imagery. Fifth International Marine Debris Conference, 20–25 March 2011, Hawaii, Honolulu.
Hinojosa, I.A., Thiel, M., 2009. Floating marine debris in fjords, gulfs and channels of southern Chile. Marine Pollution Bulletin 58, 341–350.
Hodgson, A., Kelly, N., Peel, D., 2013. Unmanned Aerial Vehicles (UAVs) for surveying marine fauna: A dugong case study. PLoS ONE 8(11): e79556.
ISPRA, 2015. Technical Annex II Marine litter & Marine macro-fauna Protocol for the agreement 'Fixed Line Transect using ferries as platform of observation for monitoring cetacean populations' Protocol for monitoring by vessel of floating marine macro-litter and marine macro-fauna along a fixed transect width. ISPRA, Rome, 10 pp.

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R., Law, K. L., 2015. Plastic waste inputs from land into the ocean. Science 347(6223), 768-771.

Koski, W. R., Allen, T., Ireland, D., Buck, G., Smith, P. R., Macrander, A. M., Halick, M. A., Rushing, C., Sliwa, D. J., McDonald T. L., 2009. Evaluation of an Unmanned Airborne System for monitoring marine mammals. Aquatic Mammals 35(3), 347-357.

Lauriano, G., Panigada, S., Casale, P., Pierantonio, N., Donovan, G. P., 2011. Aerial survey abundance estimates of the loggerhead sea turtle *Caretta caretta* in the Pelagos Sanctuary, northwestern Mediterranean Sea. Marine Ecology Progress Series 437, 291–302.

Laran S., Pettex E., Authier M., Blanck A., David L., Doremus G., Falchetto H., Monestiez P., Van Canneyt O.& Ridoux V., (2016). Seasonal distribution and abundance of cetaceans within French waters-Part I: The North-Western Mediterranean, including the Pelagos sanctuary. Deep Sea Research Part II. http://www.aires-marines.fr/Connaitre/Habitats-et-especes-pelagiques/Oiseaux-et-mammiferes-marins-en-metropole

Lazar, B., Gracan, R., 2011. Ingestion of marine debris by loggerhead sea turtle, *Caretta caretta* in the Adriatic Sea. Mar. Pollut. Bull. 62: 43-47.

Lecke-Mitchell, K. M., Mullin, K., 1992. Distribution and abundance of large floating plastic in the north-central Gulf of Mexico. Marine Pollution Bulletin 24, 598–601.

Maire, F., Mejias, L., Hodgson, A., Duclos, G., 2013. Proceedings of the 2013 IEEE/RSJ International Conference on Intelligent Robots and Systems. November 3-7, 2013. Tokyo, Japan.

MAP/UNEP, 2001. Litter Management in coastal zones of the Mediterranean Basin - Analysis of the questionnaire and proposals for guidelines. Document UNEP(DEC)/MED WG.183/4.

Marais, M., Armitage, N., Wise, C., 2004. The measurement and reduction of urban litter entering stormwater drainage systems: paper 1—quantifying the problem using the City of Cape Town as a case study. Water SA 30, 483–492.

Matiddi M. 2014. Amount and distribution of neustonic micro-plastic off the Western Sardinian coast (Central-Western Mediterranean Sea). Marine Environmental Research, 100: 10-16

Matiddi M., Hochscheid S., Camedda A., Baini M., Cocumelli C., Serena F., Tomassetti P., Travaglini A., Marra S., Campani T., Scholl F., Mancusi C., Amato E., Briguglio P., Maffucci F., Fossi MC., Flegra Bentivegna F, de Lucia GA. 2017. Loggerhead sea turtles (*Caretta caretta*): a target species for monitoring litter ingested by marine organisms in the Mediterranean Sea. Environmental Pollution. 230,199-2019 http://dx.doi.org/10.1016/j.envpol.2017.06.054

Matsumura, S., Nasu, K., 1997. Distribution of floating debris in the North Pacific Ocean: sighting surveys 1986–1991. In Marine debris: sources, impacts, and solutions (eds J. M. Coe & D. B. Rogers), pp. 15–24. New York, NY: Springer-Verlag.

McCoy, F.W., 1988. Floating megalitter in the eastern Mediterranean. Marine Pollution Bulletin 19, 25–28.

Merlino, S., Locritani, M., Muccini, F., Bianucci, M., Berta, M., Giacomazzi, F., ... Perfetti, A., 2017. Eco-drifters for a dispersion experiment at the mouth of the River Arno: the citizen-science contribution. In EGU General Assembly Conference Abstracts, Vol. 19, p. 15595.

Morris, R.J., 1980. Floating plastic debris in the Mediterranean. Marine Pollution Bulletin 11, 125.

MSFD Technical Subgroup on Marine Litter., 2013. Guidance on Monitoring of Marine Litter in European Seas. Scientific and Technical Research series, Report EUR 26113 EN.

OSPAR, 2009. Marine litter in the North-East Atlantic Region: Assessment and priorities for response. London, United Kingdom.

Panigada, S., Lauriano, G., Burt, L., Pierantonio, N., Donovan, G., 2011. Monitoring winter and summer abundances of cetaceans in Pelagos Sanctuary (Northwestern Mediterranean Sea) through aerial surveys. PLoS ONE 6(7): e22878.

