BIOINDICATOR SELECTION IN THE STRATEGIES FOR MONITORING MARINE LITTER IN THE MEDITERRANEAN SEA

UNIVERSITÀ DI SIENA 1240 | SDSN Mediterranean | PLASTIC BUSTERS | Union pour la Méditerranée / Union for the Mediterranean / الإتحاد من أجل المتوسط | The UfM Secretariat is co-funded by the EUROPEAN UNION | giz Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH

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FOREWORD

The Mediterranean Sea has long been a major tourist destination, captured in millions of photos that show pristine shorelines and sparkling waters. Today, however, this beautiful environment is becoming permanently covered in waste, making it look in many areas like an ever-growing garbage dump. Marine litter is a serious concern for the health of our oceans and worldwide, with an estimated 80% of marine litter consists of plastic materials.

This plastic problem has grown in tandem with global increases in plastic production, from less than 2 million metric tons in 1950 to 322 metric tons in 2015. The intense pollution of our oceans and seas by plastic waste is degrading marine biodiversity, damaging important ecosystems, and potentially contaminating food sources for billions of people, especially in densely populated areas such as the Mediterranean Sea.

Around 9% of the world's biodiversity is concentrated in the Mediterranean Sea; it is home to around 12,000 plant and animal species, including 650 species of fish that live within a depth of 50m, where a significant amount of plastic debris currently resides. Efforts to reduce this waste have become a defining development trend, which we hope will continue over the next several decades.

Despite the fact that plastic pollution represents most of the waste in the Mediterranean Sea, the effects of both macro- and microplastics on marine life remain poorly understood. It is within this context that we are supporting the Plastic Busters consortium of organizations that was formed and is being coordinated by the University of Siena. The University of Siena, which is also host of the Sustainable Development Solutions Network (SDSN) Mediterranean, has wide experience in the collection of data on microplastics in the Mediterranean Sea and in the ecotoxicological effects of plastic derivatives on bioindicator organisms. The dynamism of this initiative represents a major sustainable development opportunity. The initiative works through a partnership of 17 members that cooperate closely, pursue enhanced data sharing and regularly publish their achievements. It has been supported by several institutions, especially by the Federal Ministry for Economic Cooperation and Development of Germany through the German International Cooperation (GIZ).

The overarching aim of the Plastic Busters project is to tackle marine litter. The role of microplastics as potential vehicles for toxic substances and pathogens within the marine food chain is of particular interest for the investigation. It is important to understand exactly the negative impact of plastics, including microplastics, on the health of marine habitats as well as on biology and ecology of fish resources (reproductive failure, mortality increase, reduced reproductive capacity).

The Plastic Busters initiative offers evidence to all within the Mediterranean region on the hotspots of plastic waste in the sea, and opens our eyes to see where and how action is needed. The SDSN is excited to support this flagship initiative of our Mediterranean members.

María Cortés Puch

Head of the National and Regional Networks' Program for the Sustainable Development Solutions Network (SDSN)

Holger Kuhle
GIZ-advisor at the UN-SDSN secretariat

AUTHORS AND CONTRIBUTORS

AUTHORS

Matteo Baini
SDSN Mediterranean
Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente
Università di Siena, Siena, Italy

Cristina Pedà
Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA)
Laboratorio di Milazzo, Milazzo, Italy

Cristina Panti
SDSN Mediterranean
Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente
Università di Siena, Siena, Italy

Ilaria Caliani
SDSN Mediterranean
Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente
Università di Siena, Siena, Italy

Silvia Casini
SDSN Mediterranean
Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente
Università di Siena, Siena, Italy

Claudio Leonzio
Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente
Università di Siena, Siena, Italy

and

Maria Cristina Fossi
Plastic Busters Project leader and coordinator
SDSN Mediterranean
Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente
Università di Siena, Siena, Italy

CONTRIBUTORS

Alphabetic order:

Carme Alomar Mascaro
Instituto Español de Oceanografía, Centro Oceanográfico de Baleares, Palma de Mallorca, Spain

Franco Andaloro
Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA)
Laboratorio di Milazzo, Milazzo, Italy

Pietro Battaglia
Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA)
Laboratorio di Milazzo, Milazzo, Italy

Françoise Claro
Museum National D'Histoire Naturelle, Paris, France

Monserrat Compa Ferrer
Instituto Español de Oceanografía, Centro Oceanográfico de Baleares, Palma de Mallorca, Spain

Salud Deudero
Instituto Español de Oceanografía, Centro Oceanográfico de Baleares, Palma de Mallorca, Spain

François Galgani
Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), Bastia, France

Christos Ioakeimidis
UN Environment/Mediterranean Action Plan, Athens, Greece

Teresa Romeo
Istituto Superiore per la Protezione e la Ricerca Ambientale
Laboratorio di Milazzo, Milazzo, Italy

Catherine Tsangaris
Hellenic Centre for Marine Research (HCMR), Anavyssos, Greece

EXECUTIVE SUMMARY

The Mediterranean Sea has been described as one of the areas most affected by marine litter in the world. Although the effects of plastic litter on the marine environment and marine organisms have been investigated recently in several oceanic areas, the information available on the Mediterranean Sea is poor. Despite the uncertainties and gaps in knowledge concerning the exact quantities, fate and biotic impacts of marine litter, current evidence is more than sufficient to highlight the urgent need to implement mitigation measures across the entire Mediterranean basin (UNEP/MAP, 2015). Such a need has been widely recognized, for example in the UNEP/MAP Regional Plan on Marine Litter Management of the Barcelona Convention (2013), covering the whole region, and in the European Union's Marine Strategy Framework Directive (MSFD; 2008/56/EC), for European marine waters.

The main objective of this document is to review the current knowledge on the impact of marine litter (mainly ingestion) on Mediterranean biodiversity and to propose a methodological approach to assessing marine debris and its impacts in the Sea, using marine organisms as bioindicator species and applying a new integrated monitoring tool to help develop and implement future governance and mitigation actions at basin scale. Bioindicator species play an essential role in monitoring marine litter at basin scale, and a harmonized approach to the choice of selection criteria, developed by the Plastic Busters partners, is presented.

This document aims to contribute to understanding the fate and impacts of marine litter, and (i) reviews the information currently available on the impact of marine litter on Mediterranean marine organisms, (ii) defines selection criteria for the choice of bioindicator species, (iii) proposes a multiphase monitoring approach to detecting the presence and impact of marine litter in bioindicator species, and (iv) identifies gaps and further development needed.

The threefold approach to monitoring combines assessment of the amount and type of litter ingested by a range of marine organisms with analysis of tissue for contaminants - 'plastic tracers', and with the analysis of biological responses (biomarkers) present in the organism. The document also includes three technical annexes: Annex 1, which sets out methodological details for assessing the presence and effects of marine litter on bioindicator organisms; Annex 2, which looks in more detail at the IMAP and the MSFD, including the descriptors and objectives for Good Environmental Status and targets for Marine Litter; and Annex 3, a table of Mediterranean species with documented marine litter ingestion.

1 THE ISSUE OF MARINE LITTER

Concern about the occurrence, quantity and effects of marine litter in the oceans and seas of the world has grown rapidly in recent years, with increasing interest from a wide range of bodies: governments, international and national organizations, the private sector, environmental NGOs, the scientific community, the media and the general public. It is now universally recognized that marine litter, in particular plastic litter, poses a global challenge, directly affecting marine and coastal life, ecosystems and potentially also human health, as recently underlined by the G7 Summits in Germany (2015) and in Italy (2017) and from the UN Ocean Conference (New York, June 2017).
More than 10 million tonnes of debris are dumped into oceans across the world from land-based sources, maritime activities and sea-based infrastructures (Eriksen et al., 2014). Litter can be transported by ocean currents over long distances from its origin and is found in all marine environments, even in remote areas such as uninhabited islands in the open oceans or deep seas (Werner et al 2016).

Globally, plastics make up around 80% of marine litter (Thompson et al., 2009). As larger pieces of plastic debris degrade and fragment, the amount of microplastics (plastic fragments smaller than 5 mm; Thompson, 2004) in marine habitats increases, outweighing larger debris. Debris that enters the marine environment spreads and accumulates in habitats, interacting with marine organisms (Kühn et al., 2015). Information on amounts, trends, sources and impacts (including human health and socio-economic) of marine litter worldwide is incomplete; however, it is widely accepted that both the levels of marine litter and the rate of input into the oceans are rising.

One of the major concern on marine litter is the ingestion by organisms.
Ingestion of marine litter has been reported in various organisms ranging from invertebrates to vertebrates, including endangered species (Kühn et al., 2015; Werner et al., 2016; Wright et al., 2013). This phenomenon can be explained in different ways: marine organisms may ingest litter items deliberately because of their resemblance to prey (Campani et al., 2013; Cole et al., 2011; Romeo et al., 2016; Wright et al., 2013) or accidentally while feeding on their prey, e.g. by filter feeding (Fossi et al., 2014) or hunting on shoals (Battaglia et al., 2016; Romeo et al., 2015), or as a result of secondary ingestion (debris already ingested by prey). Depending on litter size and on species, marine litter (ML) particles may be excreted or accumulated in the gastrointestinal tract, but may also cause physical and mechanical damage, such as abrasion, inflammation, blockage of feeding appendages or filters, obstruction of gastrointestinal tract (Cole et al., 2011; Li et al., 2016; Pedà et al., 2016; Wright et al., 2013) or cause pseudo-satiation resulting in reduced food intake (Kühn et al., 2015). In cases where the gastrointestinal tract becomes completely blocked or severely damaged, ML ingestion may lead directly to the death of the organism (Werner et al., 2016).
ML, in particular plastic litter, may also represent a direct and indirect vehicle for the introduction of chemical substances into marine biota and into the food-web, although this issue is still debated (Koelmans et al., 2016). The sub-lethal and chronic effects of these chemicals could compromise ecosystems and have long term consequences.
Recognition of the scale of the problem has resulted in a number of initiatives at global (G7), regional (OSPAR, UNEP Regional Seas Programme, UNEP/MAP), European (the EU Marine Strategy

Framework Directive), national and local levels. More information is needed on the marine organisms that ingest litter, if and how contaminants from the litter enter the tissue and organs of the organism, the effects of those contaminants on the organism itself, but also on the overall health of marine populations and of the marine trophic web, and the potential impact on human health. Therefore, this document will give particular attention to the selection of bioindicator organisms for ML ingestion.

2

THE MEDITERRANEAN SEA: ONE OF THE AREAS MOST IMPACTED BY MARINE LITTER WORLD-WIDE

The Mediterranean Sea, or "Mare nostrum", as it was known to the Romans, was, for centuries, "the cradle of civilization", connecting and binding many different cultures. The Sea is a crucial biodiversity hotspot and has been described as one of the areas most affected by marine litter (ML) in the world (UNEP/MAP, 2015, Cózar et al., 2015). It is almost totally land-locked, with very limited exchanges with the Atlantic, and plastic debris accumulates in the Sea to a greater degree than in the open oceans (Cozar et al., 2015; UNEP/MAP, 2015).

The problem of litter is exacerbated by densely populated coastlines, highly developed coastal tourism, busy offshore waters (with 30% of the world's maritime traffic), and concentrated inputs from urban areas and large rivers. Today the Sea has become a dumping ground for the waste generated by the 22 countries (and 450 million people) bordering its shores.

As a result of the very high levels of solid waste generated annually (208-760 kg/per capita/year) by the countries of the region, the Mediterranean Sea has become highly polluted (Fossi et al. 2012, Eriksen et al., 2014; Cozar et al., 2015, Fossi et al. 2016; Suaria et al., 2016) and holds the record for the highest density of marine litter on the sea floor, with up to 100,000 items/km² (off the French coast) and the highest density of floating microplastics, up to 4,680,000 items/km² (Southern Adriatic) (UNEP/MAP, 2015).

According to Suaria and Aliani (2014), 62 million macro-litter items were estimated to be floating on the surface of the entire Mediterranean basin. ML has been detected at regional and local scales in the Mediterranean Sea, on the beaches, as floating debris on the sea surface, in the water column at different depths and on the sea floor (Aliani et al., 2003; Angiolillo et al., 2015; Bo et al., 2014; Cózar et al., 2015; Fabri et al., 2014; Fossi et al., 2016, 2017; Galgani et al., 2000; Ioakeimidis et al., 2014; Pham et al., 2014; Suaria and Aliani, 2014; UNEP, 2011; Vlachogianni and Kalampokis, 2014). Considering the high biodiversity in the Mediterranean Sea (Coll et al., 2010) and the widespread distribution of ML in its waters and on the sea floor, many species are potentially at risk of ML ingestion and its impacts.
In addition to national actions plans at country level, management of marine litter in the Mediterranean Sea is organized within the framework of two main drivers:

- the Regional Plan on Marine Litter Management in the Mediterranean in the framework of the Barcelona Convention (UN Environment/Mediterranean Action Plan), which covers the whole region. In 2016, the Contracting Parties to the Convention adopted an Integrated Monitoring and Assessment Programme (IMAP) and Related Assessment Criteria that include a number of common Ecological Objectives, and related indicators;
- the EU's Marine Strategy Framework Directive (MSFD; 2008/56/EC) and its related Decision (European Commission Decision 2017/848), covering European marine waters.

More information on IMAP and the MSFD is given in Annex 2.
Actions are also supported by the Union for the Mediterranean (UfM), through the Plastic Busters project (http://plasticbusters.unisi.it/), led by the Sustainable Development Solutions Network (SDSN) Mediterranean Regional Centre and the University of Siena (Italy).

3 CURRENT STATE OF KNOWLEDGE ON MARINE LITTER IN THE MEDITERRANEAN

Although the effects of plastic litter on the marine environment and its organisms have been investigated recently in several oceanic areas, the information available on the Mediterranean Sea remains poor. In particular, aspects such as plastic and microplastic inputs, their spatial and temporal distribution, potential accumulation areas, transport dynamics, interactions with biota and the trophic web all need deeper investigation. Recent studies in the different regions of the Mediterranean basin suggest that some areas are affected by high concentrations of ML, including microplastics and plastic additives (phthalates), representing a potential risk to endangered marine species (baleen whales, sea turtles, filter feeder sharks) and to biodiversity (Darmon et al., 2017; Fossi et al., 2016). The impact of ML and its interactions with Mediterranean marine organisms were reviewed by Deudero and Alomar (2015), who identified almost 134 species affected by ML. The data show that the presence and effects of ML represent a threat to Mediterranean marine organisms, and that a series of mitigation actions and measures are required.

There is another critical aspect to be considered in the monitoring of ML: the quantification of marine litter in the environment (particularly macro- and microplastic floating litter) will depend on several environmental factors and can change according to multiple oceanographic features (e.g. wind, currents, etc.); therefore, quantities alone cannot reflect the potential impact of litter on organisms and ecosystems.

The IMAP defines the indicator for Ecological Objective 10 on marine litter (EO10) (known as Candidate Indicator 24) as "*Trends in the amount of litter ingested by or entangling marine organisms, focusing on selected mammals, marine birds, and marine turtles*". Work is in progress to define the most representative species to be used for this indicator.

Similarly, the MSFD defines Criteria 3 of Descriptor 10 (D10.C3) as "*The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned*", and D10.C4 as "*the number of individuals of each species which are adversely affected due to litter, such as by entanglement, other types of injury or mortality, or health effects.*" The Decision also states that Member States shall establish the list of species to be assessed through regional or sub-regional cooperation. It is therefore essential to monitor ML ingestion by marine organisms and its impacts on the health of the organisms.

Some species lend themselves more easily to such monitoring than others, and can be used as (bio)indicators for ML in the marine environment. Information obtained from bioindicator species would better reflect the spatial and temporal presence of litter in the marine environment. In addition, the use of bioindicators would allow not only the occurrence of marine litter in the species and its environment to be measured, but would also identify the risk posed to the organisms by the accumulation of contaminants associated/adsorbed to plastic litter and any related biological effect. The choice and identification of the most representative bioindicator species for marine litter in the Mediterranean is one of the main goals of the multidisciplinary strategy developed by the UfM's Plastic Busters project.

The main objective of this document is to review the current knowledge on this topic and to propose a methodological approach for the assessment of marine debris in the Mediterranean Sea, using marine organisms as bioindicator species and applying a new integrated monitoring tool to support future mitigation actions for the Mediterranean basin. Selection criteria for the choice of bioindicators are suggested and harmonization of the approach to be used in the study of Mediterranean marine litter is discussed.
In particular, this document: (i) reviews existing information on the impact of marine litter on Mediterranean marine organisms, (ii) defines selection criteria for the choice of bioindicator species, (iii) proposes a multiphase monitoring approach to detect the presence and impact of marine litter in bioindicator species, and (iv) identifies gaps and suggests possible directions for further research.

3.1. Bibliographic research

In order to bring together the most recent and reliable information on the state of marine litter ingestion by Mediterranean marine organisms, a complete search of current peer-reviewed scientific literature, grey literature and reports was carried out. The search results were used to build a database of studies on litter ingestion by Mediterranean marine organisms (vertebrates and invertebrates).
The bibliographic research was performed using general search engines such as Google and Google scholar, and specialised databases such as ISI Web of Knowledge and Scopus, using the following keywords: marine plastic ingestion, marine litter ingestion, marine debris Mediterranean, marine litter Mediterranean, marine plastic Mediterranean, marine litter and vertebrates, marine debris and vertebrates, marine plastics and

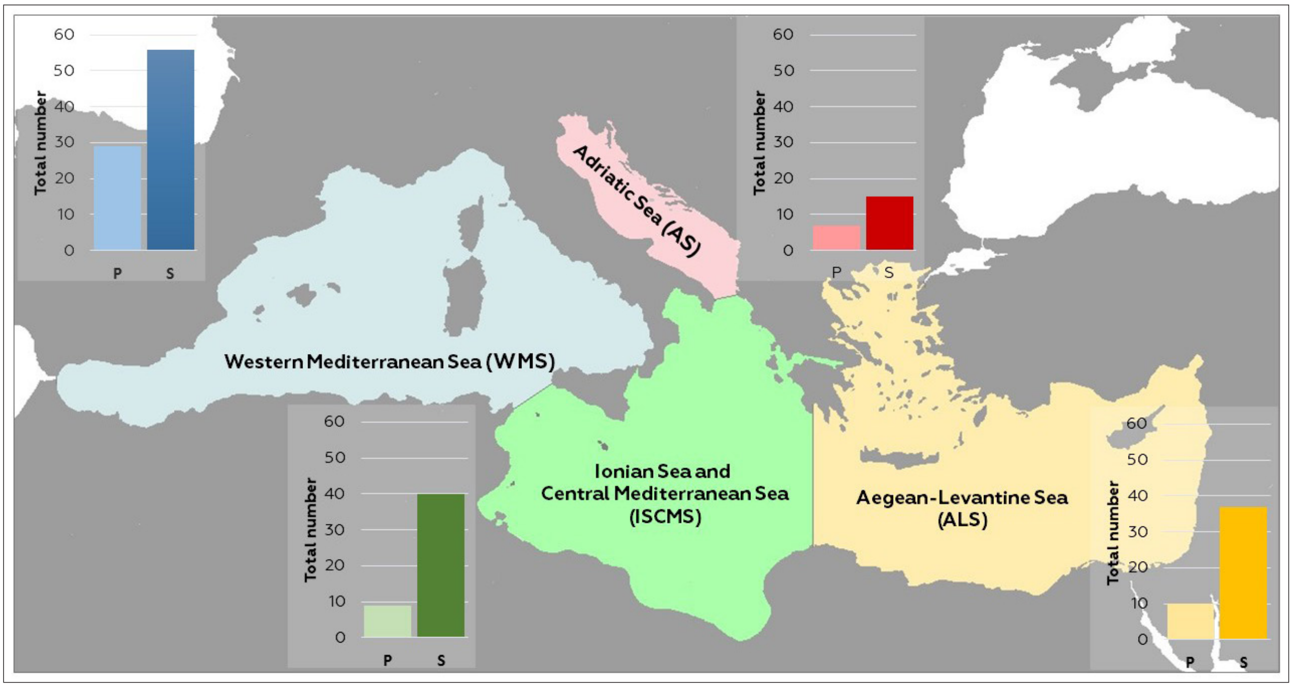


Figure 1. Number of papers (P) reporting marine litter ingestion and number of species (S) affected by marine litter in Mediterranean UN Environment/MAP sub-regions (WMS= wWestern Mediterranean Sea; ISCMS= Ionian Sea and the Central Mediterranean Sea; AS= Adriatic Sea; ALS= Aegean-Levantine Sea). (Fossi et al., in press).

vertebrates, marine litter and fish, marine debris and fish, marine plastic and fish, marine litter and invertebrates, marine debris and invertebrates and marine plastic and invertebrates.
To evaluate the number of Mediterranean species affected by marine litter ingestion and given the number of papers on litter ingestion in the Mediterranean sub-regions, the results of each search were evaluated and for the selected documents the following information was extracted and reported in the database (see Annex 3): scientific species name and taxonomic group (Annelids, Echinoderms, Crustaceans, Molluscs, Fish, Turtles, Seabirds and Marine Mammals); Mediterranean sub-region (WMS= Western Mediterranean Sea; ISCMS= Ionian Sea and the Central Mediterranean Sea; AS= Adriatic Sea; ALS= Aegean-Levantine Sea); habitat; number of specimens investigated; number of stomachs with marine litter; frequency of occurrence (%); number and weight of litter identified; reference to the source of literature.
To standardize data, where possible, missing data on the frequency of occurrence and the number of specimens with ingested marine litter were obtained from the authors of the studies. However, when these data were not available, the papers were excluded.
To date, 47 papers on the incidence of marine litter ingestion in marine organisms in the Mediterranean basin have been published (Fig. 1). Most of the research was carried out on the Western Mediterranean Sea, whereas the Ionian Sea and the Central Mediterranean Sea, the Adriatic Sea and the Aegean Levantine Sea were less investigated.
In Fig. 1, when a paper included two or more sub-regions, it was counted

Taxonomic group		**Number of species**
Invertebrates		**14**
Anellids	*Polychaeta (1)*	**1**
Crustaceans	*Decapoda (5); Amhipoda (4); Euphausiacea (1); Leptostraca (1)*	**11**
Echinoderms	*Aspidochirotida (1)*	**1**
Molluscs	*Mytilida (1)*	**1**
Vertebrates		**77**
Fish	*Anguilliformes (1); Aulopiformes (1); Clupeiformes (2); Gadiformes (3); Myctophiformes (4); Ophidiiformes (1); Perciformes (35); Pleuronectiformes (2); Scorpaeniformes (3); Tetraodontiformes (1); Carcharhiniformes (1); Lamniformes (1); Myliobatiformes (1); Squaliformes (4)*	**60**
Turtles	*Testudines (3)*	**3**
Seabirds	*Charadriiformes (5); Procellariiformes (3); Suliformes (1);*	**9**
Marine Mammals	*Cetartiodactyla (5)*	**5**
Total species		**91**

Table 1. Number of Mediterranean species with documented records of marine litter ingestion. In brackets number of species per taxonomic group

for each area. Consequently, available information concerns a higher number of species from WMS, followed by ALS. Litter ingestion has been documented for 91 Mediterranean species, belonging to different taxonomic groups including invertebrates, fish, sea turtles, seabirds and marine mammals (Table 1 and Fig. 2; Annex 3).