Panigada, S., Lauriano, G., Donovan, G., Pierantonio, N., Cañadas, A., Vázquez, J. A., Burt, L., 2017. Estimating cetacean density and abundance in the Central and Western Mediterranean Sea through aerial surveys: implications for management. Deep Sea Research Part II. Doi: 10.1016/j.dsr2.2017.04.018

Pichel, W. G., Churnside, J. H., Veenstra, T. S., Foley, D. G., Friedman, K. S., Brainard, R. E., Nicoll, J. B., Zheng, Q., Clemente-Colon, P., 2007. Marine debris collects within the North Pacific Subtropical Convergence Zone. Marine Pollution Bulletin 54, 1207–1211.

Piha, H. E., Hanke, G., Galgani, F., Werner, S., Alcaro, L., Mattidi, M., Fleet, D., Kamizoulis, G., Maes, T., Osterbaan, L., Thompson, R., Van Franeker, J. A., Mouat, J., Meacle, M., Carroll, C., Detloff, K. C., Kinsey, S., Nilsson, P., Sheavly, S., Svärd, B., Veiga, J. M., Morison, S., Katsanevakis, S. M., Lopez-Lopez, L., Palatinus, A., Scoullos, M. 2011. Marine Litter: Technical Recommendations for the Implementation of MSFD Requirements. Scientific and Technical Research Reports, EUR 25009 EN.

Ryan, P. G., 2013. A simple technique for counting marine debris at sea reveals steep litter gradients between the Straits of Malacca and the Bay of Bengal. Marine Pollution Bulletin 69(1), 128-136.

Ryan, P. G., Cooper, J., 1989. Observer precision and bird conspicuousness during counts of birds at sea. South African Journal of Marine Science 8, 271–276.

Ryan, P.G., Moore, C.J., van Franeker, J.A., Moloney, C.L., 2009. Monitoring the abundance of plastic debris in the marine environment. Philosophical Transactions of the Royal Society B: Biological Sciences 364, 1999– 2012.

Sá, S., Bastos-Santos, J., Araújo, H., Ferreira, M., Duro, V., Alves, F., Panta-Ferreira, B., Nicolau, L., Eira, C., Vingada, J., 2016. Spatial distribution of floating marine debris in offshore continental Portuguese waters, Marine Pollution Bulletin 104, 269–278.

Scheidat, M., Feindt-Herr, H., 2012. Collecting data on marine debris during cetacean aerial surveys. International Whaling Commission, SC/64/E15.

Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185: 77– 83.

Sheavly, S. B. 2007 National Marine Debris Monitoring Program: final program report, data analysis and summary. Washington, DC, USA: Ocean Conservancy.

Shiomoto, A., Kameda, T., 2005. Distribution of manufactured floating marine debris in near-shore areas around Japan. Marine Pollution Bulletin 50, 1430–1432.

Suaria, G., Aliani, S., 2014. Floating debris in the Mediterranean. Marine Pollution Bulletin 86 (1-2), 494-504.

Thiel, M., Hinojosa, I., Vásquez, N., Macaya, E., 2003. Floating marine debris in coastal waters of the SE-Pacific (Chile). Marine Pollution Bulletin 46, 224–231.

Thiel, M., Hinojosa, I.A., Joschko, T., Gutow, L., 2011. Spatio-temporal distribution of floating objects in the German Bight (North Sea). Journal of Sea Research 65, 368–379.

Thomas, L., Buckland, S. T., Rexstad, E. A., Laake, J. L., Strindberg, S., Hedley, S. L., Bishop, J. R. B., Marques, T. A., Burnham, K. P., 2010. Distance software: design and analysis of distance sampling surveys for estimating population size. Journal of Applied Ecology 47, 5–14.

Thompson, R.C., Olsen, Y., MitchellI, R.P., Davis, A ., Row land, S.J., John, A .W.G., Mcgonigle, D., Russell, A .E., 2004. Lost at sea: where is all the plastic? Science 30 4: 838 838 .

Topcu, E. N., Tonay, A. M., Öztürk, B., 2010. A preliminary study on marine litter in the Aegean Sea. Rapport Commission International Mer Méditerranée 39, 804.

UNEP, 2015. Marine Litter Assessment in the Mediterranean. UNEP/MAP Athens, 45 pp.

UNEP/MAP MEDPOL, 2011. Results of the Assessment of the Status of Marine Litter in the Mediterranean Sea, UNEP/MAP(DEPI)/MED WG.357/Inf.4.

Unger, B., Herr, H., Gilles, A., Siebert, U., 2014. Evaluation of spatio-temporal distribution patterns of marine debris in the SCI Sylt Outer Reef. 28th Conference of the European Cetacean Society, Liège (Belgium).

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015b. Microplastics are taken up by mussels (Mytilus edulis) and lugworms (Arenicola marina) living in natural habitats. Environmental Pollution 199: 10-17 .

Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R., 2015a. Microplastics in sediments: A review of techniques, occurrence and effects. Marine Environmental Research . htt p: //dx.doi.org/10.1016/j. marenvres.2015.0 6.0 07.

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environmental Pollution 193: 65-70.

Van Franeker, J., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P., Heubeck, M., Jensen, J., Le Guillou, G., Olsen, B., Olsen, K., Pedersen, J., Stienen, E. & Turner, D. 2011. Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea. Environmental Pollution 159: 2609-2615.