While fish represent 65.9% of the affected species, belonging to 14 orders, a considerable number of the studies of ML ingestion refer to endangered species (marine mammals, turtles, seabirds, elasmobranchs). All Mediterranean turtles (*Caretta caretta*, *Chelonia mydas* and *Dermochelys coriacea*) and some marine mammals (*Physeter macrocephalus*, *Balaenoptera physalus*, *Tursiops truncatus*, *Grampus griseus* and *Stenella coeruleoalba*) were found to be affected by debris ingestion. Most studies on these endangered species dealt with stranded individuals. ML ingestion in seabirds is a well-documented phenomenon on a global scale, as reported by Laist (1997) and Kühn et al. (2015), whereas in the Mediterranean basin only one paper, by Codina-García et al. (2013), has studied the presence of marine debris in several bird species belonging to the Procellariiformes, Suliformes and Charadriiformes orders (Table 1). The few studies available on ML ingestion by marine invertebrates (Alomar et al., 2016; Cristo and Cartes, 1998; Digka et al., 2016; Fossi et al., 2014; Gusmão et al., 2016; Remy et al., 2015; Vandermeersch et al., 2015) (Fig.2) investigated several species belonging to the Annelids, Crustaceans, Echinoderms and Molluscs (Table 1).

ML ingestion in Mediterranean organisms has been reported since 1988, with a clear increase in the number of scientific papers in recent years (Fig. 2). In particular, ML ingestion in fish species has gained more interest in the last decade (from 2010 to date), which is linked to concern for their value as fishery resources. The first information came from studies on the feeding ecology of Mediterranean species (Carrasón et al., 1992; Deudero, 1998;

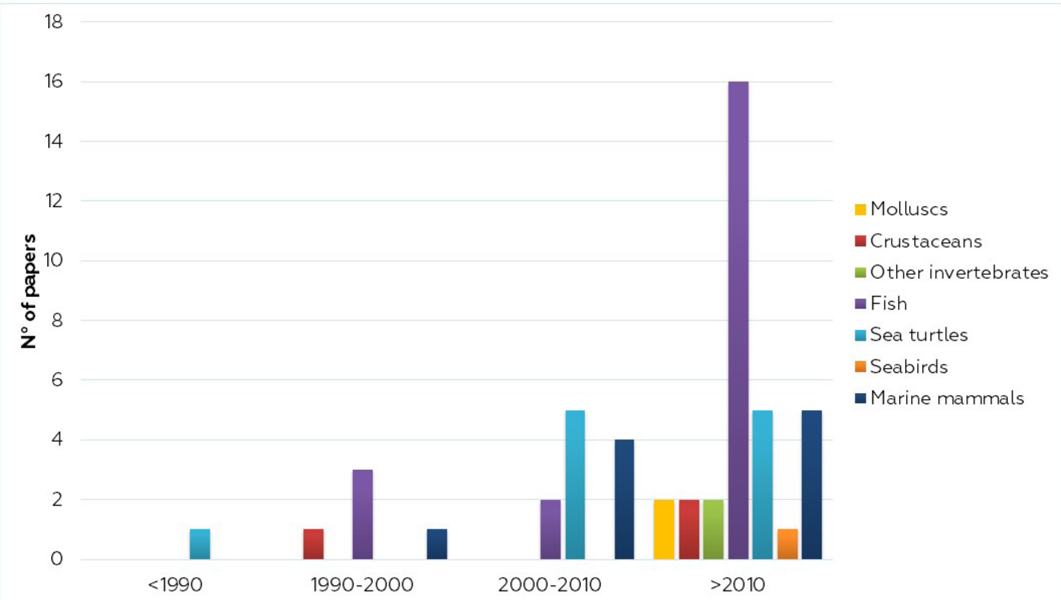


Figure 2. Number of published papers on marine litter ingestion per decade, by taxonomic group. (Fossi et al., in press).

Madurell, 2003; Massutí et al., 1998), but in recent years, the detection of debris in the stomach and intestinal tracts has been the main goal of most studies in this area.Focusing on fish, litter ingestion has been reported in species belonging to different habitats. Studies have been conducted mostly on pelagic (30%) and demersal (42%) species but also on mesopelagic (5%), benthopelagic (15%) and benthic (8%) fish (Fig. 3). In addition to the physical harm associated with marine litter, recently concern is growing regarding the chemical hazard related to the ingestion of marine litter. The concern is that plastic additives (e.g. PBDEs and phthalates) could be directly leached from plastic debris, leading to the accumulation within marine organisms of chemicals such as persistent, bioaccumulating and toxic (PBT) substances that are adsorbed and transported by marine debris.
Some studies have examined the possible link between the chemical effects of plastic ingestion and the risk of bioaccumulation along the trophic web. For instance, Fossi et al. (2014) and Baini et al. (2017) detected levels of phthalates and organochlorines in specimens of *Euphausia krohnii,* muscle samples of basking shark *Cetorhinus maximus* and in blubber samples of four cetaceans: fin whale *Balaenoptera physalus*, bottlenose dolphin *Tursiops truncatus*, Risso's dolphin *Grampus griseus* and striped dolphin *Stenella coeruleoalba*. The levels of these toxic chemicals have been used as possible tracers of exposure to plastic ingestion.
If these chemicals become bioavailable, they can penetrate cells and chemically interact with biologically important molecules. This may cause adverse effects at different levels of biological organisation, from molecular level to tissue level, including liver toxicity (Avio et al., 2015a; Rochman et al., 2013), alterations of gene expression (Karami et

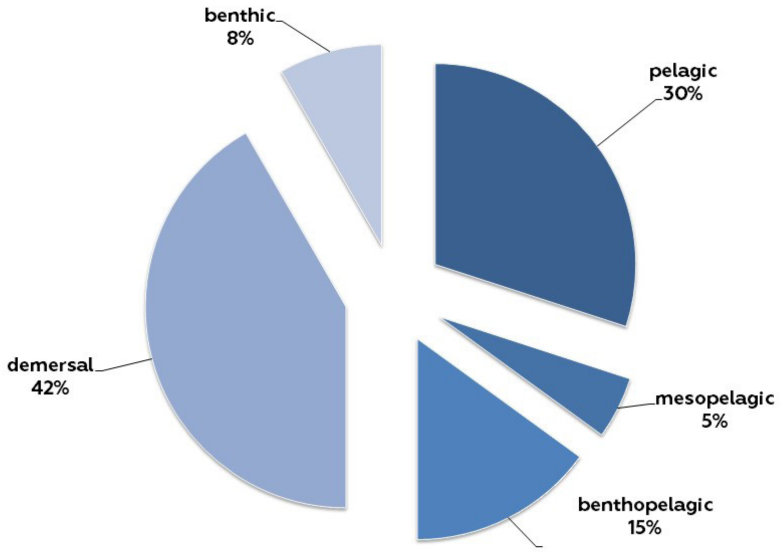


Figure 3. Fish categories with recorded debris ingestion in the Mediterranean Sea, by habitat.

al., 2017; Sleight et al., 2017), genotoxic effect (Avio et al., 2015a), endocrine disruption (Rochman et al., 2014; Teuten et al., 2009) and histological alterations (Avio et al., 2015a, 2015b; Pedà et al., 2016). However, most of these effects have been shown in laboratory studies, very few are available from field studies and particularly on Mediterranean organisms (Avio et al., 2015a).
Despite the increase in the number of studies in recent years, the information on the interaction between marine litter and Mediterranean biota remains very poor and inconsistent. As suggested by Deudero and Alomar (2015), this is in part due to the lack of standardized methods and protocols for monitoring and sampling techniques. Moreover, most studies only take into account the occurrence of macro- and meso-litter in marine organisms, which can lead to an underestimation of the impact of micro- and nano-litter.

3.2 Data analysis

A preliminary analysis was performed on the available data from literature, ranking each species with recorded marine litter ingestion. If a species was included in several papers, the available data were combined to calculate the percentage of ingestion on the total data. Only datasets with more than six specimens per species were considered, to make the analysis more reliable. The resulting data are shown below, using a colorimetric ranking, green=0% and red=100% of marine litter ingestion (Fig. 4).
Due to the wide range of species covered by the literature, an impact index was designed and assigned to each observation (study-species) for habitat type and species group. For the different Taxa groups and habitat types, a boxplot of the % occurrence of all observations was produced (Fig. 5) and an impact index dependent on the boxplot quartiles was assigned, using the following three categories: low (minimum to first quartile), medium (first quartile to

Species	% of ingestion
Balaenoptera physalus (Linnaeus, 1758)	100.00%
Caranx crysos (Mitchill, 1815)	100.00%
Engraulis encrasicolus (Linnaeus, 1758)	100.00%
Sciaena umbra Linnaeus, 1758	100.00%
Calonectris diomedea (Scopoli, 1769)	95.92%
Siganus luridus (Rüppell, 1829)	86.67%
Cetorhinus maximus (Gunnerus, 1765)	83.33%
Pagrus pagrus (Linnaeus, 1758)	77.78%
Physeter macrocephalus Linnaeus, 1758	77.78%
Argyrosomus regius (Asso, 1801)	74.51%
Puffinus yelkouan (Acerbi, 1827)	70.97%
Mullus surmuletus Linnaeus, 1758	70.59%
Puffinus mauretanicus Lowe, 1921	69.57%
Diplodus annularis (Linnaeus, 1758)	68.75%
Boops boops (Linnaeus, 1758)	67.71%
Pagellus acarne (Risso, 1827)	67.31%
Chelidonichthys lucerna (Linnaeus, 1758)	66.67%
Serranus cabrilla (Linnaeus, 1758)	66.67%
Pelates quadrilineatus (Bloch, 1790)	65.19%
Trachurus mediterraneus (Steindachner, 1868)	62.13%
Saurida undosquamis (Richardson, 1848)	55.56%
Pomadasys incisus (Bowdich, 1825)	55.17%
Nemipterus randalli Russell, 1986	54.81%
Dermochelys coriacea (Vandelli, 1761)	50.00%
Grampus griseus (G. Cuvier, 1812)	50.00%
Pteroplatytrygon violacea (Bonaparte, 1832)	50.00%
Rissa tridactyla (Linnaeus, 1758)	50.00%
Stercorarius skua (Brünnich, 1764)	50.00%

Species	% of ingestion
Scomber japonicus Houttuyn, 1782	47.73%
Upeneus moluccensis (Bleeker, 1855)	44.44%
Sparus aurata Linnaeus, 1758	43.64%
Liza aurata (Risso, 1810)	43.59%
Upeneus pori Ben-Tuvia & Golani, 1989	41.03%
Trigla lucerna Linnaeus, 1758	37.50%
Mullus barbatus Linnaeus, 1758	36.03%
Lithognathus mormyrus (Linnaeus, 1758)	34.78%
Larus michahellis J.F. Naumann, 1840	33.33%
Trachyrincus scabrus (Rafinesque, 1810)	33.33%
Polyprion americanus (Bloch & Schneider, 1801)	32.35%
Caretta caretta (Linnaeus, 1758)	31.10%
Dentex gibbosus (Rafinesque, 1810)	28.57%
Schedophilus ovalis (Cuvier, 1833)	28.57%
Larus melanocephalus Temminck, 1820	25.00%
Trachinotus ovatus (Linnaeus, 1758)	24.35%
Trachurus trachurus (Linnaeus, 1758)	24.00%
Pagellus erythrinus (Linnaeus, 1758)	22.39%
Squalus acanthias Linnaeus, 1758	21.05%
Sardina pilchardus (Walbaum, 1792)	20.41%
Naucrates ductor (Linnaeus, 1758)	18.00%
Nettastoma melanurum Rafinesque, 1810	16.67%
Coryphaena hippurus Linnaeus, 1758	14.34%
Balistes capriscus Gmelin, 1789	14.00%
Larus audouinii Payraudeau, 1826	13.33%
Thunnus alalunga (Bonnaterre, 1788)	12.90%
Thunnus thynnus (Linnaeus, 1758)	12.67%
Morus bassanus (Linnaeus, 1758)	12.50%
Xiphias gladius Linnaeus, 1758	12.28%
Stenella coerulealba (Meyen, 1833)	11.67%
Tursiops truncatus (Montagu, 1821)	11.17%

Species	% of ingestion
Cataetyx laticeps Koefoed, 1927	10.00%
Mora moro (Risso, 1810)	9.09%
Etmopterus spinax (Linnaeus, 1758)	8.82%
Merluccius merluccius (Linnaeus, 1758)	7.69%
Galeus melastomus Rafinesque, 1810	7.11%
Hygophum benoiti (Cocco, 1838)	6.85%
Electrona risso (Cocco, 1829)	6.10%
Holothuria forskali Delle Chiaje, 1823	5.71%
Myctophum punctatum Rafinesque, 1810	4.23%
Centroscymnus coelolepis Barbosa du Bocage & de Brito Capello, 1864	2.99%
Solea solea (Linnaeus, 1758)	2.27%
Seriola dumerili (Risso, 1810)	2.00%
Citharus linguatula (Linnaeus, 1758)	1.92%
Pagellus bogaraveo (Brünnich, 1768)	1.67%
Squalus blainville (Risso, 1827)	1.33%
Trachurus picturatus (Bowdich, 1825)	1.00%
Helicolenus dactylopterus (Delaroche, 1809)	0.42%
Diaphus metopoclampus (Cocco, 1829)	0.34%
Alepocephalus rostratus Risso, 1820	0.00%
Brama brama (Bonnaterre, 1788)	0.00%
Centrophorus granulosus (Bloch & Schneider, 1801)	0.00%
Chelonia mydas (Linnaeus, 1758)	0.00%
Conger conger (Linnaeus, 1758)	0.00%
Dentex dentex (Linnaeus, 1758)	0.00%
Epigonus telescopus (Risso, 1810)	0.00%
Lagocephalus spadiceus (Richardson, 1845)	0.00%
Lepidopus caudatus (Euphrasen, 1788)	0.00%
Micromesistius poutassou (Risso, 1827)	0.00%
Molva macrophthalma (Rafinesque, 1810)	0.00%
Phycis blennoides (Brünnich, 1768)	0.00%
Raja clavata Linnaeus, 1758	0.00%
Raja oxyrinchus Linnaeus, 1758	0.00%
Scorpaena elongata Cadenat, 1943	0.00%
Scyliorhinus canicula (Linnaeus, 1758)	0.00%
Sudis hyalina Rafinesque, 1810	0.00%
Umbrina cirrosa (Linnaeus, 1758)	0.00%

Figure 4. Percentage and ranking of marine litter ingestion in the species calculated on the data present in literature (February 2017). A cut-off value of a minimum of six specimens per species analysed was considered.

third quartile) and high (third quartile to maximum). Not all observations reported the percentage occurrence, reducing the number of observations from 167 to 136. To determine which species are of high concern, those whose percentages fell within the category 'third quartile to maximum' for both habitat type and species group were selected (Table 2). All of the species included in the high observation category were selected, giving a list of 30 species, spread across 5 taxa groups and 6 habitat types, as bioindicator species for monitoring marine litter ingestion by organisms in the Mediterranean Sea.

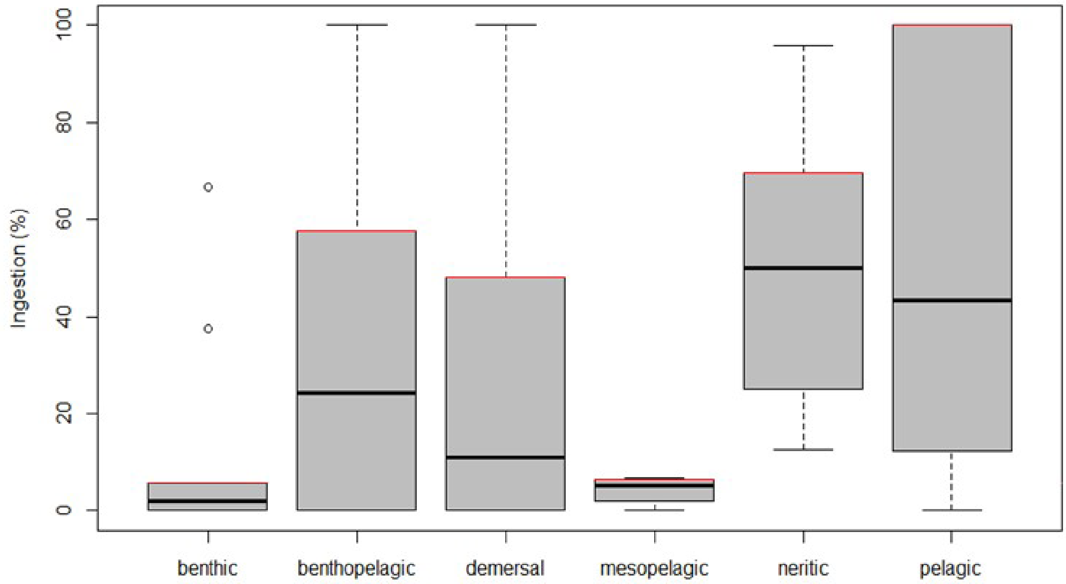


Figure 5 Boxplot for ingestion (%) for each observation by habitat (A) and by taxa groups (B).

	Benthic	Benthopelagic	Demersal	Mesopelagic	Neritic	Pelagic
Elasmobranchs			*Galeus melastomus* *Squalus acanthias*			
Teleosts	*Chelidonichthys lucerna*	*Argyrosomus regius* *Boops boops* *Pagellus acarne*	*Mullus barbatus* *Mullus surmuletus* *Saurida undosquamis* *Diplodus annularis* *Pagrus pagrus* *Pelates quadrilineatus* *Sciaena umbra* *Serranus cabrilla* *Siganus luridus*	*Hygophum benoiti*		*Caranx crysos* *Engraulis encrasicolus* *Scomber japonicus* *Trachurus mediterraneus*
Reptiles		*Caretta caretta* *Dermochelys coriacea*				
Seabirds					*Calonectris diomedea* *Puffinus yelkouan* *Puffinus mauretanicus*	
Mammals						*Physeter macrocephalus* *Balaenoptera physalus* *Tursiops truncatus* *Grampus griseus* *Stenella coeruleoalba*

Table 2. Results from the boxplots of observations with a "high" index for each boxplot.

4 BIOINDICATOR SELECTION STRATEGY

The selection of sentinel species in which to monitor the impact of marine litter on Mediterranean fauna is crucial for the assessment of this environmental threat and for the establishment of a future common/regional management policy. This aspect is essential for the development of standardized sampling methods and harmonized protocols for the establishment of a regional approach in the Mediterranean basin. The selection of sentinel (bioindicator) species has to meet specific criteria and respond to the needs of monitoring several marine habitats (from coastal areas to offshore, from benthic environments to pelagic waters) at different spatial scales (Schwacke et al., 2013).