Vlachogianni, T., Zeri, C., Ronchi, F., Fortibuoni, T., Anastasopoulou, A., 2016. Marine Litter Assessment in the Adriatic and Ionian Seas. The DeFishGear Project report.

Watts A., Lewis C., Goodhead R.M., Becket S.J., Moger J., Tyler C.R., Galloway T.S. 2014. Uptake and retention of microplastics by the shore crab *Carcinus maenas*. Environ. Sci. Technol., 48(15): 8823-8830.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impact of micro-plastics on marine organisms: A review. Environmental Pollution 178: 483-492 .

Zampoukas, N., Palialexis, A., Duffek, A., Graveland, J., Giorgi, G., Hagebro, C., Hanke, G., Korpinen, S., Tasker, M., Tornero, V., Abaza, V., Battaglia, P., Caparis, M., Dekeling, R., Frias Vega, M., Haarich, M., Katsanevakis, S., Klein, H., Krzyminski, W., Laamanen, M., Le Gac, J.C., Leppanen, J.M., Lips, U., Maes, T., Magaletti, E., Malcolm, S., Marques, J.M., Mihail, O., Moxon, R., O'Brien, C., Panagiotidis, P., Penna, M., Piroddi, C., Probst, W.N., Raicevich, S., Trabucco, B., Tunesi, L., Van der Graaf, S., Weiss, A., Wernersson, A.S., Zevenboom, W., 2014. Technical guidance on monitoring for the Marine Strategy Framework Directive. JRC Scientific and Policy Report. Scientific and Technical Research series, Report EUR 26499.

2. MONITORING MACRO AND MICRO LITTER INGESTED AT LARGE AND LOCAL MPAS SCALES

I. Sea turtles, macroplastics and diet

1) Sea turtles and macroplastics

In the Mediterranean Sea, the sea turtle represents the best indicator to monitor marine litter ingested by biota at large scale..

Due to the strong connection between the MEDSEALITTER project and the INDICIT project, both European co-financed projects with the common objective to harmonize protocols and adopt a single procedure among European and Mediterranean countries, it has been decided during the 2nd MEDSEALITTER meeting at Villasimius (Italy), to apply the same standardized protocol on sea turtles ingestion.

This protocol follows and slightly modifies the protocol proposed by the MSFD-TSG on Marine Litter report “Guidance on Monitoring of Marine Litter in European Sea” (Matiddi et al., 2011; Galganì et al., 2013), considering basic and optional parameters proposed to stakeholders according to their logistic and time constraints.

In the MEDSEALITTER project, the protocol concerns exclusively the necropsy on dead loggerhead turtles *Caretta caretta* (Linnaeus, 1758). Each portion of the gastro-intestinal tract (GI) is separated with clams in order to avoid mixing of the contents and it is analyzed separately. The contents should be emptied into a 1 mm mesh sieve, visually inspected for the presence of tar, oil or any particularly fragile material and then washed with freshwater. Items retained on the sieve are collected and preserved in 70% ethanol solution until analysis. All the materials are dried for 24h before being sorted, analyzed under a stereo-microscope and weighted.

Deriving from the Fulmar’s marine litter categories, the MSFD protocol was based on the general ‘morphs’ of plastics (sheet-like, filament, foamed, fragment, other). Following the protocol, the items were subdivided into 4 main categories (IND-Industrial plastic, USE-User plastic, RUB-Non plastic rubbish, POL-Pollutants), including 14 different subcategories, plus food remains (Foo) and natural non-food remains (Nfo). In the new protocol, the marine litter categories are reduced, as suggested by Matiddi et al. (2017), merging Pollutant and Rubbish into one category called “Other Litter (non-plastic)” (see Table 1).

Ingested Litter (Y/N)	Plastic volume (ml)					Category (dry mass mg)				
						Oesophagus /Stomach/ Intestine				
		Ind. Plastic	Use she	Use thr	Use foa	Use frag	Other (Poth)	Other Litter (non plastic)	Natural Food (Foo)	Natural No Food (Nfo)

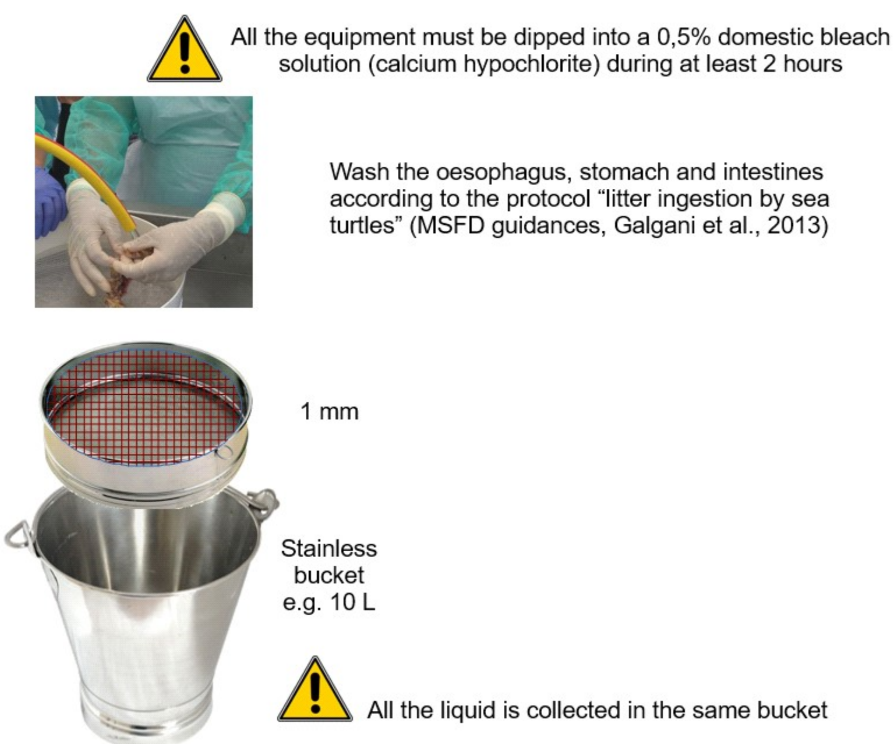
Table 1: Basic information on ingested litter in sea turtles and typologies