4.1 Biological selection criteria

A range of biological selection criteria for the choice of sentinel species, falling into some general categories, are described and summarized in Table 3:
(1) *background information*: this category includes attributes related to the biological and ecological characteristics of the species. The taxonomic identification of a sentinel species should be clearly addressed. Moreover, it is important to have enough information about the ecology and biology of the selected species, in order to relate the monitored phenomenon to specific ecological issues;
(2) *habitat information*: information on habitat and home range of the species is essential in the selection of sentinel species, as it allows monitoring to be differentiated at different spatial scales. Some species are sessile (e.g. mussels) and can give accurate information on restricted areas and areas where marine litter accumulates. Others may travel over large horizontal areas (large pelagic predators) or migrate vertically (e.g. micronekton) with excursions into the depths of the water column, and can represent integrators of marine litter over a spatial scale and of the distribution of marine litter (see more detail in the next section);
(3) *trophic information and feeding behaviour*: the feeding mechanism (e.g. filter feeding) and feeding behaviour (e.g. feeding on schoals of fish, opportunism, feeding on pleuston, bentivorous feeders, etc.) strongly influences plastic ingestion by marine organisms. Filter feeders, such as baleen whales (Fossi et al., 2012, 2014; 2016), basking sharks (Fossi et al., 2014) anchovy (Collard et al., 2015) and mussels (Galimany et al., 2009; Vandermeersch et al., 2015) are potentially exposed to the ingestion of micro-litter (Fossi et al., 2012, 2014, 2016). High percentages of plastics have been also found in the stomachs of opportunistic feeders, preying on shoals (i.e. bluefin tuna, albacore) or near the surface (*Trachinotus ovatus*) (Battaglia et al., 2016; Romeo et al., 2015). Species that feed on the seafloor (e.g. red mullet, shrimps, worms) are also at risk of litter ingestion. Trophic level is an important attribute, as species at higher levels in the food chain (e.g. large pelagic predators) are subject to chemical bioaccumulation. The monitoring of marine litter should also include sentinel species chosen from among key species of the marine trophic web, which have a crucial ecological role in maintaining the structure and integrity of the marine communities;
(4) *spatial distribution*: the monitoring of the impact of litter on Mediterranean marine biota implies the selection of sentinel species that allow an appropriate spatial coverage. The extension of sampling activities at Mediterranean scale requires the selection of widely distributed species that are found in the Mediterranean basin and sub-regions.
(5) *commercial importance and conservation status*: monitoring activity should also include commercially important

species, which are easily available through fishing activities; this would also allow the potential transfer of specific pollutants (such as plastic tracers) from marine food to humans to be estimated. It is also desirable to include protected, threatened or endangered species among selected sentinel species, in order to understand to what extent marine litter can affect species conservation. An emblematic example is the case of the loggerhead sea turtle (*Caretta caretta*), whose Mediterranean population is strongly affected by the ingestion of litter that in several cases has led to the death of the specimens (Campani et al., 2013; Lazar and Gračan, 2011; Tomás et al., 2002); (6) *Documented ingestion occurrence*: statistics and data availability on marine litter ingestion. Not all sentinel species will necessarily fit all criteria, but each taxon, selected as part of a complementary set, will satisfy multiple attributes. A whole set of sentinel species (as identified in the next sections) will allow the establishment of a comprehensive monitoring strategy for the whole Mediterranean region. Finally, the selection of sentinel species and the planning of monitoring activities should take into account some difficulties linked to the behaviour of these organisms and/or the inadequacy of sampling methods. Indeed, several authors (Duhem et al., 2003, 2008; Ramos et al., 2009) observed that the opportunistic feeding behaviour of the seabird *Larus michahellis* is strongly influenced by urbanization and, as a consequence, this species has modified its feeding behaviour, moving from marine feeding grounds to the waste dumping sites on land, where it finds food without any effort. This fact could introduce an overestimation of the amount of marine litter ingested, should this species be considered as a sentinel species. In the same way, the stomachs of fish species collected by pelagic and bottom trawl nets could contain litter particles accidentally ingested during the sampling operation, a phenomenon known as "net feeding".

GENERAL CATEGORIES	ATTRIBUTES
(1) Background information	(1a) Clear taxonomic identification (1b) Scientific knowledge on ecology and biology
(2) Habitat information	(2a) Habitat (2b) Home range
(3) Trophic information	(3a) Trophic level (3b) Keystone species (3c) Feeding behaviour (3d) Feeding mechanism
(4) Spatial distribution	(4a) Referred to sampling; spatial coverage
(5) Commercial importance and conservation status	(5a) Commercial importance (5b) Protected, threatened or endangered species
(6) Documented ingestion occurrence:	(6a) % of specimens affected by ingestion

Table 3. Summary of selection biological criteria of bioindicator species

4.2 Bioindicator selection in relation to habitat and home range

According to the data available on the interaction of marine litter with Mediterranean marine organisms and the criteria for the choice of sentinel species, different sentinel species are proposed here as sensitive indicators of the presence of marine litter and its effects in different ecological compartments (sea surface, coastal waters, open waters, seafloor, coast line and beach). The organisms have also been selected on the basis of their different home range: local scale, small-scale (FAO Geographical subareas), medium-scale (Mediterranean UN Environment/MAP sub-regions) and Mediterranean Basin scale (Table 4).

4.2.1 Local and small-scale bioindicators for microplastics along the Mediterranean coast

Several invertebrates can be considered as local-scale/spot indicators of the presence and impact of micro-litter in specific spots along Mediterranean shores. The coastline plays a vital role in the fragmentation of plastics in the marine environment, facilitating the formation of microplastics and nanoplastics. Therefore, studies on the impact of the process of fragmentation of plastic litter in coastal environments and the identification and selection of suitable bioindicators play an essential role in the monitoring of this section of the marine environment. The usefulness of bivalve molluscs, and in particular mussels (*Mytilus sp.*), as sentinel organisms for monitoring pollution in coastal environments has been established in several laboratory and field studies in the last decades. These intertidal filter-feeding invertebrates are known to accumulate high levels of contaminants (heavy metals and POPs) and microplastic (Avio et al., 2015a; von Moos et al., 2012), providing a time-integrated indication of environmental contamination. By virtue of its broad geographical distribution, abundance, basal position in the food web, accessibility, the possibility to perform in-cage studies and as its well-understood biology, the mussel (*Mytilus galloprovincialis*) can be selected as a spot-scale bioindicator of microplastics in Mediterranean coastal shores. Being a sessile suspension feeder, the mussel effectively reflects the environmental contamination of the location where it is found. It is therefore an internationally accepted sentinel early warning species for monitoring marine pollution, which is used in both the U.S. Mussel Watch and for the assessment and control of pollution in the Mediterranean region (MEDPOL). The uptake of microplastic at local scale by marine (epi)benthic organisms should also be investigated, selecting *Arenicola marina* (lugworm) as a bioindicator organism, as it is a robust and quantitatively important deposit feeder at the base of the Mediterranean food web and is commonly used in marine sediment toxicity tests (Van Cauwenberghe et al., 2015). Moreover, microplastics have been detected in *A. marina* collected from the field (Farrell and Nelson, 2013). In addition, the *Carcinus sp.* is also proposed as a suitable bioindicator species of the Mediterranean coastline. *Carcinus sp.* (which includes *Carcinus maenas*, an important invasive species, and *C. aestuarii*, a species endemic to the Mediterranean Sea) is widely distributed on coastlines with feeding activities related to ingestion of organism and litter present in the costal environment.

4.2.2 Small-scale bioindicators of microplastics and macro litter on the Mediterranean seafloor

Demersal fish live on or near sediments on the sea bottom, and depend on benthic prey for feeding. They can be used as small-scale indicators of the presence and impact of microplastic in the Mediterranean benthic environment (sea bottom). Red mullet (*Mullus barbatus*) and *Solea spp.* live on muddy and sandy bottoms, feeding mainly on benthic species. Red mullet has also been extensively used in MED-POL to monitor chemical pollution (Burgeot et al., 1996). They inhabit the whole of the Mediterranean Sea and because of their close association with the sea bottom, they are strongly exposed to the ingestion of micro-debris. Microplastic ingestion has been reported in red mullet from different Mediterranean Sea areas (Avio et al., 2015b; Bellas et al., 2016). Other sentinel species for the monitoring of plastic ingestion at small-scale level are: i) the selachian *Galeus melastomus* in whose stomachs plastic litter has already been found (Alomar and Deudero, 2017; Carrasón et al., 1992; Cartes et al., 2016); and ii) the demersal fish *Merluccius merluccius*, for its commercial importance and its trophic links between pelagic and demersal habitats.

4.2.3 Small-scale bioindicators of microplastics in Mediterranean coastal waters

According to Nadal et al. (2016) and Battaglia et al. (2016), a high level of plastic has already been found in the stomach content of the fish species *Boops boops* and *Trachinotus ovatus*, (% occurrence: 67.7% and 24.3%, respectively), and these can be considered good sentinel species at small-scale level.
The two species do not travel over large distances and are representative of fishing resources usually exploited by artisanal fishery. They are opportunistic predators and occupy an intermediate position in the marine pelagic trophic web (Cardona et al., 2012). The consumption of plastic debris by these species may also be in part determined by their predation on gregarious prey.
Moreover, *T. ovatus* tends to hunt just below the surface layer (suggested by the predation on insects and some neustonic organisms such as *Porpita porpita*) which may make it more vulnerable to the ingestion of floating plastic debris (Battaglia et al., 2016).

4.2.4 Small-scale bioindicators of microplastics in the Mediterranean open waters

Due to their trophic level and habitat use, mesopelagic fish can be used as indicators of the presence and impact of microplastics in the Mediterranean pelagic (open waters) environment at small-scale level (GSAs). A high level of microplastics were found in stomachs of mesopelagic fish species in the Atlantic Ocean (Boerger et al., 2010; Davison and Asch, 2011), and more recently, plastic ingestion in some Mediterranean myctophids (*Electrona risso, Diaphus metopoclampus, Hygophum benoiti, Myctophum punctatum*) was documented by Romeo et al. (2016). *Maurolicus muelleri* (Sternoptychidae), *Hygophum benoiti* and *Electrona risso* (Myctophidae) are mid-water small predators foraging mainly on zooplankton and micronekton (e.g. Battaglia et al., 2016; Scotto di Carlo et al., 1982). As is the case for other mesopelagic fishes, their migration is usually diel vertical, and they play an important role in the energy transfer from surface to deeper waters and from low trophic levels of the food web to top predators (Battaglia et al., 2013). The trophic model ECOPATH (Fig. 6) indicates that mesopelagic fish are among the most important prey of pelagic predators, playing a key role in the pelagic trophic web of the Tyrrhenian Sea (central Mediterranean).

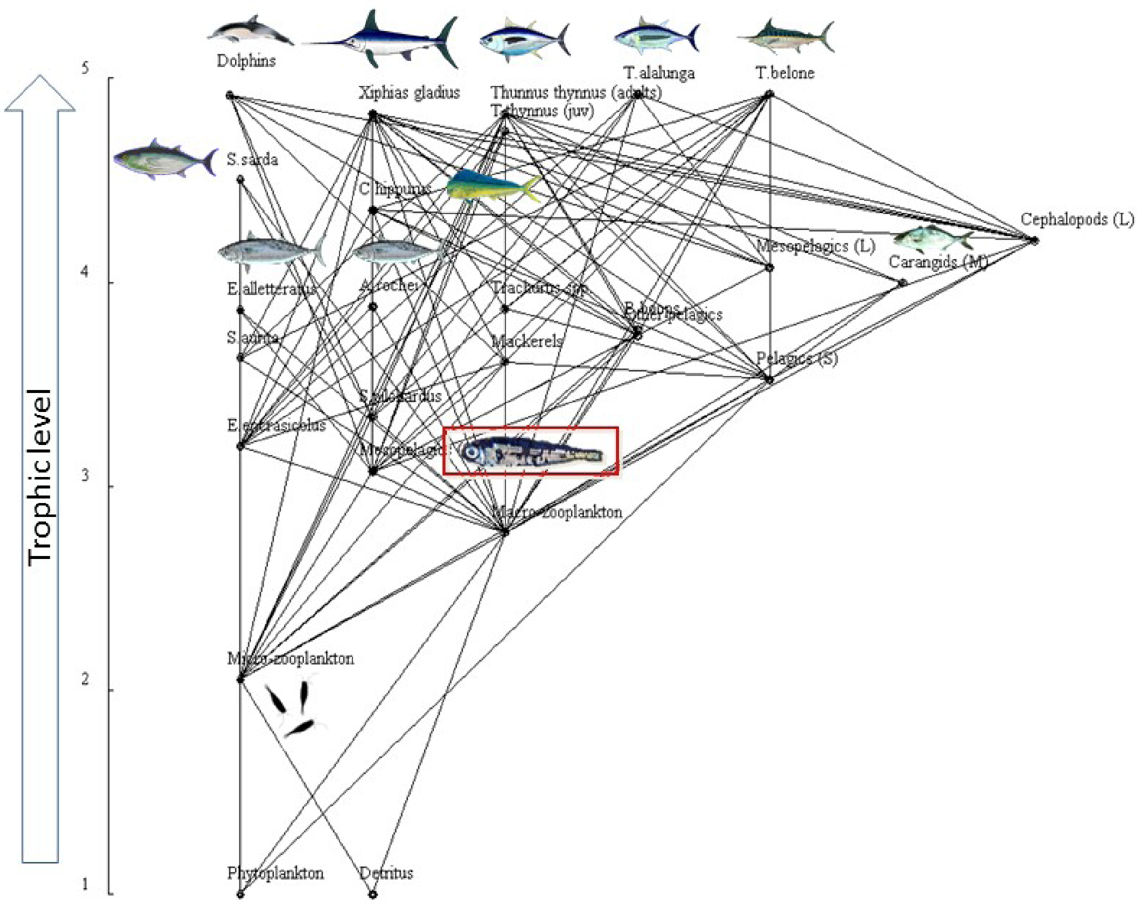


Figure 6. Reconstruction of the trophic web of the Tyrrhenian Sea.

The ingestion of plastic is a potential cause of death for vertically migrant lanternfish (myctophidae) due to the buoyancy of the material (Romeo et al., 2016) and also because the larger plastic (mesoplastics) may be retained in digestive tracts and lead to malnutrition and eventual starvation (Boerger et al., 2010; Romeo et al., 2016).

On the other hand, *Engraulis encrasicolus* and *Sardina pilchardus* are small pelagic fish, distributed in all Mediterranean waters, and are proposed as sentinel species because they are filter feeders and may potentially be impacted by microplastics in the water column. They are also among the most important commercial fishing resources of Mediterranean Sea and represent the main prey of several pelagic predators.

4.2.5 Medium-scale bioindicators of microplastics in Mediterranean open waters

At medium scale, large pelagic predators *Thunnus alalunga* and *Coryphaena hippurus* represent the most suitable sentinel species for monitoring microplastic input into the Mediterranean trophic web; and because of their wide-ranging migration in the basin, they are suitable bioindicators at medium-scale. These important fishing resources are widely distributed in Mediterranean sub-regions and have been already been identified as species impacted by plastic ingestion (Deudero and Alomar, 2015; Romeo et al., 2015).

4.2.6 Wide-scale bioindicators of of microplastics in Mediterranean open waters

Large filtrating marine organisms, such as baleen whales and filter feeder sharks are proposed as wide-scale indicators of the presence and impact of microplastic in the whole Mediterranean pelagic environment. The fin whale (*Balaenoptera physalus*), one of the largest filter feeders in the world, feeds primarily on planktonic euphausiid species. The basking shark (*Cetorhinus maximus*) is a large, filter-feeding pelagic species (Sims, 2008) that is both migratory and widely distributed. Basking sharks feed on zooplankton captured by forward swimming with an open mouth, so that a passive water flow passes across the gill-raker (filter) apparatus. Both species are at risk from the ingestion and degradation of microplastics (Fossi et al. 2014; Fossi et al 2016).
The large pelagic predators *Thunnus thynnus* and *Xiphias gladius* are also included in this sentinel species, according to the findings of Romeo et al. (2015). They are migratory species widely distributed in the Mediterranean and are considered important top predators in the pelagic trophic web. Their high commercial value and importance for food consumption is an additional reason for their inclusion in the monitoring strategy. However, for *T. thynnus* it is important to take into consideration the migration between the Atlantic Ocean and the Mediterranean when assessing the impact of plastic ingestion.

4.2.7 Wide-scale bioindicators of macro-litter in the Mediterranean open waters

The loggerhead sea turtle (*Caretta caretta*), which ingests macro-litter while feeding, can be considered as a wide-scale indicator of the presence and impact of macro-plastics (large plastic fragments) in the whole Mediterranean pelagic environment. Depending on its stage of development and on the availability of food, this species uses different ecological marine compartments from the surface to the bottom. Due to its widespread distribution and propensity to ingest marine debris, the loggerhead turtle has been proposed as a target indicator species within the MSFD (D10 C3 indicator) to evaluate the impact of debris in the Mediterranean Sea; the sea turtle was also selected as a candidate indicator by OSPAR in 2016 (Claro, 2016) and it has been identified as a candidate species to be used for the development of the UN Environment/MAP IMAP Candidate Indicator 24. Furthermore, the use of this indicator species as EI 18 for debris ingestion is recommended at the Mediterranean scale by the MedPol Marine Debris Action Plan.
Being carnivorous to omnivorous, the loggerhead turtle can ingest a high amount of debris that may be mistaken for gelatinous prey or be encrusted by food, causing, in the worst case, the death of the animal by occlusion of gastro-intestinal tract. Within the Mediterranean basin, before 2013 the occurrence of ingestion varied, from 35 % in Adriatic Sea to nearly 80 % in Mediterranean Spain (Galgani, 2017); more recent results suggest that the occurrence may have increased with time. In France for example, this ingestion occurrence increased from 35% to 76% between 2003 and 2008 (Darmon and Miaud, 2016). However, several biological constraints and sources of biases have been identified (Casale et al., 2016; Claro et al., 2014) and further tests are necessary in order to clarify the conditions in which this bioindicator could be used. Monitoring of this species may be done on stranded/dead organisms or on loggerhead turtles hospitalized in rescue centres (after stranding or caught as bycatch) by analyzing i) the

	SEA SURFACE	COASTAL WATERS	OPEN WATERS	SEAFLOOR	COAST LINE AND BEACH SEDIMENT
BASIN SCALE **(*Mediterranean Sea*)**	*Calonectris diomedea Puffinus yelkouan*	*Calonectris diomedea Puffinus yelkouan*	*Balaenoptera physalus Cetorhinus maximus Xiphias gladius Thunnus thynnus Xiphias gladius Thunnus thynnus Caretta caretta Physeter macrocephalus*		
MEDIUM SCALE **(*Mediterranean UN Environment/ MAP sub-regions)***			*Thunnus alalunga Coryphaena hippurus Caretta caretta Thunnus alalunga*		
SMALL-SCALE **(*FAO GSA)***		*Boops boops Trachinotus ovatus*	*Maurolicus muelleri Engraulis encrasicolus Sardina pilchardus Myctophids*	*Mullus barbatus Nephrops norvegicus Galeus melastomus, Merluccius merluccius, Solea spp. Galeus melastomus, Scyliorhinus canicula*	
LOCAL SCALE				Holothurians	*Mytilus galloprovincialis Arenicola marina* Decapods (e.g. *Carcinus sp.*)

Table 4. Bioindicator proposed in relation to habitat and home range. In blue bioindicators for macro-litter, in red bioindicators for microplastic

gastro-intestinal tract contents of dead animals or feces excreted by alive animals in tanks, and ii) the accumulation of contaminants in the tissue and responses of a set of biomarkers. The monitoring of ML ingestion is already going on in several Mediterranean European countries using the standardized protocols included in the European monitoring guidance (MSFD Technical Subgroup on Marine Litter, 2013), and training sessions are being organised (CORMON, MedPol action Plan). Similarly, sperm whales (*Physeter macrocephalus*) seems to be one of the most affected species among cetaceans in the Mediterranean Sea. As a high occurrence of marine litter ingestion has been reported in whales stranded along the Mediterranean coast (de Stephanis et al., 2013; Mazzariol et al., 2011), a harmonized and standardized protocol for the analysis of marine litter for large marine mammals needs to be further validated.

4.2.8 Wide-scale bioindicators of macro-litter in the marine environment (sea surface coastal shores)

Worldwide, ingestion of litter has been most studied in birds. In some oceanic regions, over 50% of the species ingest litter (NOAA, 2014). Because some species are abundant and present high rates of ingestion, they are interesting candidates to be indicators for monitoring microplastics and also mesoplastics (between 5 and 25 mm in size). Compared with other regions, the work in the Mediterranean Sea has unfortunately been limited to some rare studies (Steen et al., 2016), with just one describing the relevance of ingestion of plastics by seabirds for monitoring the impact of litter in this region (Codina-García et al., 2013). The results of the analysis of birds accidentally caught by fishermen in the western Mediterranean between 2003 and 2010 show very significant differences in rates of ingestion, but no difference in the features of the plastic ingested or between the sexes. The puffins *Calonectris diomedea*, *Puffinus yelkouan* and *Puffinus mauretanicus* presented the highest occurrence of debris ingestion (70-94% of individuals depending on species) and the greatest number of tiny particles of plastic per affected bird. However, these species have a restricted distribution in the Mediterranean. Other species, such as the Audouin's gull and the yellow legged gull *(Larus audouinii, Larus michahellis*), great skua *(Catharacta skua*), and the northern gannet *(Morus bassanus*) are less affected (10-33%). The kittiwake *(Rissa tridactylus)*, with an ingestion rate of nearly 50%, represents a locally interesting target species but its distribution in the Mediterranean remains fairly restricted.

5 THE THREEFOLD MONITORING APPROACH TO DETECTING MARINE LITTER INGESTION AND THE RELATED IMPACT IN BIOINDICATOR ORGANISMS

Deciphering the impact of litter on marine organisms is a challenging task. Physical and ecotoxicological effects strictly related to marine litter and, in particular, to plastics can be directly addressed in only a very few cases; therefore an integrated approach is needed. The impact of litter on marine organisms should be assessed using a threefold monitoring approach, which links the detection of the ingested marine litter to the physical and toxicological effects related to the ingestion of contaminated plastic litter.