According to this protocol, the dry mass (weight in mg) and the abundance (number of items) ingested are the main information useful for the monitoring program.

Other information such as the color of items, the categories of litter, and the incidence of litter in oesophagus, intestine and stomach, are useful for research activities as well as management decisions.

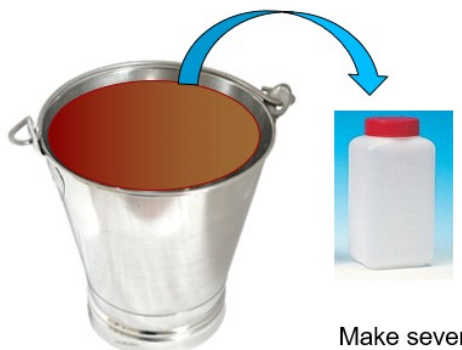
2) Diet of sea turtles

The knowledge of sea turtle diet is based on the analysis of gut content (*e.g*. Pegard *et al*., 2009; Soininen *et al*. 2009; Shehzad *et al*., 2012). Gut content is mainly identified visually. In this case, diet studies directly depend on the ability to identify ingested items. When these items possess hard parts (*e.g.* gastropod or bivalve shells, crab exoskeletons, cephalopod beaks, fish scales, etc.), visual identification can be performed. However, these hard items can be quickly degraded during digestion processes, preventing correct and precise identification. Furthermore, some potential preys do not have solid parts (*e.g*. starfishes, holothuries, plants, algae, etc.). The use of molecular methods to identify diet, through DNA fragments of ingested items (so called eDNA), has solved this methodological bottleneck. It has been used to identify plant ingested by small rodents (Soininen *et al*., 2009) and chamois (Pegard *et al*., 2009) or for predator species such as snow panthers (Shehzad *et al*., 2012). EPHE/CEFE proposed a protocol (figure 1) to use the eDNA method during necropsies of gastro-intestinal tracks realized for the analysis of plastic ingestion by sea turtles.



If the bucket is full while the digestive track washing is not finish…

Homogenize the contain with a stainless spatula, then collect 1 L of the liquid and keep it in a separate bottle (= subsamples).



1 L

10 L

Make several subsamples if the washing lead to the filling of several buckets

The sieve is washed and items are analysed following the MSFD protocol.

The biological part is referenced and stored in freezer bags at -20°C for future visual determination

Add subsample(s) in the collecting bucket

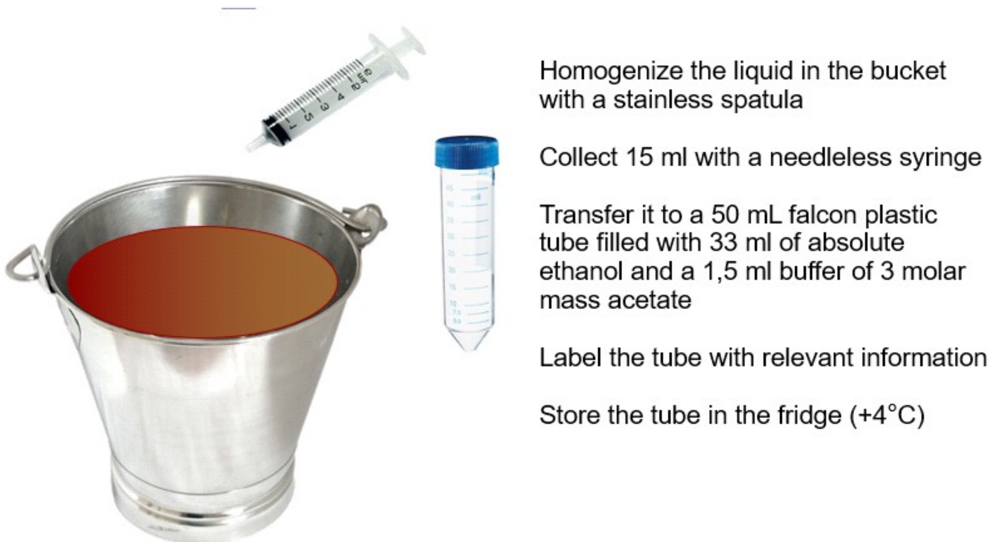


Figure 1. Protocol for the eDNA analysis

II. Fishes and microplastics

At local scale (eg: inside AMPs) the target species should reflect the environmental condition in which it has been collected. For this reason animals with a long transit time should be avoided (e.g.: Turles).
During the studying phase of the MEDSEALITTER project, a workshop was organized in Greece with all the partners.
The objective of the workshop was to test the methods proposed for the detection of microplastic ingestion to select and adopt a common methodology among MEDSEALITTER partners. To achieve this, a short experiment for the detection of microplastics in fish or mussel microplastic-enriched tissues has been designed.
Four methods for microplastic extraction from fish and mussel tissue have been tested:

a) Tissue digestion using H_2O_2 15%- 5 samples
b) Tissue digestion using H_2O_2 30%- 5 samples
c) Tissue digestion using KOH 10%- 5 samples
d) Ultrasonic extraction of microplastics- 2 samples