The monitoring approach relies on the following three kinds of data (Fig. 7):

i. **analysis of the gastro-intestinal content in vertebrates/invertebrates** (or of the whole organism, in the case of small invertebrates) to evaluate the marine litter ingested by the bioindicator species organisms, with a particular focus on plastics and microplastics. The results of this analysis must focus on assessing the occurrence (%) of individuals that have ingested marine litter among a subpopulation/population/species, the abundance (n°) of marine litter ingested per individual, weight (g) of marine litter ingested as a total and per category of litter, colour of litter items, polymer characterization of the plastic litter and microplastics ingested by the different individuals/species analysed (see details in Annex 1). Information on the degree to which biota ingest marine litter (including microplastics) is essential in order to determine and monitor threshold levels to define 'good environmental status' (GES) for marine litter and plastic pollution (as recommended by the EU MSFD and other regional and international regulations such as, specifically: Descriptor 10-MSFD, EO 10 and IMAP Common indicator 24-IMAP; see details in Annex 2). The development of robust legislation is reliant on toxicological studies with ecological relevance, requiring an accurate measure of marine litter and microplastic loads in organisms in the field. As such, it is essential that researchers are able to accurately isolate, identify, quantify, and characterize debris consumed by biota.

ii. **quantitative and qualitative analysis of plastic additives (eg. phthalates and PBDEs) and PBT compounds** in the tissues of bioindicators, used as "plastic tracers". The detection of plastics additives and PBT compounds that can migrate from plastic litter to the tissues of organisms can represent the degree of accumulation of compounds related to the ingested plastic litter and its putative ecotoxicological effects;

iii. **analysis of the effects based on biomarker responses** at different biological levels (from gene/protein expression variations to histological alterations). Assessing the undesirable biological responses (alteration of a set of biomarkers by the measurement of endpoints) related to the ingestion of marine litter and the accumulation of plastic associated compounds is crucial to understanding and evaluating the extent of the threat of marine litter and plastic ingestion to marine organisms at individual and, ultimately, at population level.

There is clear evidence of the harm caused by marine litter ingestion to marine organisms, since a large number of marine species ingest plastic, with lethal effect; though the extent of harm can be underestimated, because of difficulties in obtaining samples and performing necropsies.

It is more difficult to determine the chemical harm related to plastic ingestion and to ascertain related sub-lethal impacts. The application of the threefold approach can elucidate not only the rate of ingestion among the different

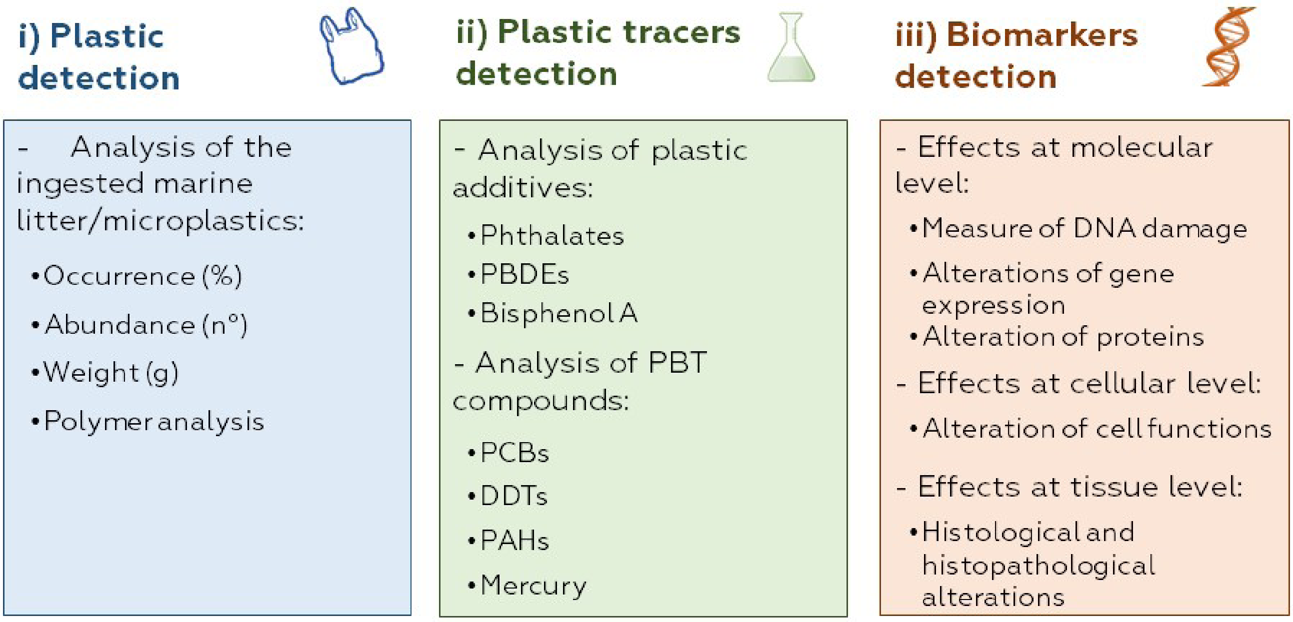
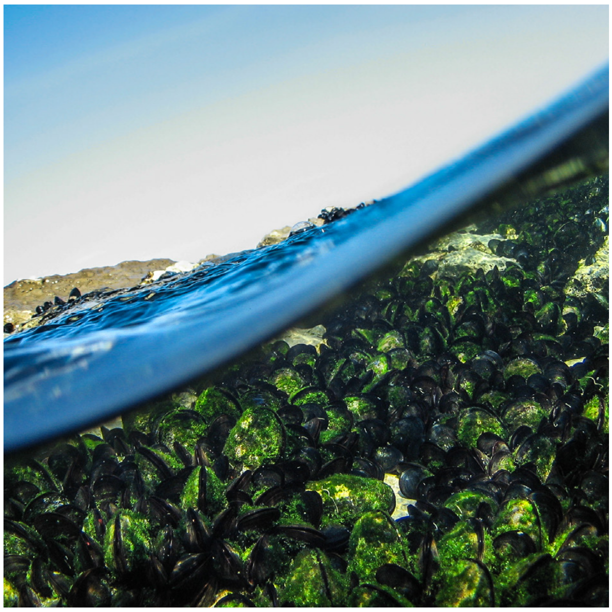


Figure 7. Threefold monitoring approach to detecting marine litter ingestion and its impact in bioindicator organisms.

bioindicators, but also the multiple sub-lethal stresses that marine litter ingestion can cause in the short and long term. Each of the three investigation tools that make up the threefold approach can be applied independently or simultaneously to the selected bioindicator species. The tools analysis that can be done in the different groups is described below.
For reliable information on changes (or stability) in quantities of ingested litter and their effect, a statistical sample size is recommended, as well as continuous sampling in order to collect the background information needed to define 'good environmental status' and to evaluate possible temporal trends.

5.1 Invertebrates

A range of invertebrates including filter feeders, deposit feeders and detritivores have been shown to ingest microplastic in the laboratory and in the field studies (Annex 3). In many cases it may be possible to examine organisms that are dead at the time of collection, for example, invertebrates from trawls or other sampling. Live specimens must be sacrified, adhering to prevailing ethical legislation. For each specimen a sampling sheet should be completed, covering: species, dimension (according to the standards for each species), gender (if possible), alive/injured or dead at time of collection, reproductive state, quantity of food present in digestive tract (if possible), location collected, method of sampling, sampling campaign or from commercial fishing activities. Small individuals can be stored whole. For larger individuals, the gut can



be dissected but may otherwise be stored intact. Examination of the gut is facilitated by a dissecting microscope. Any fragments of an unusual appearance are removed with forceps and placed on clean filter paper in petri dishes which are then sealed prior to further examination, for example, via spectroscopy. The soft tissue of the organisms can be analysed for microplastics following the procedure described in Annex 1.1. During the sampling and analytical procedure, several blanks must also be tested, in order to determine the level of airborne and environmental contamination.

In the case of small invertebrates, where it is not possible to extract the gut, analysis will involve digestion of the whole organism, to analyse the ingested plastic. Digesting the whole organisms does not allow analysis of biomarkers and contaminants. However, simultaneous analyses can be carried out during the same monitoring programme, devoting a statistically significant number of the samples to the detection of plastics and a statistically significant number of the samples to contaminants analysis (Annex 1.2) and biomarkers (Annex 1.3 table A1.4).

5.2 Fish

Live or dead fish can be obtained from existing active monitoring programmes, for example the MEDITS programme, or from *ad hoc* monitoring campaigns. In dead specimens it is possible to detect the occurrence and rate of marine litter ingestion and to quantify the possible accumulation of contaminants, while in the live specimens, in addition to marine litter ingestion and contaminant analysis, it may be possible to evaluate the relative biological effects (biomarker responses). Immediately after sampling the following parameters should be recorded: sampling site, date, trawl/fishery type, species, total length and standard length, weight, sex, visible deformations and external

Dead organisms: after the recording of the biological parameters, the stomach and intestine are removed and stored in tin foil. If the analysis of the stomach contents cannot be done immediately, the specimens can be frozen to -20°C. The procedure for litter ingested by fish is not standardized, but for large fish (adult animals) see Annex 1.1 (Macro-litter), while for smaller fish or juvenile life stages the procedure is indicated in Annex 1.1 (Microplastic).

Live organisms: After recording the biological parameters listed above, the specimens should be sacrified and the stomach and intestine collected and processed, as in the procedure for 'dead organisms', ready for the analysis of marine litter (Annex 1.1). The blood should be withdrawn from the caudal vein using a disposable heparinized syringe. An aliquot of the whole blood should be used for the evaluation of biological responses, part to obtain blood smears and the rest kept in liquid nitrogen or at -80°C; another whole blood aliquot should be kept at -20°C for analysis of the level of contaminants (Annex 1.2). The remaining part of the blood should be centrifuged to obtain plasma, kept at -80°C or in liquid nitrogen, for biomarker analysis (Annex 1.3, Table A1.5). The organs, liver, kidney, gills, bile and brain, should be collected in aluminium foil and immediately stored in liquid nitrogen or at -80°C until biomarker analysis. Muscle should be removed and stored at -80°C for biomarker analysis or kept at -20°C for contaminant analysis.

5.3 Sea Turtles

The Loggerhead sea turtle (*Caretta caretta*) is a protected species, and can only be handled by authorized persons. The handling and tissue collection procedure must be carried out in strict accordance with the relevant national and international guidelines and permits. At first, morphologic parameters such as curve carapace length (CCL), curve carapace width (CCW) and total body weight should be measured for each specimen, and the apparent health status and/or cause of death in stranded animals noted. The sex of the specimens should be determined visually, where possible.

Stranded organisms: Loggerhead sea turtles that are stranded or caught accidentally (as by-catch to commercial fishing) should be used for the analysis of contaminants and gastro-intestinal content. The animal should be transported to an authorized centre for necropsy. If the necropsy cannot be carried out immediately after recovery, the carcass should be frozen at -20°C. External examination of the animal should be conducted, including inspection of the oral cavity for the possible presence of foreign material. To remove and separate the plastron from the carapace, an incision is made on the outside edge. Once the inside of the plastron is accessed, the ligament attachments of the pectoral and pelvic girdle are cut. Qualitative evaluation of the trophic status of the animal is recommended. An aliquot of fatty tissue, muscle and liver should be collected and kept in aluminium foil at -20°C for the evaluation of contaminant levels (Annex 1.2). Pectoral muscles and the heart should be removed to expose the gastrointestinal tract (GI) which should be processed following the procedure described in Annex 1.1.

Hospitalized and free-ranging organisms: Live loggerhead turtles can be sampled in Marine Turtle rescue centres, or free-ranging, where permitted by national legislation. A sampling sheet should be completed for each specimen sampled. Non-destructive collection of biological tissues should be carried out with the support of a veterinarian. Blood (a few ml, amount depending on the size of the turtle) should be collected from the cervical venous sinus of each animal using a disposable heparinised syringe. An aliquot of the whole blood should be used for the evaluation of biological responses, part to obtain blood smears and the rest kept in liquid nitrogen or at -80°C; a second whole blood aliquot will be kept at -20°C for analysis of the level of contaminants (see Annex 1.2). The remaining part of the blood should be centrifuged to obtain plasma, kept at -80°C, for the evaluation of biological responses (see Annex 1.3 table A1.6). When possible, a skin biopsy sample should be collected, using a biopsy punch with a size ranging from 0.4 to 0.8 mm diameter according to the size of the turtle, and kept in liquid nitrogen or at -80°C until evaluation of biological responses. Approximately 0.250 g of carapace should be removed using a plastic scraping tool and frozen in a whirl pack bag. Scute scrapings should be taken from the areas of the carapace free of barnacles and algae. Only the most superficial keratinous layer should be taken, with no penetration to the keratinous layer-bone interface below. Carapace sub-samples should be kept at – 20°C until analysis. From the day the animal arrives at the recovery centre, the faeces should be collected daily for at least the first 20 days, then once a week. Faeces should be kept at -20°C until the analysis of marine litter (see Annex 1.1) and biological responses (see Annex 1.3, table A1.6).

5.4 Seabirds

Several seabird species feed on the surface of the sea. Therefore, the water column and especially the water surface is the marine compartment addressed when detecting marine litter ingestion or quantifying litter in the stomachs of dead seabirds, and analysing contaminants and relative biological effects in live seabirds.

A full series of data on the seabirds in the sample should be recorded, covering sex, age, breeding status, origin, likely cause of death (if the specimen is dead) and other issues. Age, the only variable found to influence litter quantities in stomach contents, is largely determined on the basis of development of sexual organs (size and shape) and presence of Bursa of Fabricius (a gland-like organ positioned near the end of the gut which is involved in immune systems of young birds; it is well developed in chicks, but disappears within the first year of life or shortly after).

Dead organisms: dead seabirds collected from beaches or accidental mortalities such as long-line victims, fledgling road kills, etc. can be used. The methodology of this tool follows the OSPAR Ecological Quality Objective (EcoQO) methods for monitoring litter particles in the stomachs of northern fulmars (*Fulmarus glacialis*), and the background information and the technical requirements are described in detail in documents related to the fulmar EcoQO methodology. If the specimen cannot be processed when it arrives at the laboratory, it can be frozen at -20°C. Bird dissection procedures including characteristics for age, sex, cause of death etc. have been specified in van Franeker (2011). Some information about dissection, in a condensed form, is given below. After dissection, the contents of the stomachs of the birds should be processed according the Annex 1.1, while the fat and muscle is collected in tin foil and stored at -20°C for later contaminant analysis (Annex 1.2).

Hospitalized and free -ranging organisms: several seabird species are protected, and can only be handled by authorized persons. The handling and tissue collection procedure must be carried out in strict accordance with the relevant national and international guidelines and permits. Non-destructive collection of biological tissues should be done with the support of a veterinarian. Blood (a few ml, amount depending on the size of the seabird) should be collected from a brachial vein using an insulin syringe. To improve the visibility of the vein, it may help to wet the feathers with a cotton ball soaked in mild disinfectant. An aliquot of the whole blood should be used for the evaluation of biological responses, part to obtain blood smears and the rest kept in liquid nitrogen or at -80°C; another whole blood aliquot for analysing the level of contaminants should be kept at -20°C (see Annex 1.2). The remaining part of the blood should be centrifuged to obtain plasma for the evaluation of biological responses, and kept at -80°C (see Annex 1.3, Table A1.6). A few mg of feathers should be collected from each sample and frozen at -20°C for contaminant analysis. A few mg of excreta should be collected from the burrow (shearwaters) or close to the nest (gulls) with a spoon (taking care to avoid collecting vegetables, roots, pebbles, guano), and the samples put into individual plastic bags and refrigerated (within 24-48 hours) at -20°C for later contaminant analysis. The collection of deserted or addled eggs should be useful to test for contaminants. Considering the difficulties in sampling fresh excrement from the target species, only dry excrement can be collected. Excrement collected from soil close to the nest is not suitable to analyse for trace metals (as these may be contaminated by trace elements naturally present in soil), but is optimal to evaluate biological responses.

5.5 Marine mammals

The Mediterranean marine mammal fauna are composed of eight resident species of cetaceans and only one species of pinnipeds, the monk seal (*Monachus monachus*). However, given the fragmented distribution and the relatively small breeding subpopulations of the monk seal, for the purpose of the protocol, sampling procedures are proposed only for the cetacean species.

Stranded organisms: For stranded cetaceans, it is possible to analyse the gastro-intestinal contents and the contaminants in the tissues. Small stranded cetaceans may be transported to an authorized centre for necropsy, while for large animals dissection is usually done directly at the stranding site by authorized institutions. Before the necropsy is carried out, morphometric measurements should be collected, in particular the total length, which allows the age of the specimens to be estimated. During the necropsy, the trophic status should be noted and blubber collected for the analysis of levels of contaminant (Annex 1.2). The contents of the G.I. tract should be examined to determine the diet of the animal and for the analysis of ingested plastic (Annex 1.1).

Free-ranging organisms: Skin biopsies from free-ranging cetaceans can be obtained by a non-destructive, remote dart sampling method, using a modified dart with an aluminium tip (8mm diameter) launched with a crossbow, with the sample being immediately stored in liquid nitrogen as described by Fossi et al. (2008). To avoid any possible infection, the tip is sterilized each time with alcohol before shooting. The biopsy sample should be taken from the dorsal area close to the dorsal fin and on the upper part of the caudal peduncle. Each biopsy (1-2 g of epidermal, dermal and blubber tissue) needs to be subdivided into different aliquots according to the different analysis: blubber tissue for contaminants analysis (plastic tracers) (Annex 1.2) and epidermal, dermal part for ecotoxicological biomarkers (Annex 1.3, Table A1.6) All the aliquots should be stored in liquid nitrogen or at -80°C until the analysis.

6 RISK ASSESSMENT MODELS AND ACCUMULATION AREAS

Most environmental risks are spatially and temporally limited, so a critical early need is to establish the risk of what is happening to whom, or which part of the environment, where and when (Werner et al., 2016). Conceptually, risk assessment provides a structured process to inform judgments about the risks posed by an activity or various activities and their significance. It involves four stages (Werner et al., 2016) including: (1) assessing the potential consequences after exposure at a particular level (hazard identification/ characterisation); (2) the assessment of the exposure (probability that a hazard will be realised); (3) the characterisation of the risk, combining hazard and exposure; and (4) the evaluation of uncertainties.

In the case of the ingestion of marine litter, the risk assessment should indicate where and when harm may occur. This is not only defined by the potential encounter of marine organisms with litter items, but also takes into account an assessment of the potential harmfulness of litter items, such as the nature and shape of litter.

Risk assessment has been used recently to investigate areas where species may suffer from the presence of litter and more precisely to predict areas of high risk of ingestion. Schuyler and colleagues (2014, 2016) investigated whether plastic litter ingestion prevalence in marine turtles has changed over time, what types of litter are most commonly ingested, the geographic distribution of litter ingestion by marine turtles relative to global litter distribution, and which species and life-cycle stages are most likely to ingest litter. The ecological threats to marine biota at a population level are often unclear, as is the geographical extent of their impact. To address this knowledge gap, Hardesty et al. (2015) identified a broader suite of items of concern for ingestion, with plastic bags and plastic utensils ranked as the greatest threats. Birds (Wilcox et al., 2015) and sea turtles (Schuyler et al., 2014) in nearly all the regions studied ingest litter, and models of global marine plastic distribution combined with habitat maps and species distributions have enabled levels of exposure to plastic pollution to be predicted. The authors modelled the probability of litter ingestion by incorporating exposure to litter and consequences of exposure. In the Mediterranean Sea, only one study using real data has been described for sea turtles, in the northern part of the western basin (Darmon et al., 2017). Based on aerial surveys, distribution of both litter and sea turtles were investigated, enabling the probabilities of sea turtles encountering floating litter to be mapped and areas at risk to be defined.

In a recent paper Fossi and collaborators (Fossi et al., 2017) investigated the possible overlap between microplastic, mesoplastic and macro-litter accumulation areas and the fin whale feeding grounds in the pelagic Specially Protected Area of Mediterranean Importance, the Pelagos Sanctuary. Models of ocean circulation and potential fin whale habitat were merged to compare marine litter accumulation with the presence of whales. Field data on the abundance of microplastics, mesoplastics and macro-litter, and on the presence of cetaceans were collected simultaneously in that study. The resulting data were compared, as a multi-layer, with the simulated distribution of plastic concentration and the whale habitat model. This is the first study in the Pelagos Sanctuary in which the simulated microplastic distribution has been confirmed by field observations and where the overlap between the fin whale feeding habitat and the microplastic hot spots is reported as an important contribution to the risk assessment of fin whale exposure to microplastics (Fig. 8).

The approaches used in these two papers (Darmon et al., 2017; Fossi et al., 2017) predicted where species were the most affected, enabled sensitive areas for species-specific ingestion to define, and provided a basis on which to decide on the mapping of areas to be protected. Based on data or outputs from models on both litter, macro or microplastics, and species distribution, from plankton to large vertebrates, the same approach could be largely used to predict areas where the risk of ingestion occurs, with possible consequences on the quality of fish and associated risk, including after human consumption.