Microplastic enrichment of samples for used to test digestion methods

In order to test the efficiency of each digestion method, gastrointestinal tracts of 25 anchovy (*Engraulis encrasicolus*) specimen were extracted. Fish tissue (aprox. 0.60g) was enriched with a fixed number of microplastic particles (10 microplastic particles/sample). Microplastics were first sieved between 300μm and 1000μm. Sizes of all microplastics used were recorded before the experiment. Five different chemical types of plastic were used: PE, PP, PVC, PS and PET (Table 2).

Method	Types of plastic				
H_2O_2 15%	SAMPLE 1 (PE)	SAMPLE 2 (PP)	SAMPLE 3 (PVC)	SAMPLE 4 (PS)	SAMPLE 5 (PET)
H_2O_2 30%	SAMPLE 6 (PE)	SAMPLE 7 (PP)	SAMPLE 8 (PVC)	SAMPLE 9 (PS)	SAMPLE 10 (PET)
KOH 10%	SAMPLE 11 (PE)	SAMPLE 12 (PP)	SAMPLE 13 (PVC)	SAMPLE 14 (PS)	SAMPLE 15 (PET)
Ultrasonic	SAMPLE 16 (PE)	SAMPLE 17 (PE)			

Table 2. Sample distribution with varying plastic types and digestion methods

Protocol applied for tissue digestion

- Add 20ml of H_2O_2 (15% or 30%) or 10 ml of KOH 10% per 1g of tissue to each sample
- Prepare a blank sample to check airborne contamination
- Cover samples with foil paper
- Incubate samples on hot plate (60˚C approximately for H2O2 digestion, 40˚C approximately for KOH digestion) until all organic matter is removed
- Add 100 ml of H_2O and stir it at high intensity using a magnetic stirrer
- Filter samples in GF/C 0.2μm 47 mm diam. in a glove box using vacuum pump- Rinse glass above the filter with distilled water
- Store filters in glass petri dishes (or plastic petri dishes covered by foil paper) and dry overnight in the oven (45°C).
  - Examine filters for micro plastics under stereomicroscope

Microplastic enrichment of samples for used to test ultrasonic extraction of microplastics

In order to check the efficiency of ultrasonic separation of microplastic from tissue, a short experiment was performed. Two mussels (*Mytilus galloprovincialis*) were exposed to 0.060g of PE particles (ranging from 300-1000 μm) individually in 1L tanks for one day. Digestive gland and gills were extracted and ultrasonic separation protocol was followed in general accordance with Wager et al. 2016, with some alterations in the ultrasonic treatment (lower burst power and larger time intervals between bursts) due to the different capacities of the available ultrasonic instrument.

Protocol applied for ultrasonic extraction of microplastics

- Add 40ml of H_2O in each sample
- Sonicate samples for 5 min (stop for 30 sec after each minute) in water bath
- Sieve samples through a 1 mm sieve
- Filter samples in GF/C 0.2μm 47 mm diam. using vacuum pump- Rinse glass above the filter with distilled water
- Store filters in glass petri dishes (or plastic petri dishes covered by foil paper) and dry for 24 hours at room temperature
- Examine sieved tissue and filters for micro plastics under stereomicroscope

Contamination Precautions

In order to prevent airborne contamination, samples were covered by foil paper throughout the whole digestion process, while blank samples were also processed at the same time. During the procedure of dissection and filtration, samples were processed under a glove box, while during stereoscopical observation of the filters Petri dishes were covered by a glass dish.

Results

Microplastic collection

After digestion and filtration, samples were observed under stereoscope and microplastics were counted. As indicated below in Table 3, digestion by H2O2 15% shows the highest average percentage of recovery (from 80 to 100%), followed by KOH (from 80 to 100%) and H2O2 30% (from 60 to 100%).

Sample	Method	Plastic	MPS before digestion	MPS after digestion	% recovery	% Average per method
1	**H_2O_2 15%**	PE	10	9	90	
2	**H_2O_2 15%**	PP	10	10	100	
3	**H_2O_2 15%**	PVC	10	10	100	
4	**H_2O_2 15%**	PS	10	10	100	
5	**H_2O_2 15%**	PET	10	8	80	**94**
6	**H_2O_2 30%**	PE	10	8	80	
7	**H_2O_2**	PP	10	10	100	

	30%					
8	**H_2O_2 30%**	PVC	10	9	90	
9	**H_2O_2 30%**	PS	10	6	60	
10	**H_2O_2 30%**	PET	10	10	100	**86**
11	**KOH 10%**	PE	10	10	100	
12	**KOH 10%**	PP	10	10	100	
13	**KOH 10%**	PVC	10	9	90	
14	**KOH 10%**	PS	10	9	90	
15	**KOH 10%**	PET	10	8	80	**92**

Table 3. Percentages of microplastic recovery for each method.