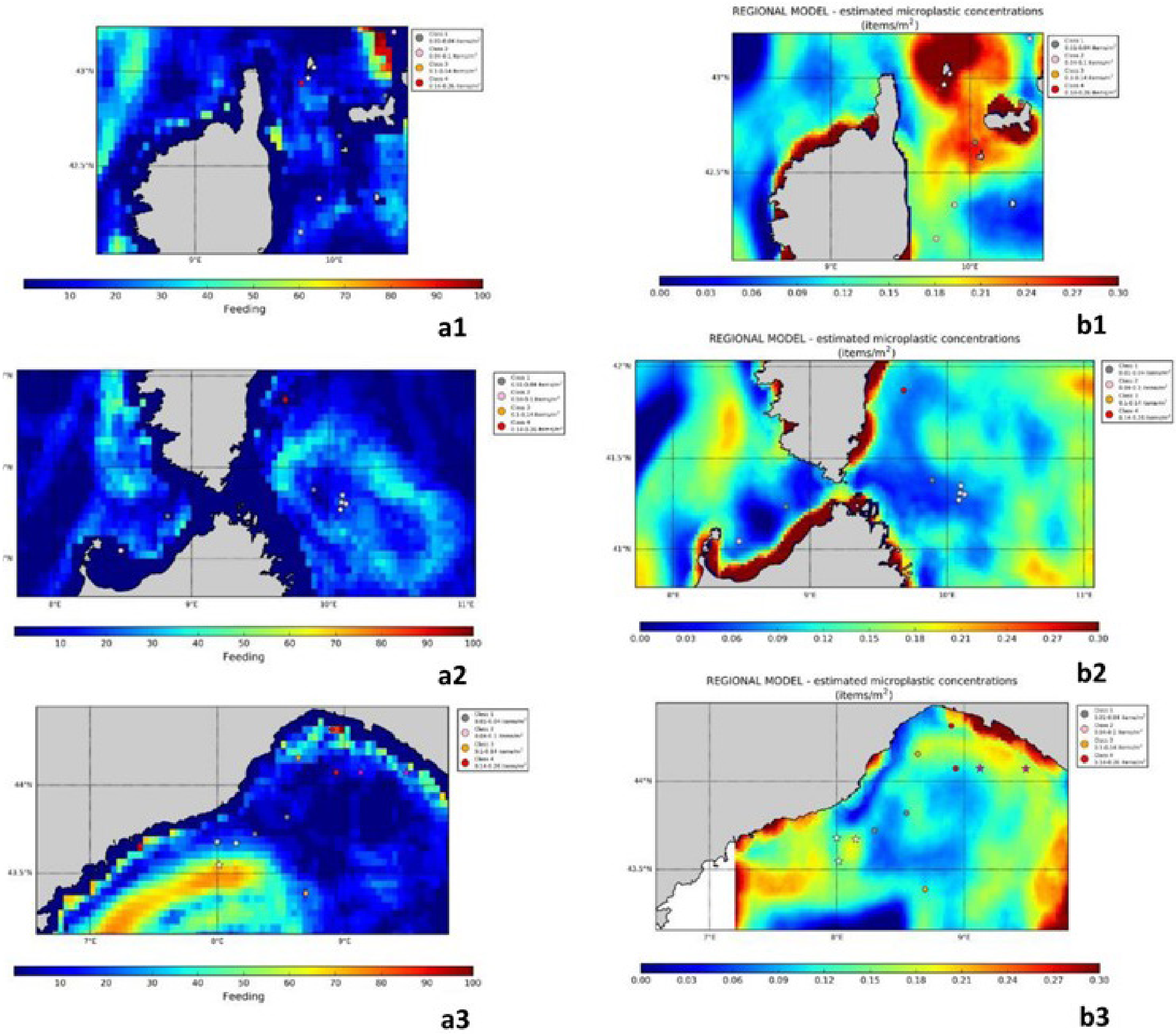


Figure 8 . Fin whale habitat maps (Feeding Habitat Occurrence) (A1–A3) and simulated distribution of microplastics (items/m^2) (B1–B3) in north-western Mediterranean Sea. Microplastic concentrations (circle), and cetacean sighting data (white star: *Balaenoptera physalus*, pink star: *Stenella coeruleolba, Tursiops truncatus, Grampus griseus*) plotted in the two maps. (From: Fossi et al. 2017).

7 GAPS AND FURTHER DEVELOPMENTS

As reported by the recent report of UNEP/MAP (Galgani, 2017), monitoring the impacts of marine litter on marine biota depends on the availability of specific indicator species in which to measure the prevalence and effects of ingestion of litter. It was also underlined that monitoring of effects can be designed within a multi-species approach in order to cover impacts linked to both the diverse types of litter - of varied size (micro-particles and macro-litter) and nature (plastics, metal, glass, etc.) - and to the varied ways of life (sedentary, benthic, nekto-benthic, pelagic, aerial) and feeding (detritus-eaters, suspension-eaters, omnivores, carnivores) of the species that interact with it, as reported extensively in this review. Several gaps and potential further development in this field are reported below.

7.1 Harmonization of methods to detect ingested plastic, chemicals and effects

Researchers and agencies around the world are currently highlighting the urgent need for the harmonization of methods to detect the presence of plastics and its effects (Rochman et al., 2017). Most methods on plastic ingestion are not standardized as they have been developed only recently and have not yet been applied to monitoring, except for litter ingestion by Northern Fulmars in the North Sea (Lusher et al., 2017; Provencher et al., 2017). Sampling protocols and plastic detection methods as well as reported metrics vary between different research teams. For example, methods for the detection of microplastic ingestion include direct microscopical examination of gastrointestinal contents, isolation of microplastics from tissues or gastrointestinal contents using digestion by different chemicals or enzymes, ultrasonic treatment and density separation, and subsequent polymer identification again by various techniques such as Fourier Transform Infrared (FTIR) and Raman spectroscopy (Lusher et al., 2017; Vandermeersch et al., 2015; Wagner et al., 2017). Methods used to detect plastic-associated chemicals and effects (biomarkers) are also variable; however, some methods are standardized as they have been previously developed and applied to monitoring of chemical contamination (Hylland et al., 2017). Standardized protocols and harmonised monitoring methods are needed to allow spatial and temporal comparisons and to enable the presence of plastic and its effects in marine biota to be assessed. Intercalibration exercises can help validate and harmonise methods used across different research teams and laboratories.

7.2 Food chain and human health

Microplastics ingestion by aquatic organisms has been confirmed in laboratory and field work, including in commercially important edible species (Avio et al. 2015; Pedà et al. 2016). The number of microplastics observed in the gut contents of farmed and wild aquatic animals is usually low, but overall exposure levels are not known. The presence of plastic debris in the stomach of Mediterranean fish represents a potential risk to human health, due to the potential effects of the transfer of environmental contaminants from debris to edible fish tissue. Although the presence of these persistent pollutants in several fish species has been already studied, the origin and the pathways that introduce pollutants in the trophic web has not yet been well clarified. Plastics could themselves be a direct source of contamination as well as an indirect vector of several pollutants and adsorbed substances. The main goal of future studies and research should be the understanding of how these plastics are transferred and made bioavailable in fish and shellfish tissue, and ultimately to the final consumers (top predators, humans).

7.3 Future developments

The quantification of the transfer of chemicals associated with plastic particles from the plastic into the tissues of the organisms, as a result of uptake or ingestion, is very poorly quantified (Koelmans et al., 2016). This is due in large measure to the difficulty in separating the contaminant load due to consumption of natural prey items from similar chemicals derived from plastic. This makes it extremely difficult to estimate the proportion of contaminant loading derived from ingested or otherwise incorporated plastic particles. In order to cover these knowledge gaps, future investigation needs to be carried out in this field, such as:

- The marine food chain and particularly fish consumed by humans should be evaluated for chemicals that are added to plastics during manufacturing, e.g. phthalates and PBDEs. The future investigation of specific "plastic tracers" is highly recommended in order to correctly identify and define the toxicological impacts associated with the ingestion of plastic material (see details in Annex 2).
- Further data on the translocation of different polymers should be developed for aquatic organisms and studies need to be carried out on microplastics as a source of chemicals and pathogens to fishery and aquaculture products, and to humans, when consumed.
- The potential transfer of microplastics and related contaminants in the marine food-chain, focusing on top predators (e.g., tuna, swordfish), should be investigated to explain both contamination in fishery products and also in marine mammals as a sentinel for the health of the marine environment and food safety.
- Further development and application of the threefold monitoring approach proposed in this document to detect marine litter presence and impact in bioindicator organisms are highly recommended. The simultaneous analysis in several bioindicator species of i) the gastro-intestinal content, to evaluate the marine litter ingested by the organisms; ii) plastic additives and PBT compounds used as plastic tracers; and iii) the effects identified by biomarkers responses at different level of biological organization, will allow a more complete assessment of the real impact related to plastic debris ingestion by marine organisms (see details in Annex 2).

ANNEX 1
Methodology

A1.1 MARINE LITTER DETECTION

Macro-litter count and characterization

Gastrointestinal tract - The different portions of the GI tract (oesophagus, stomach(s), intestine) of the selected bioindicator organism should be separated (see Chapter 4), by means of clamps fixed on the esophagus proximal to the mouth, on the esophageal valve, on the peg and close to the anal orifice, to prevent mixing of the contents. During the analysis of small-sized animals the entire GI tract should be removed and placed on the examination surface, while for large-size animals it is preferable to remove the portions separately.
The following sampling procedure of GI contents should be applied to all sections of the GI. The sections of the GI should be opened and the contents collected. The contents should be inspected for the presence of any tar, oil, or particularly fragile material, which should be removed and treated separately. The liquid portion, mucus and digested unidentifiable matter should be removed by washing the contents with filtered freshwater through a 1 mm sieve. The food organic component should be separated from any other items or material (marine litter) for diet analysis. The fraction of macro-litter should be dried at room temperature, weighed and categorised according to the MSFD protocol, modified by van Franeker et al. (2011) (Table A1.1).

Faecal pellet analysis - Once in the lab, the excreta should be lyophilized. The faecal part should be separated from the plastic and used for biomarker analysis. The litter present in the faecal excreta should be characterized according to Table A1.1. Other information such as colour of items, surface of litter, different type of litter, and polymer analysis are useful for impact analysis and for designing future mitigation measures.

Microplastic count and characterization

Analysis of microplastics ingested by biota is a challenging task, and for that reason an increasing number of techniques have been developed in recent years(Lusher et al., 2017b, 2017a). The use of harmonized methods will allow more accurate assessment of the impacts and risks that microplastics pose to biota and will increase comparability between studies. For these reasons, the methods that appear, on balance, to be among the most widely tested and effective digestive treatments currently available are described here. In all cases, the costs, strengths and weaknesses, and applicability of each method to the organism being studied should be carefully considered.
The gastro-intestinal tract or the whole organism is rinsed with deionized filtered (0.45 μm) water to avoid contamination of the sample. To degrade organic matter, the sample is digested using a 10% KOH solution at 60°C for 12 hours (Rochman et al., 2015) or using enzymatic digestion (Cole et al., 2014). After digestion of the organic matter, the obtained solution is filtered onto glass fibre filters (1.6 μm) for analysis by stereomicroscope of the residual inorganic particles (e.g. non-digested tissue particles, inorganic residues, microplastics, etc.) which are characterised by their type, size, and colour, and then weighed and subdivided into the categories listed in Table A1.2. Given the difficulty of identifying marine microlitter on a visual basis, further analysis is used to confirm the isolated particle. Such analysis can be performed by spectroscopy (FT-IR or Raman) or by the “hot needle” technique, if spectroscopy instruments could not be available. Spectroscopic analysis is also used to determine the nature of the polymer that makes up the particles, in order to better define the nature and

Table A1.1. Categories of macro-litter

Marine litter			Description	TSG_ML General-Code (1)
PLASTICS	Industrial	Pellets	industrial plastic granules with different shapes (cylindrical, oval, spherical or cubical)	G112
		Spheres		G118
	User plastics	Sheet-like	remains of bags, cling-foil, agricultural sheets, rubbish bags, etc.	G119
		Thread-like	pieces of nylon wire, net fragments, woven clothing, etc.	G120
		Foamed	all foamed plastics, i.e. polystyrene foam, foamed soft rubber, etc.	G121
		Fragments	fragments, broken pieces of thicker types of plastic, (can be bit flexible, but not sheet-like materials)	G122
		Other	any other objects, incl. elastics, dense rubber, cigarette filters, pieces of balloons, soft air-gun bullets; etc.	
RUBBISH	Paper		newspaper, packaging, cardboard; includes multi-layered material (e.g. tetrapack pieces) and aluminium foil	G157
	Kitchen food		human food waste (galley waste) such as onion, beans, chicken bones, bacon, tomato, grape, pepper, and melon seeds, etc.	G215
	Various rubbish		other consumer waste, such as processed wood, pieces of metal, metal air-gun bullet; lead shot, paint chips.	G216
	Fish hook		fishing hook remains (not for hooks on which longline victims were caught)	G183
POLLUTANTS	Slag/coal		industrial oven slags ('looks like non-natural pumice) or coal remains	G212
	Oil/tar		lumps of oil or tar (also not n=1 and g=0.0001g if other particles smeared with tar but cannot be sampled separately)	G214
	Paraffin/chemical		lumps or mash of unclear paraffin wax-like substances (NOT stomach oil!). If needed, subsample and estimate mass	G213
	Feather lump		lump of feathers from excessive preening of fouled feathers (n=1 with dry mass) (NOT for few normal own feathers)	

(1) TSG_ML: MSFD GES Technical Subgroup on Marine Litter

source of contamination in bioindicator species. During all analytical procedures, particular attention must be paid to the prevention of possible environmental contamination due to the ubiquitous nature of certain types of particles (e.g. synthetic fibres). For this reason, together with the samples, it is recommended to include experimental blanks. In addition, all analytical procedures should be carried out with glass material where possible, minimizing the use of plastic laboratory material.

A1.2 Contaminant analysis

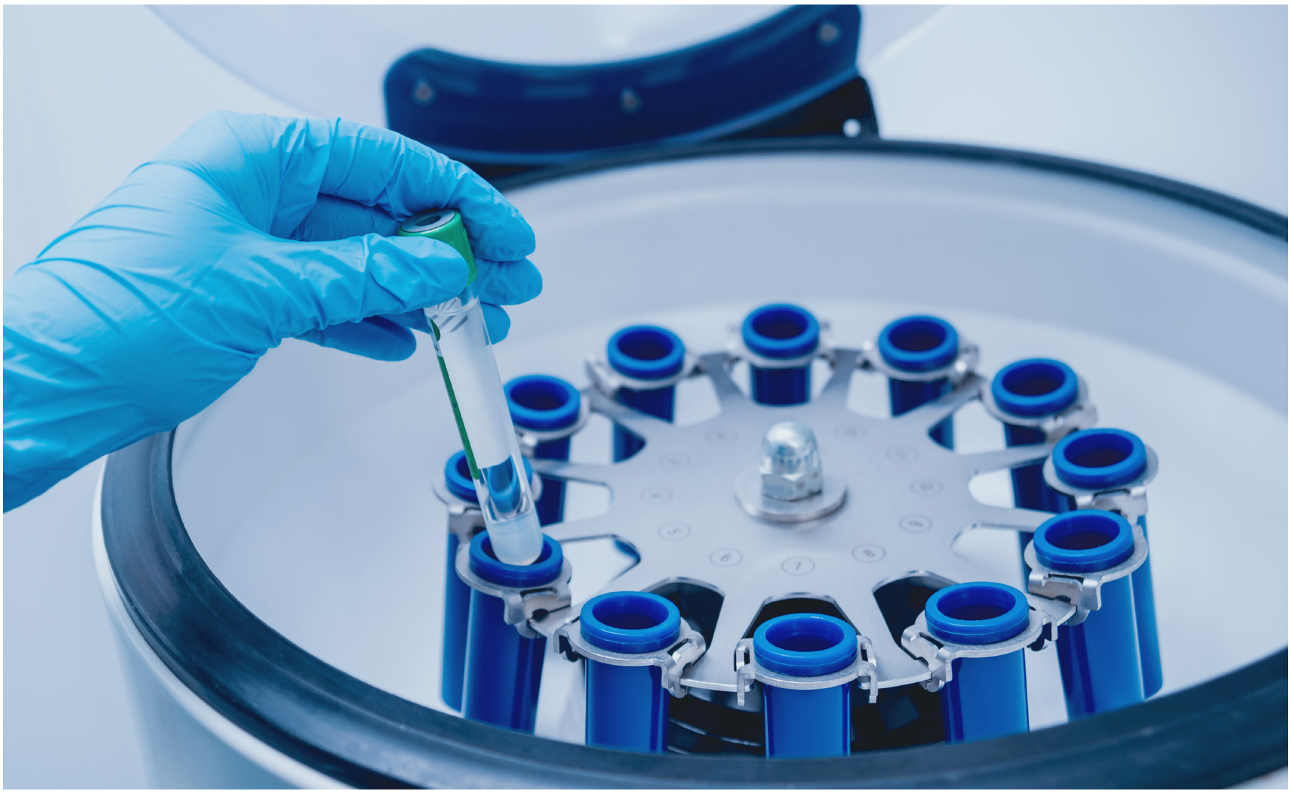
Because of their physical and chemical properties marine plastic debris is associated with a 'cocktail of chemicals', including those that are ingredients of the plastic material (e.g. monomers and additives) and those present in the marine environment that adsorb onto plastic when it becomes marine debris (e.g. persistent organic pollutants (POPs) and metals). Depending on the compounds and the tissue, different methods should be applied (see Table A1.3).

Table A1.2. Categories of microplastics

Microplastics			TSG_ML General- Code
PLASTICS	Fragments	Rounded	G103
		Subrounded	G104
		Subangular	G105
		Angular	G106
	Pellets	Cylindrical	G107
		Disks	G108
		Flat	G109
		Ovoid	G110
		Spheruloids	G111
	Filaments		G113
	Films		G114
	Foamed plastic		G115
	Granules		G116
	Styrofoam		G117

Table A1.3 Contaminants analysis

	Chemical compound	Tissue/Sample	Analytical Method
PLASTIC ADDITIVES	Phthalates	Blubber, muscle, liver	Baini et al. (2017), Fossi et al. (2014)
		Blood	Takatori et al. (2004)
		Uropygium gland	Hardesty et al. (2015)
	Bisphenol A	Muscle	Ballesteros-Gòmez et al. (2009)
		Blubber	Xue and Kannan (2016)
		Blood	Cobellis et al. (2009)
	Polybrominated diphenyl ethers	Blubber, muscle, liver, blood	Muñoz-Arnanz et al. (2016)
ADSORBED COMPOUNDS	Polycyclic aromatic hydrocarbons	Blubber, muscle, liver, blood	Marsili et al. (2001)
	Organochlorine contaminants	Blubber, muscle, liver, blood	Marsili and Focardi (1997)
	Mercury	Blood, skin	Correa et al. (2013)



A1.3 Biomarker detection

A more comprehensive evaluation of the actual ecotoxicological risk for the bioindicator species, associated with the presence of marine litter in the Mediterranean area, can be performed using a set of extremely sensitive diagnostic and prognostic methodologies, the so-called biomarkers. Such methodologies are based on an evaluation of the "response" - at the organism, population or community level - induced by a source of environmental chemical stress. A biomarker therefore produces an integrated outlook at the pollution level in a given area and, consequently, is a content-rich indicator of the ecotoxicological risk faced by a natural population (Fossi et al., 2000). The aim of such methodologies is to foresee, and hence mitigate, negative outcomes at the ecological level. In order to evaluate the possible effects on the bioindicator species, from molecular to cellular level, a set of biomarkers can be applied that integrate the data obtained from the detection of marine litter and the plastic tracers with a more complex ecotoxicological evaluation of the health status of the selected bioindicator organisms. Some of the potential biomarker techniques applicable for the different systematic groups are reported in Table A1.4-5-6.