Digestion efficiency

In order to test the digestion efficiency of each method, filters were weighted before and after filtration. In both cases, filters were dried overnight in the oven (45ºC). Table 4 indicates that KOH is the most efficient method in terms of tissue digestion which agrees with previous studies (Karami et al., 2017), although pieces of tissues were visible in the sample after digestion.

Sample	Method	Plastic	Filter weight before (g)	Filter weight after (g)	Difference	Tissue weight (g)	% recovery	% average per methodology
1	**H_2O_2 15%**	PE	0,092	0,123	0,031	0,62	5,000	
2	**H_2O_2 15%**	PP	0,124	0,121	-0,003	0,65		
3	**H_2O_2 15%**	PVC	0,081	0,105	0,024	0,645	3,721	
4	**H_2O_2 15%**	PS	0,094	0,111	0,017	0,621	2,738	
5	**H_2O_2 15%**	PET	0,094	0,106	0,012	0,6	2,000	**3,365**
6	**H_2O_2 30%**	PE	0,080	0,115	0,035	0,57	6,140	
7	**H_2O_2 30%**	PP	0,074	0,115	0,041	0,63	6,508	

8	**H_2O_2 30%**	PVC	0,091	0,111	0,02	0,603	3,317	
9	**H_2O_2 30%**	PS	0,093	0,110	0,017	0,618	2,751	
10	**H_2O_2 30%**	PET	0,090	0,103	0,013	0,601	2,163	**4,176**
11	**KOH 10%**	PE	0,080	0,095	0,015	0,58	2,586	
12	**KOH 10%**	PP	0,090	0,086	-0,004	0,6		
13	**KOH 10%**	PVC	0,081	0,094	0,013	0,61	2,131	
14	**KOH 10%**	PS	0,089	0,088	-0,001	0,6		
15	**KOH 10%**	PET	0,085	0,096	0,011	0,602	1,827	**2,182**

Table 4. Average of digestion efficiency for each method.

Ultrasonic extraction of microplastics

Sonification of the mussels results in a non completely separation of the plastic items from the tissue. Stereoscopic observation showed microplastic retained by the filters but also a few microplastics in the tissue. The remained tissue has been degraded with $H_2 0_2$ and microplastics appeared also in the sieved tissue after digestion as shown in Table 5.

Samples	M1	M2
Mussel exposure time	1 day	1 day
Volume of seawater in exposure tank (L)	1	1
Plastic type	PE	PE
Plastic Size (μm)	300-1000	300-1000
Plastic Weight (g)	0,0061	0,0062
Tissue Weight Gills & Digestive gland (g)	0,85	1,72
Ultrasonic separation Time (min)	5	5
Filters used	4	2
Plastics on filters (items)	2	1
Plastics in tissue (before digestion)	4	1

Plastics in tissue (after digestion) (items)	1	1
Total plastics in the Mussels (items)	7	3

Table 5. Ultrasonic separation of plastic ingested by mussels.

Discussion

Four different digestion or separation methods were tested and compared (Table 6).

Among the three digestion methods, H_2O_2 15% seems to be the most efficient in terms of both digestion efficiency and time consumed. More precisely, according to results on microplastic recovery, H_2O_2 15% indicated the highest percentage of recovery (94%), followed by that of KOH (92%) and that of H_2O_2 30% (86%). In terms of time needed for each digestion method, both H_20_2 15% and 30% were the fastest with no significant difference between them, although KOH 10% needed more than 7 days to sufficiently digest organic matter.

Ultrasonic separation method is a new method for microplastic separation from tissue. Its efficiency cannot be estimated as we are not able to determine the amount of microplastic ingested, so a comparison to the other 3 methods is not possible. In addition, the characteristics of the ultrasonic machine used, vary in comparison to those described in the literature, most likely impacting the efficiency of the demonstrated method.

Digestion Method	**Digestion Time needed**	**Digestion efficiency %**	**Microplastic recovery %**						**Microplastic appearance alteration**
			PE	**PP**	**PVC**	**PS**	**PET**	**Average**	
H2O2 15%	8-12 hours	**3,365**	90	100	100	100	80	**94**	colour alteration (PE)
H2O2 30%	8-12 hours	**4,176**	80	100	90	60	100	**86**	colour alteration (PE) , fragmentation (PP)
KOH 10%	> 7 days	**2,182**	100	100	90	90	80	**92**	colour alteration (PE) , fragmentation (PP)

Table 6. Comparison of digestion and separation method to quantify plastic ingestion.

Conclusions and recommendations for the WP4 testing phase

Results obtained in this workshop lead to the proposal of using digestion with 15% H_2O_2 as recommended extraction method for the detection of microplastics in fish.

From this workshop until nowadays, analyses performed as pre-testing have showed some problems of clogging membrane.

When fish gastro intestinal tract weights more than 2 g, or there is sand or shell fragment or other undigested residual, the material cannot be filtered with only one membrane.

In this case, different solutions could be applied:

1 Use more than one membrane for a single fish
2 Digest only the GI content without fish tissue and inspect tissue separately
3 Pre-filter the solution on a sieve around 50/100 µm mesh and then filter
4 Use membrane with mesh larger than 0.2µm
5 other proposals can be added by partners along the application of this protocol

Proposed solutions should be tested by the partners in their own laboratory during the testing phase, and will be shared among partners.