Table A1.4 Biomarker techniques in invertebrates

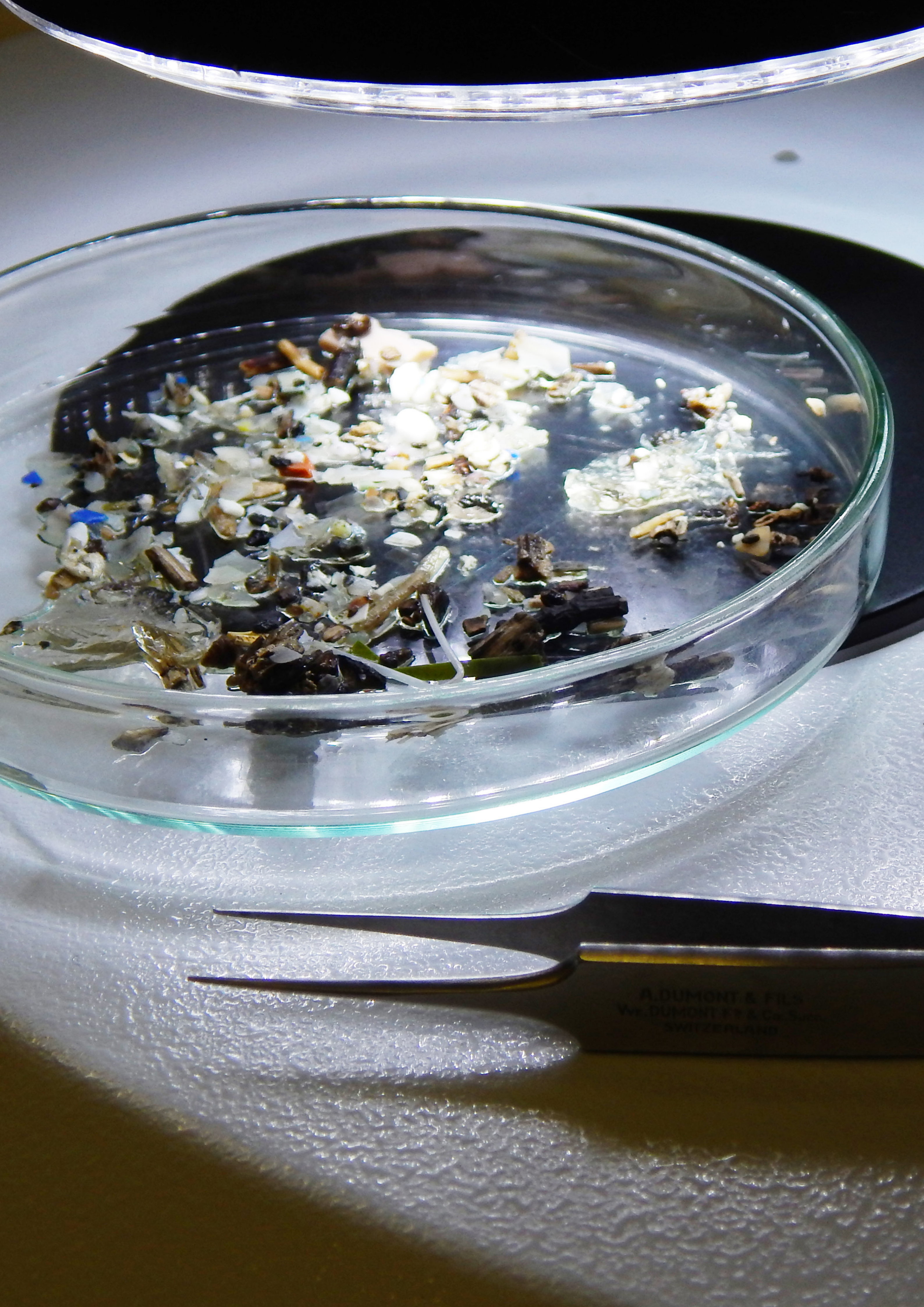
Effect	Tissue	Test
Gene expression/Trascriptomics/ Proteomics	Digestive gland/whole organisms	GADD45A, GADD45G; GSTP1, GSTP2; GPX2 and GPX3; SOD2, CASP, TRAF (Avio et al., 2015a; Sussarellu et al., 2016)
Genotoxicity	Haemolymph, digestive gland	Comet assay (Kwok et al., 2013) Mn test (Rothfuss et al., 2011)
Oxidative stress	Digestive gland	LPO, CAT, SOD, GSH, GPX (Prego-Faraldo et al., 2017)
Immunotoxicity	Digestive gland	(Hannam et al., 2010)
Reproduction	Gonads	Gamete Quality, and Larval Development (Sussarellu et al., 2016)
Inflammation/morphology	Digestive gland	Histopathology, histology (Avio et al., 2015a)

Table A1.5 Biomarker techniques in fish

EFFECT	TISSUE	TEST
Gene expression	Plasma, brain	PPARs, bcl-2, tp53, casp8, casp9, casp3a sod1, gpx4b, gstp, ACHE, TRHs (Karami et al. 2017; Mathieu-Denoncourt et al., 2015).
Genotoxicity	Blood, liver, kidney, gill	Comet assay (Frenzilli et al., 2009) Mn test, ENA assay (Pacheco and Santos, 1997)
Oxidative stress	Liver, kidney, gill	Cat, LPO, GPX, GSH (Caliani et al., 2009)
Xenobiotic metabolism and cellular stress	Liver, blood, bile	Porphyrins (Casini et al., 2003) Bile metabolites (Casini et al., 2006)
Neurotoxicity	Brain, blood	AChE activity (Ellman et al., 1961)
Reproduction	Plasma, Gonads	CYP17A, CYP19, ERs, VTG, StAR (Mathieu-Denoncourt et al., 2015). Vitellogenin (Fossi et al., 2004)
Inflammation/morphology	Liver, kidney, gill	Histopathology,histology (Pedà et al. 2016; Karami et al. 2017)

Table A1.6. Biomarker techniques in sea turtles, seabirds and marine mammals

EFFECT	TISSUE	TEST
Genotoxicity	Blood, skin	Comet assay (Caliani et al., 2014) Mn test, ENA assay (Borghesi et al., 2016)
Oxidative stress	Blood, plasma, skin	LPO, CAT (Fossi et al., 2013)
Reproduction	Plasma, skin	Vitellogenin (Herbst et al., 2003) CYP17A, CYP19, ERs, VTG, StAR (Mathieu-Denoncourt et al., 2015; Panti et al., 2011)
Cellular stress	Blood, skin	PPARA, PPARG, HSP70, GPX, E2F1 (Mathieu-Denoncourt et al., 2015; Panti et al., 2011)
Xenobiotic metabolism	Blood, skin, excreta	CYP1A; AHR, CYP3A (Fossi et al., 2014, Panti et al., 2011), Porphyrins (Casini et al., 2003)
Neurotoxicity	Plasma	AChE, BChE (Casini et al. 2003)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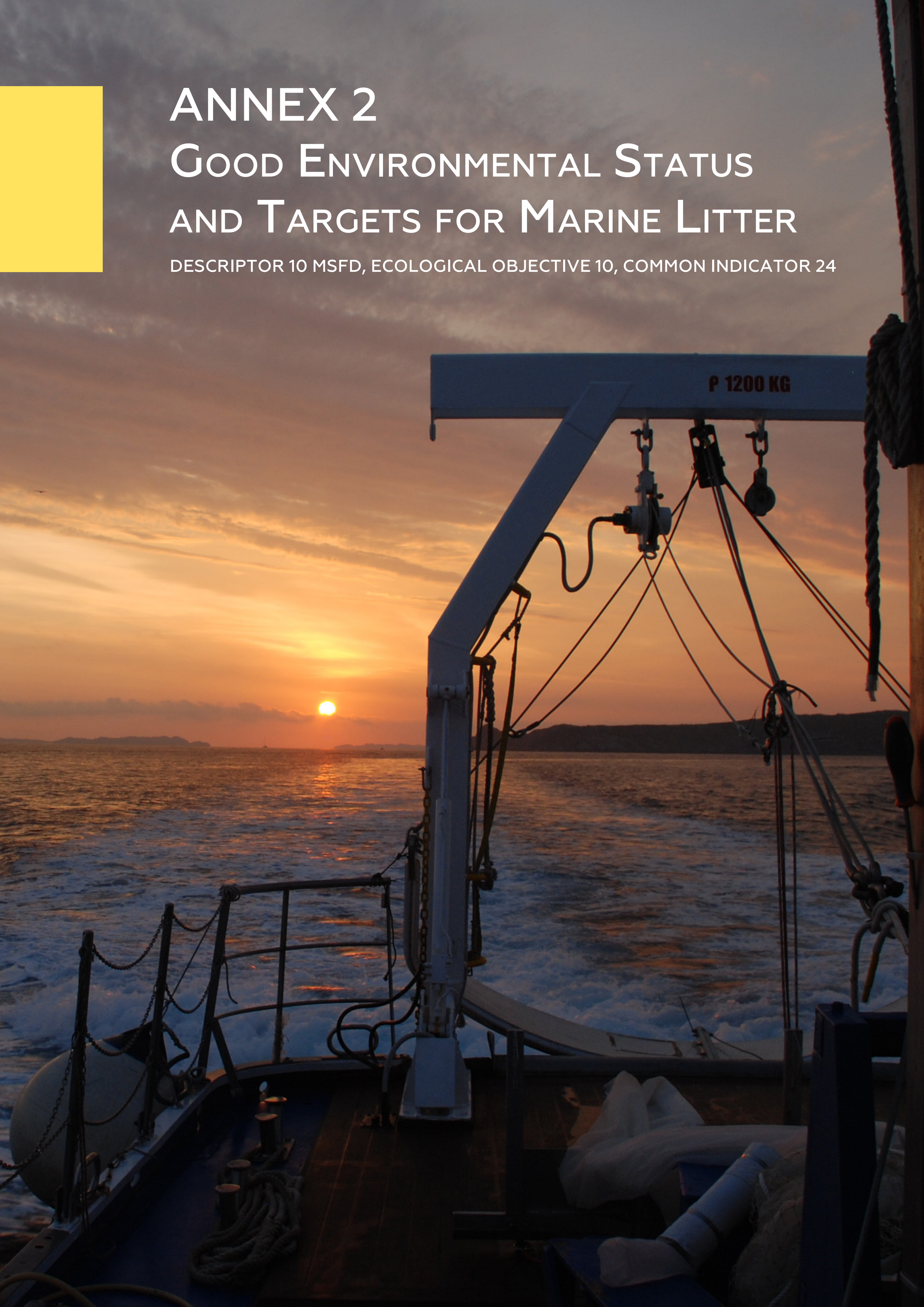
SWITZERLAND

ANNEX 2
Good Environmental Status and Targets for Marine Litter

DESCRIPTOR 10 MSFD, ECOLOGICAL OBJECTIVE 10, COMMON INDICATOR 24



Regional Seas Conventions and Action Plans (RSCAPs) play a critical role in encouraging cooperation and coordination between countries sharing a common resource. There are 18 Regional Seas Conventions and Action Plans, six of which are administered directly by UNEP: Mediterranean (Barcelona Convention), Wider Caribbean (WCR), East Asia Seas, Eastern Africa (Nairobi Convention), Northwest Pacific (NOWPAP), and West and Central Africa (WACAF). The RSCAPs are instrumental in supporting the implementation of the Global Programme of Action for the Protection of the Marine Environment from Land-based Activities (GPA) at regional levels. Several RSCAPs have developed or are in the process of developing regional action plans on marine litter as reported by UNEP, 2016:

- Strategic framework on the management of marine litter in the Mediterranean, adopted in 2012; Regional Plan on the Management of Marine litter in the Mediterranean, adopted in 2013, entered into force in June 2014; Barcelona Convention for the protection of the marine environment and the coastal region of the Mediterranean.
- Regional Action Plan on Marine Litter for the OSPAR Convention for the Protection of the Marine Environment of the Northeast Atlantic. Marine litter also forms a key part of OSPAR's regional action, monitoring and assessment programme. A specific Action Plan for marine litter was agreed in 2014. The initiative 'fishing for litter' forms part of OSPAR's Regional Action Plan, mostly as a process to highlight the issue to fisheries stakeholders, although in the process, litter is being removed from the seabed when it is brought up in nets. (www.ospar.org/html_documents/ospar/html/marine_litter_unep_ospar.pdf).
- Regional Action Plan on Marine Litter for the Helsinki Convention: Convention on the Protection of the Marine Environment of the Baltic Sea Area, adopted in March 2015. Several of the recommendations are directly or indirectly related to marine litter (www.helcom.fi).
- Regional Action Plan on Marine Litter for the Wider Caribbean Region (RAPMaLi), approved in 2008 and revised in 2014.
- Northwest Pacific Action Plan on Marine Litter (2008).
- South Pacific: CLEANER PACIFIC 2025: Pacific Regional Waste and Pollution Management Strategy 2016-2025. Marine debris has been identified as a priority area in this strategy.

The regional action plans have been developed taking account of the specific environmental, social and economic context of each region. They vary in the degree of detail and the extent to which actions are required or recommended by States.

THE EU'S MARINE STRATEGY FRAMEWORK DIRECTIVE (MSFD)

The European Union (EU) has adopted a number of measures on waste management, packaging and environmental protection that are relevant to the reduction of marine plastic debris (Werner et al., 2016). These apply to all 28 Member States of the EU. One of the most relevant pieces of European legislation is the Marine Strategy Framework Directive (Directive 2008/56/EC) in which marine litter is one of eleven 'descriptors' of the environmental state of European seas. The MSFD includes provision for setting targets and indicators for litter reduction. The principal aim of the MSFD is to achieve Good Environmental Status (GES) of EU marine waters by 2020. The Directive defines GES as: "The environmental status of marine waters where these provide ecologically diverse and dynamic oceans

and seas which are clean, healthy and productive".
Descriptor 10 of the MSFD calls on EU Member States (MS) to achieve Good Environmental Status (GES), defined as "marine litter does not cause harm to the coastal and marine environment."
Building upon this definition according to the MSFD GES Technical Group on Marine Litter (TG ML, 2013), GES is achieved, when:

1. Litter and its degradation products present in, and entering into EU waters do not cause harm to marine life and damage to marine habitats;
2. Litter and its degradation products present in, and entering into EU waters do not pose direct or indirect risks to human health;
3. Litter and its degradation products present in, and entering into EU waters do not lead to negative socioeconomic impacts.

Following the initial assessment by MS of their marine waters under the MSFD, the European Commission Decision of 17 May 2017 (EU 2017/848) established the criteria and methodological standards on good environmental status of marine waters, and specifications and standardised methods for monitoring and assessment, replacing the earlier MSFD standards, etc. set out in Decision 2010/477/EU.

INTEGRATED MONITORING AND ASSESSMENT PROGRAMME (IMAP) - ECOLOGICAL OBJECTIVE 10, COMMON INDICATOR 24

In February 2016, at the 19th COP Meeting, the Contracting Parties to the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention) adopted an Integrated Monitoring and Assessment Programme

SPECIFICATIONS AND STANDARDISED METHODS FOR MONITORING AND ASSESSMENT

1. For D10C1: litter shall be monitored on the coastline and may additionally be monitored in the surface layer of the water column and on the seabed. Information on the source and pathway of the litter shall be collected, where feasible;
2. For D10C2: micro-litter shall be monitored in the surface layer of the water column and in the seabed sediment and may additionally be monitored on the coastline. Micro-litter shall be monitored in a manner that can be related to point-sources for inputs (such as harbours, marinas, waste-water treatment plants, storm-water effluents), where feasible.
3. For D10C3 and D10C4: the monitoring may be based on incidental occurrences (e.g. strandings of dead animals, entangled animals in breeding colonies, affected individuals per survey).

Units of measurement for the criteria:

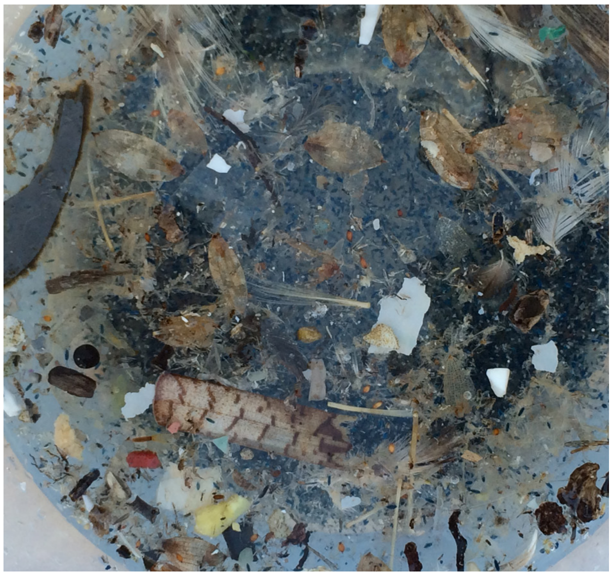
- D10C1: amount of litter per category in number of items:
- per 100 metres (m) on the coastline,
- per square kilometre (km2) for surface layer of the water column and for seabed,
- D10C2: amount of micro-litter per category in number of items and weight in grams (g):
- per square metre (m2) for surface layer of the water column, — per kilogram (dry weight) (kg) of sediment for the coastline and for seabed,
- D10C3: amount of litter/micro-litter in grams (g) and number of items per individual for each species in relation to size (weight or length, as appropriate) of the individual sampled,
- D10C4: number of individuals affected (lethal; sub-lethal) per species.

Table A2. Criteria and Methodological standards for Decriptor 10.

Criteria elements	Criteria	Methodological standards
Litter (excluding micro-litter), classified in the following categories[1]: artificial polymer materials, rubber, cloth/textile, paper/ cardboard, processed/worked wood, metal, glass/ceramics, chemicals, undefined, and food waste. Member States may define further sub-categories.	**D10C1 —** Primary: The composition, amount and spatial distribution of litter on the coastline, in the surface layer of the water column, and on the seabed, are at levels that do not cause harm to the coastal and marine environment. Member States shall establish threshold values for these levels through cooperation at Union level, taking into account regional or sub-regional specificities.	*Scale of assessment:* Subdivisions of the region or sub-region, divided where needed by national boundaries. *Use of criteria:* The extent to which good environmental status has been achieved shall be expressed for each criterion separately for each area assessed as follows: (a) the outcomes for each criterion (amount of litter or micro-litter per category) and its distribution per matrix used under D10C1 and D10C2 and whether the threshold values set have been achieved; (b) the outcomes for D10C3 (amount of litter and micro-litter per category per species) and whether the threshold values set have been achieved. The use of criteria D10C1, D10C2 and D10C3 in the overall assessment of good environmental status for Descriptor 10 shall be agreed at Union level. The outcomes of criterion D10C3 shall also contribute to assessments under Descriptor 1, where appropriate.
Micro-litter (particles < 5mm), classified in the categories 'artificial polymer materials' and 'other'.	**D10C2 — Primary**: The composition, amount and spatial distribution of micro-litter on the coastline, in the surface layer of the water column, and in seabed sediment, are at levels that do not cause harm to the coastal and marine environment. Member States shall establish threshold values for these levels through cooperation at Union level, taking into account regional or sub-regional specificities.	
Litter and micro-litter classified in the categories 'artificial polymer materials' and 'other', assessed in any species from the following groups: birds, mammals, reptiles, fish or invertebrates. Member States shall establish that list of species to be assessed through regional or sub-regional cooperation.	**D10C3 — Secondary**: The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned. Member States shall establish threshold values for these levels through regional or sub-regional cooperation.	
Species of birds, mammals, reptiles, fish or invertebrates which are at risk from litter. Member States shall establish that list of species to be assessed through regional or sub-regional cooperation.	**D10C4 — Secondary**: The number of individuals of each species which are adversely affected due to litter, such as by entanglement, other types of injury or mortality, or health effects. Member States shall establish threshold values for the adverse effects of litter, through regional or sub-regional cooperation.	*Scale of assessment:* As used for assessment of the species group under Descriptor 1. *Use of criteria:* The extent to which good environmental status has been achieved shall be expressed for each area assessed as follows: — for each species assessed under criterion D10C4, an estimate of the number of individuals in the assessment area that have been adversely affected. The use of criterion D10C4 in the overall assessment of good environmental status for Descriptor 10 shall be agreed at Union level. The outcomes of this criterion shall also contribute to assessments under Descriptor 1, where appropriate.

[1]**These are the 'Level 1 —** Material' categories from the Master List of categories of litter items from the Joint Research Centre 'Guidance on Monitoring of marine litter in European seas' (2013, ISBN 978-92-79-32709-4). The Master List specifies what is covered under each category, for instance 'Chemicals' refers to paraffin, wax, oil and tar. http://publications.jrc.ec.europa.eu/repository/bitstream/JRC83985/lb-na-26113-en-n.pdf

(IMAP) and Related Assessment Criteria that set out the principles and targets of the programme, and include a specific list of common indicators for good environmental status.
The IMAP lays down the principles for integrated monitoring, which will, for the first time, monitor biodiversity and non-indigenous species, pollution and marine litter, coast, and hydrography in an integrated manner. The IMAP aims to facilitate the implementation of Article 12 of the Barcelona Convention and several monitoring related provisions under different protocols, with the main objective to assess GES.



The series of Marine Litter Baseline Values and Environmental Targets adopted by the 19th Meeting of Contracting Parties (Decision IG.22/10) are shown in the "Baseline Values and Environmental Targets" table.

Baseline values and Environmental targets	
Baseline Values for Affected Sea Turtles (%):	• Minimum value: 14% • Maximum value: 92.5% • Mean value: 45.9% • Proposed Baseline: 40-60%
Environmental Targets for Affected Sea Turtles (%):	• Types of Target: % of decrease in the rate of affected animals • Minimum: - • Maximum: - • Reduction Targets: Statistically Significant
Baseline Values for Ingested Marine Litter (gr):	• Minimum value: 0 gr • Maximum value: 14 gr • Mean value: 1.37 gr • Proposed Baseline: 1-3 gr
Environmental Targets for Ingested Marine Litter (gr):	• Types of Target: % decrease in quantity of ingested weight (gr) • Minimum: - • Maximum: - • Reduction Targets: Statistically Significant

ANNEX 3
Mediterranean species studied for marine litter ingestion

Table of Mediterranean species studied for marine litter ingestion with details on area of study, habitat and references.