Contamination should be prevent or strongly reduced, for this reason all the steps should be performed under laminar flow cabinet or captair pyramid, covering the membrane with glass dish. Blank samples should be processed at the same time to detect airborne contamination.

Fish target species

Regarding the fish species to use as target in MEDSEALITTER, experimental results do not show any particular abundance in one species respect to the others.

During the meeting in Athens, partners discussed about the criteria to be used to select the best species to evaluate microplastic contamination..

This discussion lead to the selection of *Boops boops* as target species because :

- This species is one of the fish with the main reported FO% (Deudero and Alomar 2015).
- Nadal et al., 2016 results affirm that *B. boops* gastro-intestinal contents show spatial variability.

In the UNEP/Map report WG.439/Inf.12 *Boops boops* has been suggested as target species, together with Myctophidans, the *Schedophilus ovalis* and the *Naucrates ductor*, because among the most affected species in terms of plastic ingestion.

B. boops is an omnivorous species and in the case of micro-plastics, it seems that omnivorous species present higher rates of ingestion than herbivores or carnivores (Mizraji et al., 2017).

During the testing phase,100 individuals of *Boops boops* will be collected and analyzed by each partner, 50 caught inside MPA and 50 caught outside, if possible near a massively polluted area (e.g. river mouth).Others species could also be analyzed by each partner, in order to better understand the process of microplastic ingestion in fish.

III. Polychaeta and microplastics

Microplastic ingestion by marine invertebrates was studied mainly under experimental conditions by using mesocosms and microplastic supply for ingestion. 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, 2015) and De Witte et al. (2014). Applying 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. Microplastic ingestion has been reported primarily in the vertebrates. Investigations examining field impacts of plastics on benthic invertebrates are virtually nonexistent. To study the interaction of Polychaeta with marine microplastics, some ecological and pragmatical aspects have to be considered, such as feeding guild, habitats and sampling availability. This process led to the selection of some Polychaeta families and some species that, better than others, are supposed to have some adaptation for microplastic ingestion. Due to the varying size, buoyancy and composition of marine litter, ingestion of different litter types can vary between feeding guilds and ways of life (sessile, vagile fauna). Planktivores and filter feeders will encounter low-density litter fragments suspended in the upper water column whereas high density fragments are more likely available to deposit feeders and detrivores (Thompson et al., 2004; Setala et al., 2016). Some Polychaeta can preferentially select plastic particles as filter feeders whereas deposit and suspension feeders can ingest plastic together with other material that they ingest without selection. Polychaeta scavengers feeding on litter in the benthic zone can ingest large volumes of sediment and the associated litter items and microorganisms could be retained (Thompson et al., 2004; Setala et al., 2016). The marine polychaete *Arenicola marina* also demonstrates size-based selectivity, whereby smaller particles stick to the mucus-lined proboscis papillae and are retained, whilst larger particles are rejected (Van Cauwenberghe et al., 2015).

Polychaeta target species

Based on the above considerations, some guidelines referring to the selection process of best Polychaeta family/species to be used as target can be outlined. Under an ecological point of view preferentially families/species with feeding guild and ways of life that maximize interactions with marine microplastics should be selected and studied. In any case, in the framework of MEDSEALITTER project, this consideration should refer to pragmatic issues such as availability of the family/species at the right scale, sampling feasibility, size of organisms. Besides, also the availability of previous studies on certain species, reported in scientific articles or papers, could define the selection process and optimize results interpretation. In the corresponding task of the MEDSEALITTER project on the ingestion of microplastics by the Polychaeta, the above studies led to the selection of some families that will help to reach the aims of the project.

The selected families are: Arenicolidae, Maldanidae, Orbinidae, Flabelligeridae, Sternaspidae, Ampharetidae, Pectinariidae, Terebellidae, Oweniidae, Sabellariidae, Chaetopteridae, Amphinomidae, Euphrosinidae, Eunicidae, Onuphidae, Aphroditidae, Chrysopethidae, Glyceridae, Nephtydae, Polynoidae, Polynoidae, Sigalionidae, Sphinteridae. The selected species are: *Arenicola marina, Dasybranchus caducus, Aphrodita aculeate, Laetmonice hystrix, Harmothoe* ss.pp., *Sternaspis scutata, Sabella pavonina, Sabella spallanzanii, Sabellaria alveolata*. In the framework of Medsealitter project a second level of selection was applied to start the testing activities for the evaluation of microplastic ingestion in marine organisms. A species was selected based on its feeding guild, its wide distribution, both geographically and at habitat level, its availability to be sampled during all the seasons, and its presence in different habitats along the marine coastal areas. The species is *Sabella spallanzanii* belonging to Sabellidae family. The most studied Polychaeta species, the lugworm *Arenicola marina,* was not selected due to its scarceness in the natural habitats along marine coastal areas. The abundance of this species decreased in the last decades and it is currently not easy to find it and sample it. Within the planned Medsealitter activities about Polychaeta microplastic ingestion some organisms of *S. spallanzanii* were sampled in different sites along the Italian coasts: Porto Torres (Sardinia), Piombino (Tuscany), considering different habitats, including harbour and marine fish farm. During next months, other sampling locations will be selected and samples will be collected for further analysis. The collected *S. spallanzazii* specimen were kept frozen till laboratory analysis. Then, samples underwent to three different digestion processes: tissue digestion using H_2O_2 15%; tissue digestion using H_2O_2 30%; tissue digestion using KOH 10%. Results have still to be processed and conclusions will be reported in the next reports.