Taxa	*Species*	*MED sub-regions*	*Habitat*	*References*
Mollusca				
Mytilida	*Mytilus galloprovincialis* Lamarck, 1819	WMS/AS/ISCMS	benthic	Digka et al., 2016; Vandermeersch et al., 2015
Arthropoda				
Amphipoda	*Gammarella fucicola* (Leach, 1814)	WMS	benthic	Remy et al., 2015
	Gammarus aequicauda (Martynov, 1931)	WMS	benthic	Remy et al., 2015
	Melita hergensis Reid, 1939	WMS	benthic	Remy et al., 2015
	Nototropis guttatus Costa, 1853	WMS	benthic	Remy et al., 2015
Decapoda	*Nephrops norvegicus* (Linnaeus, 1758)	WMS/AS/ALS	benthic	Cristo and Cartes, 1998
	Palaemon xiphias Risso, 1816	WMS	benthic	Remy et al., 2015
	Liocarcinus navigator (Herbst, 1794)	WMS	benthic	Remy et al., 2015
	Athanas nitescens (Leach, 1813 [in Leach, 1813-1814])	WMS	benthic	Remy et al., 2015
	Galathea intermedia Lilljeborg, 1851	WMS	benthic	Remy et al., 2015
Euphausiacea	*Euphausia krohnii* (Brandt, 1851)	WMS	pelagic	Fossi et al., 2014
Leptostraca	*Nebalia strausi* Risso, 1826	WMS	benthic	Remy et al., 2015
Anellida				
Polychaeta	*Saccocirrus papillocercus* Bobretzky, 1872	WMS	benthic	Gusmão et al., 2016
Echinodermata				
Aspidochirotida	Holothuria (Panningothuria) forskali Delle Chiaje, 1823	WMS	benthic	Alomar et al., 2016

	Taxa	Species	MED sub-regions	Habitat	References
VERTEBRATES	**Teleosts**				
	Anguilliformes	*Conger conger* (Linnaeus, 1758)	ISCMS	demersal	Anastasopoulou et al., 2013
		Nettastoma melanurum Rafinesque, 1810	ISCMS/WMS	demersal	Anastasopoulou et al., 2013; Cartes et al., 2016
	Aulopiformes	*Sudis hyalina* Rafinesque, 1810	ISCMS	mesopelagic	Anastasopoulou et al., 2013
		Saurida undosquamis (Richardson, 1848)	ALS	demersal	Güven et al., 2017
	Clupeiformes	*Engraulis encrasicolus* (Linnaeus, 1758)	WMS	pelagic	Collard et al., 2015
		Sardina pilchardus (Walbaum, 1792)	AS/ALS/ISCMS	pelagic	Avio et al., 2015; Digka et al., 2016; Güven et al., 2017;Vlachogianni et al., 2017
	Gadiformes	*Merluccius merluccius* (Linnaeus, 1758)	AS/ISCMS	benthopelagic	Anastasopoulou et al., 2013; Avio et al., 2015
		Micromesistius poutassou (Risso, 1827)	ISCMS	benthopelagic	Anastasopoulou et al., 2013
		Molva macrophthalma (Rafinesque, 1810)	ISCMS	demersal	Anastasopoulou et al., 2013
		Mora moro (Risso, 1810)	WMS/ISCMS	demersal	Anastasopoulou et al., 2013; Cartes et al., 2016
		Phycis blennoides (Brünnich, 1768)	WMS/ISCMS	demersal	Anastasopoulou et al., 2013; Cartes et al., 2016
		Trachyrincus scabrus (Rafinesque, 1810)	WMS	demersal	Cartes et al., 2016
	Myctophiformes	*Diaphus metopoclampus* (Cocco, 1829)	ISCMS	benthopelagic	Romeo et al., 2016
		Electrona risso (Cocco, 1829)	ISCMS	mesopelagic	Romeo et al., 2016
		Hygophum benoiti (Cocco, 1838)	ISCMS	mesopelagic	Romeo et al., 2016
		Myctophum punctatum Rafinesque, 1810	WMS/ISCMS	mesopelagic	Collignon et al., 2012; Romeo et al., 2016
	Ophidiiformes	*Cataetyx laticeps* Koefoed, 1927	WMS	demersal	Cartes et al., 2016
	Osmeriformes	*Alepocephalus rostratus* Risso, 1820	WMS	demersal	Cartes et al., 2016
	Perciformes	Argyrosomus regius (Asso, 1801)	ALS	benthopelagic	Güven et al., 2017
		Boops boops (Linnaeus, 1758)	WMS	benthopelagic	Nadal et al., 2016
		Brama brama (Bonnaterre, 1788)	ISCMS	pelagic	Anastasopoulou et al., 2013
		Caranx crysos (Mitchill, 1815)	ALS	pelagic	Güven et al., 2017
		Coryphaena hippurus Linnaeus, 1758	WMS	pelagic	Deudero, 1998; Massutí et al., 1998; Deudero and Alomar, 2015
		Dentex dentex (Linnaeus, 1758)	ALS	benthopelagic	Güven et al., 2017

	Taxa	Species	MED sub-regions	Habitat	References
VERTEBRATES	**Teleosts**				
	Perciformes	*Dentex gibbosus* (Rafinesque, 1810)	ALS	benthopelagic	Güven et al., 2017
		Diplodus annularis (Linnaeus, 1758)	ALS	demersal	Güven et al., 2017
		Epigonus telescopus (Risso, 1810)	ISCMS	benthopelagic	Anastasopoulou et al., 2013
		Lepidopus caudatus (Euphrasen, 1788)	ISCMS	pelagic	Anastasopoulou et al., 2013
		Lithognathus mormyrus (Linnaeus, 1758)	ALS	demersal	Güven et al., 2017
		Liza aurata (Risso, 1810)	ALS	benthopelagic	Güven et al., 2017
		Mullus barbatus Linnaeus, 1758	WMS/ISCMS/AS/ALS	demersal	Avio et al., 2015; Bellas et al., 2016; Digka et al., 2016; Güven et al., 2017; Vlachogianni et al., 2017
		Mullus surmuletus Linnaeus, 1758	ALS	demersal	Güven et al., 2017
		Naucrates ductor (Linnaeus, 1758)	WMS	pelagic	Deudero, 1998; ; Deudero and Alomar, 2015
		Nemipterus randalli Russell, 1986	ALS	demersal	Güven et al., 2017
		Pagellus acarne (Risso, 1827)	ALS	benthopelagic	Güven et al., 2017
		Pagellus bogaraveo (Brünnich, 1768)	ISCMS	benthopelagic	Anastasopoulou et al., 2013
		Pagellus erythrinus (Linnaeus, 1758)	ISCMS/AS/ALS	demersal	Digka et al., 2016; Güven et al., 2017; Vlachogianni et al., 2017
		Pagrus pagrus (Linnaeus, 1758)	ALS	demersal	Güven et al., 2017
		Pelates quadrilineatus (Bloch, 1790)	ALS	demersal	Güven et al., 2017
		Polyprion americanus (Bloch & Schneider, 1801)	WMS/ISCMS	pelagic	Deudero, 1998; Anastasopoulou et al., 2013; Deudero and Alomar, 2015
		Pomadasys incisus (Bowdich, 1825)	ALS	demersal	Güven et al., 2017
		Schedophilus ovalis (Cuvier, 1833)	WMS/ISCMS	pelagic	Deudero, 1998; Anastasopoulou et al., 2013; Deudero and Alomar, 2015
		Sciaena umbra Linnaeus, 1758	ALS	demersal	Güven et al., 2017
		Scomber japonicus Houttuyn, 1782	AL/ALS	pelagic	Güven et al., 2017; Vlachogianni et al., 2017

	Taxa	***Species***	***MED sub-regions***	***Habitat***	***References***
VERTEBRATES	**Teleosts**				
	Perciformes	*Seriola dumerili* (Risso, 1810)	WMS	pelagic	Deudero, 1998; Deudero and Alomar, 2015
		Serranus cabrilla (Linnaeus, 1758)	ALS	demersal	Güven et al., 2017
		Siganus luridus (Rüppell, 1829)	ALS	demersal	Güven et al., 2017
		Sparus aurata Linnaeus, 1758	ALS	demersal	Güven et al., 2017
		Thunnus alalunga (Bonnaterre, 1788)	WMS/ISCMS	pelagic	Romeo et al., 2015
		Thunnus thynnus (Linnaeus, 1758)	WMS/ISCMS/ALS	pelagic	de la Serna et al., 2012; Karakulak et al., 2009; Romeo et al., 2015
		Trachinotus ovatus (Linnaeus, 1758)	ISCMS	pelagic	Battaglia et al., 2016
		Trachurus mediterraneus (Steindachner, 1868)	WMS/ALS	pelagic	Deudero, 1998; Deudero and Alomar, 2015; Güven et al., 2017
		Trachurus picturatus (Bowdich, 1825)	WMS	pelagic	Deudero, 1998; ; Deudero and Alomar, 2015
		Trachurus trachurus (Linnaeus, 1758)	AS	pelagic	Vlachogianni et al., 2017
		Umbrina cirrosa (Linnaeus, 1758)	ALS	demersal	Güven et al., 2017
		Upeneus moluccensis (Bleeker, 1855)	ALS	demersal	Güven et al., 2017
		Upeneus pori Ben-Tuvia & Golani, 1989	ALS	demersal	Güven et al., 2017
		Xiphias gladius Linnaeus, 1758	WMS/ISCMS	pelagic	Anastasopoulou et al., 2013; Romeo et al., 2015
	Pleuronectiformes	*Citharus linguatula* (Linnaeus, 1758)	ISCMS	benthic	Vlachogianni et al., 2017
		Solea solea (Linnaeus, 1758)	ISCMS/AS	benthic	Vlachogianni et al., 2017
	Scorpaeniformes	*Chelidonichthys lucerna* (Linnaeus, 1758)	AS	benthic	Avio et al., 2015
		Helicolenus dactylopterus (Delaroche, 1809)	ISCMS/ALS	benthic	Madurell, 2003; Anastasopoulou et al., 2013; Deudero and Alomar, 2015
		Scorpaena elongata Cadenat, 1943	ISCMS	benthic	Anastasopoulou et al., 2013
		Trigla lucerna Linnaeus, 1758	ALS	benthic	Güven et al., 2017
	Tetraodontiformes	*Balistes capriscus* Gmelin, 1789	WMS	benthopelagic	Deudero, 1998; Deudero and Alomar, 2015
		Lagocephalus spadiceus (Richardson, 1845)	ALS	benthopelagic	Güven et al., 2017

VERTEBRATES

Taxa	*Species*	*MED sub-regions*	*Habitat*	*References*
Elasmobranchs				
Carcharhiniformes	*Galeus melastomus* Rafinesque, 1810	WMS/ISCMS/ALS	demersal	Carrasón et al., 1992; Madurell, 2003; Anastasopoulou et al., 2013; Deudero and Alomar, 2015; Cartes et al., 2016; Alomar and Deudero, 2017
	Scyliorhinus canicula (Linnaeus, 1758)	ISCMS	demersal	Anastasopoulou et al., 2013
Lamniformes	*Cetorhinus maximus* (Gunnerus, 1765)	WMS	pelagic	Fossi et al., 2014
Myliobatiformes	*Pteroplatytrygon violacea* (Bonaparte, 1832)	ISCMS	pelagic	Anastasopoulou et al., 2013
Squaliformes	*Centrophorus granulosus* (Bloch & Schneider, 1801)	ISCMS	demersal	Anastasopoulou et al., 2013
	Centroscymnus coelolepis Barbosa du Bocage & de Brito Capello, 1864	WMS	demersal	Carrassón et al.1992; Cartes et al., 2016
	Etmopterus spinax (Linnaeus, 1758)	WMS/ISCMS/ALS	demersal	Madurell, 2003; Anastasopoulou et al., 2013; (Avio et al., 2015b); Deudero and Alomar, 2015; Cartes et al., 2016
	Squalus acanthias Linnaeus, 1758	ISCMS/AS	demersal	Anastasopoulou et al., 2013; (Avio et al., 2015b)
	Squalus blainville (Risso, 1827)	ISCMS	demersal	Anastasopoulou et al., 2013
Rajiformes	*Raja clavata* Linnaeus, 1758	ISCMS	demersal	Anastasopoulou et al., 2013
	Raja oxyrinchus Linnaeus, 1758	ISCMS	demersal	Anastasopoulou et al., 2013

VERTEBRATES

Taxa	*Species*	*MED sub-regions*	*Habitat*	*References*
Sea Turtles				
Testudines	*Caretta caretta* (Linnaeus, 1758)	WMS/ISCMS/AS/ALS	benthopelagic	Camedda et al., 2014; Campani et al., 2013; Casale et al., 2016, 2008; Gramentz, 1988; Kaska et al., 2004; Lazar and Gračan, 2011; Revelles et al., 2007; Russo et al., 2003; Tomás et al., 2002
	Chelonia mydas (Linnaeus, 1758)	WMS	benthopelagic	Russo et al., 2003
	Dermochelys coriacea (Vandelli, 1761)	WMS	benthopelagic	Poppi et al., 2012; Russo et al., 2003
Seabirds				
Procellariiformes	*Calonectris diomedea* (Scopoli, 1769)	WMS	neritc	Codina-García et al., 2013
	Puffinus yelkouan (Acerbi, 1827)	WMS	neritc	Codina-García et al., 2013
	Puffinus mauretanicus Lowe, 1921	WMS	neritc	Codina-García et al., 2013
Pelecaniformes	*Morus bassanus* (Linnaeus, 1758)	WMS	neritc	Codina-García et al., 2013
Charadriiformes	*Larus audouinii* Payraudeau, 1826	WMS	neritc	Codina-García et al., 2013
	Larus michahellis J.F. Naumann, 1840	WMS	neritc	Codina-García et al., 2013
	Larus melanocephalus Temminck, 1820	WMS	neritc	Codina-García et al., 2013
	Rissa tridactyla (Linnaeus, 1758)	WMS	neritc	Codina-García et al., 2013
	Stercorarius skua (Brünnich, 1764)	WMS	neritc	Codina-García et al., 2013
Marine Mammals				
Cetartiodactyla	*Physeter macrocephalus* Linnaeus, 1758	WMS/AS/ALS	pelagic	de Stephanis et al., 2013; Katsanevakis, 2008; Mazzariol et al., 2011; Roberts, 2003; Vitale et al., 1992
	Balaenoptera physalus Linnaeus, 1758	WMS	pelagic	Baini et al., 2017; Fossi et al., 2014
	Tursiops truncatus (Montagu, 1821)	WMS/AS/ALS	pelagic	Baini et al., 2017; Baulch and Perry, 2014; Levy et al., 2009
	Grampus griseus (G. Cuvier, 1812)	WMS	pelagic	Baini et al., 2017
	Stenella coerulealba (Meyen, 1833)	WMS/AS	pelagic	Baini et al., 2017; Baulch and Perry, 2014

LIST OF ABBREVIATIONS

ALS	Aegean-Levantine Sea
AS	Adriatic Sea
COP	Conference of the Parties
CORMON	Correspondence Group on Monitoring
D10.C3	Criteria 3 of the MSFD's Descriptor 10, on amount of litter ingested by marine animals
D10.C4	Criteria 4 of the MSFD's Descriptor 10, on number of animals adversely affected
EcoQO	(OSPAR) Ecological Quality Objective
EO10	(IMAP) Ecological Objective 10 on marine litter
EU	European Union
FAO	(UN) Food and Agriculture Organisation
GES	Good Environmental Status
GI	Gastrointestinal tract
GSA	(FAO) Geographical sub-area
ISCMS	Ionian Sea and the Central Mediterranean Sea
KOH	Potassium Hydroxide
MEDITS	International bottom trawl survey in the Mediterranean
MEDPOL	Programme for the Assessment and Control of Marine Pollution in the Mediterranean
ML	Marine litter
MS	(EU) Member State
MSFD	(EU) Marine Strategy Framework Directive
NGO	Non-governmental organisation
NOAA	(USA) National Oceanic and Atmospheric Administration
OSPAR	(Convention) for the Protection of the Marine Environment of the North-East Atlantic
PBDEs	Polybrominated diphenyl ethers
PBTs	Persistent, bioaccumulating and toxic chemicals
POPs	Persistent organic pollutants
RSCAPs	Regional Seas Conventions and Action Plans
SDSN	Sustainable Development Solutions Network
TG ML	(MSFD) GES Technical Group on Marine Litter
UfM	Union for the Mediterranean
UNEP	United Nations Environment Programme
UNEP/MAP	UNEP Mediterranean Action Plan
WMS	Western Mediterranean Sea

REFERENCES

Alomar, C., Deudero, S., 2017. Evidence of microplastic ingestion in the shark Galeus melastomus Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. Environ. Pollut. doi:10.1016/j.envpol.2017.01.015

Alomar, C., Estarellas, F., Deudero, S., 2016. Microplastics in the Mediterranean Sea: Deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar. Environ. Res. 115, 1–10. doi:10.1016/j.marenvres.2016.01.005

Anastasopoulou, A., Mytilineou, C., Smith, C.J., Papadopoulou, K.N., 2013. Plastic debris ingested by deep-water fish of the Ionian Sea (Eastern Mediterranean). Deep Sea Res. Part Oceanogr. Res. Pap. 74, 11–13. doi:10.1016/j.dsr.2012.12.008

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015a. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222. doi:10.1016/j.envpol.2014.12.021

Avio, C.G., Gorbi, S., Regoli, F., 2015b. 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

Baini, M., Martellini, T., Cincinelli, A., Campani, T., Minutoli, R., Panti, C., Finoia, M.G., Fossi, M.C., 2017. First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber. Anal Methods 9, 1512–1520. doi:10.1039/C6AY02674E

Ballesteros-Gòmez, A., Rubio, S., Pérez-Bendito, D., 2009. Analytical methods for the determination of bisphenol A in food. J. Chromatogr. A 1216, 449–469. doi:10.1016/j.chroma.2008.06.037

Battaglia, P., Pedà, C., Musolino, S., Esposito, V., Andaloro, F., Romeo, T., 2016. Diet and first documented data on plastic ingestion of Trachinotus ovatus L. 1758 (Pisces: Carangidae) from the Strait of Messina (central Mediterranean Sea). Ital. J. Zool. 83, 121–129. doi:10.1080/11250003.2015.1114157

Baulch, S., Perry, C., 2014. Evaluating the impacts of marine debris on cetaceans. Mar. Pollut. Bull. 80, 210–221. doi:10.1016/j.marpolbul.2013.12.050

Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Mar. Pollut. Bull. 109, 55–60. doi:10.1016/j.marpolbul.2016.06.026

Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar. Pollut. Bull. 60, 2275–2278. doi:10.1016/j.marpolbul.2010.08.007

Borghesi, F., Abdennadher, A., Baccetti, N., Baini, M., Bianchi, N., Caliani, I., Marsili, L., Thévenet, M., 2016. Developing sampling protocols for biomonitoring contaminants in Mediterranean seabirds. doi:10.13140/RG.2.1.1654.6807

Caliani, I., Campani, T., Giannetti, M., Marsili, L., Casini, S., Fossi, M.C., 2014. First application of comet assay in blood cells of Mediterranean loggerhead sea turtle (Caretta caretta). Mar. Environ. Res. 96, 68–72. doi:10.1016/j.marenvres.2013.09.008

Caliani, I., Porcelloni, S., Mori, G., Frenzilli, G., Ferraro, M., Marsili, L., Casini, S., Fossi, M.C., 2009. Genotoxic effects of produced waters in mosquito fish (Gambusia affinis). Ecotoxicology 18, 75–80. doi:10.1007/s10646-008-0259-0

Camedda, A., Marra, 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 Mediterranean Sea). Mar. Environ. Res. 100, 25–32. doi:10.1016/j.marenvres.2013.12.004

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, 225–230. doi:10.1016/j.marpolbul.2013.06.053

Cardona, L., Álvarez de Quevedo, I., Borrell, A., Aguilar, A., 2012. Massive consumption of gelatinous plankton by Mediterranean apex predators. PLoS ONE 7, e31329. doi:10.1371/journal.pone.0031329

Carrasón, M., Stefanescu, C., Cartes, J.E., 1992. Diets and bathymetric distributions of two bathyal sharks of the Catalan deep sea (western Mediterranean). Mar. Ecol. Prog. Ser. 82, 21–30.

Cartes, J.E., Soler-Membrives, A., Stefanescu, C., Lombarte, A., Carrassón, M., 2016. Contributions of allochthonous inputs of food to the diets of benthopelagic fish over the northwest Mediterranean slope (to 2300m). Deep Sea Res. Part Oceanogr. Res. Pap. 109, 123–136. doi:10.1016/j.dsr.2015.11.001

Casale, P., Abbate, G., Freggi, D., Conte, N., Oliverio, M., Argano, R., 2008. Foraging ecology of loggerhead sea turtles Caretta caretta in the central Mediterranean Sea: evidence for a relaxed life history model. Mar. Ecol. Prog. Ser. 372, 265–276. doi:10.3354/meps07702

Casale, P., Freggi, D., Paduano, V., Oliverio, M., 2016. Biases and best approaches for assessing debris ingestion in sea turtles, with a case study in the Mediterranean. Mar. Pollut. Bull. 110, 238–249. doi:10.1016/j.marpolbul.2016.06.057

Casini, S., Fossi, M.C., Leonzio, C., Renzoni, A., 2003. Review: porphyrins as biomarkers for hazard assessment of bird populations: destructive and non-destructive use. Ecotoxicol. Lond. Engl. 12, 297–305.

Casini, S., Marsili, L., Fossi, M.C., Mori, G., Bucalossi, D., Porcelloni, S., Caliani, I., Stefanini, G., Ferraro, M., di Catenaja, C.A., 2006. Use of biomarkers to investigate toxicological effects of produced water treated with conventional and innovative methods. Mar. Environ. Res. 62, S347–S351. doi:10.1016/j.marenvres.2006.04.060

Claro, F., 2016. Développement d'une stratégie en vue du renforcement d' un réseau de surveillance mesurant les déchets ingérés par les tortues marines., Rapport MNHN IFREMER. Bastia, France.