References micro and macro litter ingested

Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. Mar. Environ. Res. 111, 18–26. doi:10.1016/j.marenvres.2015.06.014

De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., Robbens, J., 2014. Quality assessment of the blue mussel (Mytilus edulis): comparison between commercial and wild types. Mar. Pollut. Bull. 85 (1): 146 – 155 .

Deudero S, and Alomar C. 2015. Mediterranean marine biodiversity under threat: Reviewing influence of marine litter on species. Mar. Pollut. Bull. 98:58–68.

Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R.C., Van Franeker, J., Vlachogianni, T., Scoullos, M., Mira Veiga, J., Palatinus, A., Matiddi, M., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J., Liebezeit, G., 2013a. Guidance on Monitoring of Marine Litter in European Seas. Scientific and Technical Research series, Report EUR 26113 EN.

Graham, E.R., Thompson, J.T., 2009. Deposit and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. J. Exp. Mar. Biol. Ecol. 36: 822-8 29 .

Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B., Salamatinia, B., 2017. A high-performance protocol for extraction of microplastics in fish. Sci. Total Environ. 578, 485–494. doi:10.1016/j.scitotenv.2016.10.213

Mathalon, A., Hill, P., 2014. Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. Mar. Pollut. Bull. 81, 69–79. doi:10.1016/j.marpolbul.2014.02.018

Matiddi M., Hochscheid S., Camedda A., Baini M., Cocumelli C., Serena F., Tomassetti P., Travaglini A., Marra S., Campani T., Scholl F., Mancusi C., Amato E., Briguglio P., Maffucci F., Fossi MC., Flegra Bentivegna F, de Lucia GA. 2017. Loggerhead sea turtles (Caretta caretta): A target species for monitoring litter ingested by marine organisms in the Mediterranean Sea. Environmental Pollution. 230,199-2019 http://dx.doi.org/10.1016/j.envpol.2017.06.054

Matiddi, M., van Franeker, J.A., Sammarini, V., Travaglini, A., Alcaro, L., 2011. Monitoring litter by sea turtles: an experimental protocol in the Mediterranean. In: Proceedings of the 4th Mediterranean Conference on Sea Turtles. 7e10 November, Naples, p. 129.

Mizraji, R., Ahrendt, C., Perez-Venegas, D., Vargas, J., Pulgar, J., Aldana, M., Patricio Ojeda, F., Duarte, C., Galbán-Malagón, C., 2017. Is the feeding type related with the content of microplastics in intertidal fish gut? Mar. Pollut. Bull. 116, 498–500. doi:10.1016/j.marpolbul.2017.01.008

Nadal M., C.Alomar, S.Deudero .2016. High levels of microplastic ingestion by the semipelagic fish bogue Boops boops (L.) around the Balearic Islands. Env. Poll., 214, 517–523.

Pegard, A., Miquel, C., Valentini, A., Coissac, E., Bouvier, F., François, D., Taberlet, P., Engel, E., Pompanon, F., 2009. Universal DNA-Based Methods for Assessing the Diet of Grazing Livestock and Wildlife from Feces. J. Agric. Food Chem. 57 (13): 5700–5706.

Setala O., Norkko J., Lehtiniemi M. 2016. Feeding type affects microplastic ingestion in a coastal invertebrate community. Marine Pollution Bulletin, 102: 95-101.

Shehzad, W., Riaz, T., Nawaz, M.A., Miquel, C., Poillot, C., Shah S.A., Pompanon, F., Coissac, E., Taberlet, P., 2012. Carnivore diet analysis based on next-generation sequencing: application to the leopard cat (Prionailurus bengalensis) in Pakistan. Mol. Ecol 21 (8): 1951–1965.

Soininen, E.M., Valentini, A., Coissac, A., Miquel, C., Gielly, L., Brochmann, C., Brysting, A.K., Sønstebø, J.H., Ims, R.A., Yoccoz, N. G., Taberlet, P., 2009. Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures. Frontiers in Zoology 6: 16.

Thompson, R.C., Olsen, Y., MitchellI, R.P., Davis, A ., Row land, S.J., John, A .W.G., Mcgonigle, D., Russell, A .E., 2004. Lost at sea: where is all the plastic? Science 30 4: 838 838 .
UNEP(DEPI)/MED WG.439/Inf.12.2017. Defining the most representative species for IMAP Candidate Indicator 24. Meeting of the MED POL Focal Points Rome, Italy, 29-31 May.
Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M. B. and Janssen, C. R. (2015). Microplastics are taken up by mussels (Mytilus edulis) and lugworms (Arenicola marina) living in natural habitats. Environmental Pollution, 199: 10 – 17.
Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environmental Pollution 193: 65-70.
Wagner, J., Wang, Z.-M., Ghosal, S., Rochman, C., Gassel, M., Wall, S., 2017. Novel method for the extraction and identification of microplastics in ocean trawl and fish gut matrices. Anal. Methods 9, 1479–1490. doi:10.1039/C6AY02396G.