Claro, F., Darmon, G., Miaud, C., Galgani, F., 2014. « Project of EcoQO/GES for marine litter ingested by Sea Turtles (MSFD D10.2.1.)", Minutes of the european workshop, October 13th, 2014- Marseille (Mediterranean Instiute of Oceanology),16 pp.

Cobellis, L., Colacurci, N., Trabucco, E., Carpentiero, C., Grumetto, L., 2009. Measurement of bisphenol A and bisphenol B levels in human blood sera from healthy and endometriotic women. Biomed. Chromatogr. 23, 1186–1190. doi:10.1002/bmc.1241

Codina-García, M., Militão, T., Moreno, J., González-Solís, J., 2013. Plastic debris in Mediterranean seabirds. Mar. Pollut. Bull. 77, 220–226. doi:10.1016/j.marpolbul.2013.10.002

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4. doi:10.1038/srep04528

Collard, F., Gilbert, B., Eppe, G., Parmentier, E., Das, K., 2015. Detection of anthropogenic particles in fish stomachs: an isolation method adapted to identification by Raman spectroscopy. Arch. Environ. Contam. Toxicol. 69, 331–339. doi:10.1007/s00244-015-0221-0

Collignon, A., Hecq, J.-H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar. Pollut. Bull. 64, 861–864. doi:10.1016/j.marpolbul.2012.01.011

Correa, L., Castellini, J.M., Wells, R.S., O'Hara, T., 2013. Distribution of mercury and selenium in blood compartments of bottlenose dolphins (Tursiops truncatus) from Sarasota Bay, Florida: Hg and Se distribution in blood of bottlenose dolphins. Environ. Toxicol. Chem. n/a-n/a. doi:10.1002/etc.2327

Cristo, M., Cartes, J.E., 1998. A comparative study of the feeding ecology of Nephrops norvegicus (L.), (Decapoda: Nephropidae) in the bathyal Mediterranean and the adjacent Atlantic. Sci. Mar. 62, 81–90.

Darmon, G., Miaud, C., 2016. Elaboration d'un indicateur de déchets ingérés par les tortues marines (D10-2-1) et d'un bon état écologique (BEE) pour la Directive Cadre Stratégie pour le Milieu Marin (DCSMM), et d'un objectif de qualité écologique (EcoQO) pour la convention internationale pour la protection du milieu marin de l'atlantique nord-est (OSPAR). Rapport final de contrat d'étude CNRS-IFREMER. Montpellier, France.

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 Res. Part II Top. Stud. Oceanogr. 141, 319–328. doi:10.1016/j.dsr2.2016.07.005

de la Serna, J.M., Godoy, M.D., Olaso, I., Zabala, J., Majuelos, E., Báez, J.C., 2012. Preliminary study on the feeding of bluefin tuna (Thunnus thynnus) in the Mediterranean and the Strait of Gibraltar area. Collect. Vol. Sci. Pap. ICCAT 68, 115–132.

de Stephanis, R., Giménez, J., Carpinelli, E., Gutierrez-Exposito, C., Cañadas, A., 2013. As main meal for sperm whales: Plastics debris. Mar. Pollut. Bull. 69, 206–214. doi:10.1016/j.marpolbul.2013.01.033

Deudero, S., 1998. Relaciones tróficas en las comunidades ícticas asociadas a dispositivos agregadores de peces. (PhD thesis). University of the Balearic Islands.

Deudero, S., Alomar, C., 2015. Mediterranean marine biodiversity under threat: Reviewing influence of marine litter on species. Mar. Pollut. Bull. 98, 58–68. doi:10.1016/j.marpolbul.2015.07.012

Digka, N., Tsangaris, C., Torre, M., Anastasopoulou, A., Zeri, C., 2016. Microplastic ingestion in marine biota: a case study in the Nothern Ionian Sea. 51st Eur. Mar. Biol. Symp. EMBS.

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95. doi:10.1016/0006-2952(61)90145-9

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., 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

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). Environ. Pollut. 177, 1–3. doi:10.1016/j.envpol.2013.01.046

Fossi, M.., Marsili, L., Neri, G., Casini, S., Bearzi, G., Politi, E., Zanardelli, M., Panigada, S., 2000. Skin biopsy of Mediterranean cetaceans for the investigation of interspecies susceptibility to xenobiotic contaminants. Mar. Environ. Res. 50, 517–521. doi:10.1016/S0141-1136(00)00127-6

Fossi, M.C., Casini, S., Marsili, L., Ancora, S., Mori, G., Neri, G., Romeo, T., Ausili, A., 2004. Evaluation of ecotoxicological effects of endocrine disrupters during a four-year survey of the Mediterranean population of swordfish (Xiphias gladius). Mar. Environ. Res. 58, 425–429. doi:10.1016/j.marenvres.2004.03.026

Fossi, M.C., Coppola, D., Baini, M., Giannetti, M., Guerranti, C., Marsili, L., Panti, C., de Sabata, E., Clò, S., 2014. Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: The case studies of the Mediterranean basking shark (Cetorhinus maximus) and fin whale (Balaenoptera physalus). Mar. Environ. Res. 100, 17–24. doi:10.1016/j.marenvres.2014.02.002

Fossi, M.C., Panti, C., Marsili, L., Maltese, S., Spinsanti, G., Casini, S., Caliani, I., Gaspari, S., Muñoz-Arnanz, J., Jimenez, B., Finoia, M.G., 2013. The Pelagos Sanctuary for Mediterranean marine mammals: Marine Protected Area (MPA) or marine polluted area? The case study of the striped dolphin (Stenella coeruleoalba). Mar. Pollut. Bull. 70, 64–72. doi:10.1016/j.marpolbul.2013.02.013

Fossi, M.C., Romeo, T., Baini, M., Panti, C., Marsili, L., Campani, T., Canese, S., Galgani, F., Druon, J.-N., Airoldi, S., Taddei, S., Fattorini, M., Brandini, C., Lapucci, C., 2017. Plastic Debris Occurrence, Convergence Areas and Fin Whales Feeding Ground in the Mediterranean Marine Protected Area Pelagos Sanctuary: A Modeling Approach. Front. Mar. Sci. 4. doi:10.3389/fmars.2017.00167

Fossi, M.C, Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M. Biondicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Env. Poll., in press

Frenzilli, G., Nigro, M., Lyons, B., 2009. The Comet assay for the evaluation of genotoxic impact in aquatic environments. Mutat. Res. Mutat. Res. 681, 80–92. doi:10.1016/j.mrrev.2008.03.001

Galgani, F., 2017. Specially Protected Areas Protocol Regional Activity Centre (Barcelona Convention), 2017, Defining the most representative species for IMPA common indicator 24. SPA/RAC, Tunis.

Gramentz, D., 1988. Involvement of loggerhead turtle with the plastic, metal, and hydrocarbon pollution in the central Mediterranean. Mar. Pollut. Bull. 19, 11–13. doi:10.1016/0025-326X(88)90746-1

Gusmão, F., Domenico, M.D., Amaral, A.C.Z., Martínez, A., Gonzalez, B.C., Worsaae, K., Ivar do Sul, J.A., Cunha Lana, P. da, 2016. In situ ingestion of microfibres by meiofauna from sandy beaches. Environ. Pollut. 216, 584–590. doi:10.1016/j.envpol.2016.06.015

Güven, O., Gökdağ, K., Jovanović, B., Kıdeyş, A.E., 2017. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. Environ. Pollut. 223, 286–294. doi:10.1016/j.envpol.2017.01.025

Hannam, M.L., Bamber, S.D., John Moody, A., Galloway, T.S., Jones, M.B., 2010. Immunotoxicity and oxidative stress in the Arctic scallop Chlamys islandica: Effects of acute oil exposure. Ecotoxicol. Environ. Saf. 73, 1440–1448. doi:10.1016/j.ecoenv.2010.06.012

Hardesty, B.D., Good, T.P., Wilcox, C., 2015. Novel methods, new results and science-based solutions to tackle marine debris impacts on wildlife. Ocean Coast. Manag. 115, 4–9. doi:10.1016/j.ocecoaman.2015.04.004

Herbst, L.H., Siconolfi-Baez, L., Torelli, J.H., Klein, P.A., Kerben, M.J., Schumacher, I.M., 2003. Induction of vitellogenesis by estradiol-17β and development of enzyme-linked immunosorbant assays to quantify plasma vitellogenin levels in green turtles (Chelonia mydas). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 135, 551–563. doi:10.1016/S1096-4959(03)00141-6

Karakulak, F.S., Salman, A., Oray, I.K., 2009. Diet composition of bluefin tuna (Thunnus thynnus L. 1758) in the Eastern Mediterranean Sea, Turkey. J. Appl. Ichthyol. 25, 757–761. doi:10.1111/j.1439-0426.2009.01298.x

Kaska, Y., Celik, A., Bag, H., Aureggi, M., Ozel, K., Elci, A., Kaska, A., Elca, L., 2004. Heavy metal monitoring in stranded sea turtles along the Mediterranean coast of Turkey. Fresenius Environ. Bull. 13, 769–776.

Katsanevakis, S., 2008. Marine debris, a growing problem: sources, distribution, composition and impact., in: Marine Pollution: New Research. Nova Science Publishers, pp. 53–100.

Kwok, A., Lyons, B.P., Hodges, N.J., Bean, T.P., 2013. Cryopreservation and storage of mussel (Mytilus spp.) haemocytes for latent analysis by the Comet assay. Mutat. Res. Toxicol. Environ. Mutagen. 750, 86–91. doi:10.1016/j.mrgentox.2012.09.010

Lazar, B., Gračan, R., 2011. Ingestion of marine debris by loggerhead sea turtles, Caretta caretta, in the Adriatic Sea. Mar. Pollut. Bull. 62, 43–47. doi:10.1016/j.marpolbul.2010.09.013

Levy, A.M., Brenner, O., Scheinin, A., Morick, D., Ratner, E., Goffman, O., Kerem, D., 2009. Laryngeal snaring by ingested fishing net in a common bottlenose dolphin (Tursiops truncatus) off the

Israeli shoreline. J. Wildl. Dis. 45, 834–838. doi:10.7589/0090-3558-45.3.834

Lusher, A.L., Hollman, P.C.H., Mendoza-Hill, J.J., 2017a. Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety., FAO Fisheries and Aquaculture Technical Paper No. 615. FAO, Rome, Italy.

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017b. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal Methods 9, 1346–1360. doi:10.1039/C6AY02415G

Marsili, L., Caruso, A., Cristina Fossi, M., Zanardelli, M., Politi, E., Focardi, S., 2001. Polycyclic aromatic hydrocarbons (PAHs) in subcutaneous biopsies of Mediterranean cetaceans. Chemosphere 44, 147–154. doi:10.1016/S0045-6535(00)00206-X

Marsili, L., Focardi, S., 1997. Chlorinated hydrocarbon (HCB, DDTS and PCBS levels in cetaceans stranded along the Italian coasts: an overview. Environ. Monit. Assess. 45, 129–180. doi:10.1023/A:1005786627533

Massutí, E., Deudero, S., Sánchez, P., Morales-Nin, B., 1998. Diet and feeding of dolphin (Coryphaena hippurus) in western Mediterranean waters. Bull. Mar. Sci. 62, 329–341.

Mathieu-Denoncourt, J., Wallace, S.J., de Solla, S.R., Langlois, V.S., 2015. Plasticizer endocrine disruption: Highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species. Gen. Comp. Endocrinol. 219, 74–88. doi:10.1016/j.ygcen.2014.11.003

Mazzariol, S., Guardo, G.D., Petrella, A., Marsili, L., Fossi, C.M., Leonzio, C., Zizzo, N., Vizzini, S., Gaspari, S., Pavan, G., Podestà, M., Garibaldi, F., Ferrante, M., Copat, C., Traversa, D., Marcer, F., Airoldi, S., Frantzis, A., Quirós, Y.D.B., Cozzi, B., Fernández, A., 2011. Sometimes sperm whales (Physeter macrocephalus) cannot find their way back to the high seas: A Multidisciplinary Study on a Mass Stranding. PLOS ONE 6, e19417. doi:10.1371/journal.pone.0019417

MSFD Technical Subgroup on Marine Litter, 2013. Guidance on monitoring of marine litter in European seas. Publications Office, Luxembourg.

Muñoz-Arnanz, J., Roscales, J.L., Ros, M., Vicente, A., Jiménez, B., 2016. Towards the implementation of the Stockholm Convention in Spain: Five-year monitoring (2008–2013) of POPs in air based on passive sampling. Environ. Pollut. 217, 107–113. doi:10.1016/j.envpol.2016.01.052

Nadal, M.A., Alomar, C., Deudero, S., 2016. High levels of microplastic ingestion by the semipelagic fish bogue Boops boops (L.) around the Balearic Islands. Environ. Pollut. 214, 517–523. doi:10.1016/j.envpol.2016.04.054

NOAA, 2014. Report on the Occurrence and Health Effects of Anthropogenic Debris Ingested by Marine Organisms. N. Silver Spring, MD.

Pacheco, M., Santos, M.A., 1997. Induction of EROD Activity and Genotoxic Effects by Polycyclic Aromatic Hydrocarbons and Resin Acids on the Juvenile Eel (Anguilla anguillaL.). Ecotoxicol. Environ. Saf. 38, 252–259. doi:10.1006/eesa.1997.1585

Panti, C., Spinsanti, G., Marsili, L., Casini, S., Frati, F., Fossi, M.C., 2011. Ecotoxicological diagnosis of striped dolphin (Stenella coeruleoalba) from the Mediterranean basin by skin biopsy and gene expression approach. Ecotoxicology 20, 1791–1800. doi:10.1007/s10646-011-0713-2

Poppi, L., Zaccaroni, A., Pasotto, D., Dotto, G., Marcer, F., Scaravelli, D., Mazzariol, S., 2012. Post-mortem investigations on a leatherback turtle Dermochelys coriacea stranded along the Northern Adriatic coastline. Dis. Aquat. Organ. 100, 71–76. doi:10.3354/dao02479

Prego-Faraldo, M.V., Vieira, L.R., Eirin-Lopez, J.M., Méndez, J., Guilhermino, L., 2017. Transcriptional and biochemical analysis of antioxidant enzymes in the mussel Mytilus galloprovincialis during experimental exposures to the toxic dinoflagellate Prorocentrum lima. Mar. Environ. Res. 129, 304–315. doi:10.1016/j.marenvres.2017.06.009

Remy, F., Collard, F., Gilbert, B., Compère, P., Eppe, G., Lepoint, G., 2015. When Microplastic Is Not Plastic: The Ingestion of Artificial Cellulose Fibers by Macrofauna Living in Seagrass

Macrophytodetritus. Environ. Sci. Technol. 49, 11158–11166. doi:10.1021/acs.est.5b02005

Revelles, M., Cardona, L., Aguilar, A., Fernández, G., 2007. The diet of pelagic loggerhead sea turtles (Caretta caretta) off the Balearic archipelago (western Mediterranean): relevance of long-line baits. J. Mar. Biol. Assoc. UK 87, 805. doi:10.1017/S0025315407054707

Roberts, S.M., 2003. Examination of the stomach contents from a Mediterranean sperm whale found south of Crete, Greece. J. Mar. Biol. Assoc. UK 83, 667–670. doi:10.1017/S0025315403007628h

Rochman, C.M., Lewison, R.L., Eriksen, M., Allen, H., Cook, A.-M., Teh, S.J., 2014. Polybrominated diphenyl ethers (PBDEs) in fish tissue may be an indicator of plastic contamination in marine habitats. Sci. Total Environ. 476–477, 622–633. doi:10.1016/j.scitotenv.2014.01.058

Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.-C., Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci. Rep. 5, 14340. doi:10.1038/srep14340

Romeo, T., Pedà, C., Fossi, M.C., Andaloro, F., Battaglia, P., 2016. First record of plastic debris in the stomach of Mediterranean lanternfishes. Acta Adriat. 57, 115–124.

Romeo, T., Pietro, B., Pedà, C., Consoli, P., Andaloro, F., Fossi, M.C., 2015. First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Mar. Pollut. Bull. 95, 358–361. doi:10.1016/j.marpolbul.2015.04.048

Rothfuss, A., Honma, M., Czich, A., Aardema, M.J., Burlinson, B., Galloway, S., Hamada, S., Kirkland, D., Heflich, R.H., Howe, J., Nakajima, M., O'Donovan, M., Plappert-Helbig, U., Priestley, C., Recio, L., Schuler, M., Uno, Y., Martus, H.-J., 2011. Improvement of in vivo genotoxicity assessment: Combination of acute tests and integration into standard toxicity testing. Mutat. Res. Toxicol. Environ. Mutagen. 723, 108–120. doi:10.1016/j.mrgentox.2010.12.005

Russo, G., Di Bella, C., Insacco, G., Palazzo, P., Violani, C., Zava, B., 2003. Notes on the influence of human activities on sea Chelonians in sicilian waters. J. Mt. Ecol. 7, 37–41.

Schuyler, Q., Hardesty, B.D., Wilcox, C., Townsend, K., 2014. Global Analysis of Anthropogenic Debris Ingestion by Sea Turtles. Conserv. Biol. 28, 129–139. doi:10.1111/cobi.12126

Schuyler, Q.A., Wilcox, C., Townsend, K.A., Wedemeyer-Strombel, K.R., Balazs, G., van Sebille, E., Hardesty, B.D., 2016. Risk analysis reveals global hotspots for marine debris ingestion by sea turtles. Glob. Change Biol. 22, 567–576. doi:10.1111/gcb.13078

Sims, D.W., 2008. Sieving a living: a review of the biology, ecology and conservation status of the plankton-feeding basking shark Cetorhinus maximus., in: Advances in Marine Biology. Elsevier, pp. 171–220. doi:10.1016/S0065-2881(08)00003-5

Steen, R., Torjussen, C.S., Jones, D.W., Tsimpidis, T., Miliou, A., 2016. Plastic mistaken for prey by a colony-breeding Eleonora's falcon (Falco eleonorae) in the Mediterranean Sea, revealed by camera-trap. Mar. Pollut. Bull. doi:10.1016/j.marpolbul.2016.02.069

Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. Sci. Rep. 6. doi:10.1038/srep37551

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. 113, 2430–2435. doi:10.1073/pnas.1519019113

Takatori, S., Kitagawa, Y., Kitagawa, M., Nakazawa, H., Hori, S., 2004. Determination of di (2-ethylhexyl)phthalate and mono(2-ethylhexyl)phthalate in human serum using liquid chromatography-tandem mass spectrometry. J. Chromatogr. B 804, 397–401. doi:10.1016/j.jchromb.2004.01.056

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Bjorn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2027–2045. doi:10.1098/rstb.2008.0284

Thompson, R.C., 2004. Lost at Sea: Where Is All the Plastic? Science 304, 838–838. doi:10.1126/science.1094559

Thompson, R.C., Moore, C.J., vom Saal, F.S., Swan, S.H., 2009. Plastics, the environment and human health: current consensus and future trends. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364, 2153–2166. doi:10.1098/rstb.2009.0053

Tomás, J., Guitart, R., Mateo, R., Raga, J.A., 2002. Marine debris ingestion in loggerhead sea turtles, Caretta caretta, from the Western Mediterranean. Mar. Pollut. Bull. 44, 211–216. doi:10.1016/S0025-326X(01)00236-3

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

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (Mytilus edulis) and lugworms (Arenicola marina) living in natural habitats. Environ. Pollut. 199, 10–17. doi:10.1016/j.envpol.2015.01.008

van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P.-L., Heubeck, M., Jensen, J.-K., Le Guillou, G., Olsen, B., Olsen, K.-O., Pedersen, J., Stienen, E.W.M., Turner, D.M., 2011. Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea. Environ. Pollut., Nitrogen Deposition, Critical Loads and Biodiversity 159, 2609–2615. doi:10.1016/j.envpol.2011.06.008

Vandermeersch, G., Van Cauwenberghe, L., Janssen, C.R., Marques, A., Granby, K., Fait, G., Kotterman, M.J.J., Diogène, J., Bekaert, K., Robbens, J., Devriese, L., 2015. A critical view on microplastic quantification in aquatic organisms. Environ. Res., Non-regulated environmental contaminants in seafood: contributions of the ECsafeSEAFOOD EU project 143, Part B, 46–55. doi:10.1016/j.envres.2015.07.016

Vitale, D., Varneau, N., Tison, Y., 1992. Stomach obstruction in a sperm whale beached on the Lavezzi islands: macropollution in the Mediterranean. J Rech. Oceanogr. Paris 16, 100–102.

Vlachogianni, T., Anastasopoulou, A., Fortibuoni, T., Ronchi, F., Zeri, C., 2017. Marine Litter Assessment in the Adriatic and Ionian Seas. IPA-Adriatic DeFishGear Project, MIO-ECSDE, HCMR and ISPRA.

von Moos, N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis L. after an experimental exposure. Environ. Sci. Technol. 46, 11327–11335. doi:10.1021/es302332w

Wilcox, C., Sebille, E.V., Hardesty, B.D., 2015. Threat of plastic pollution to seabirds is global, pervasive, and increasing. Proc. Natl. Acad. Sci. 112, 11899–11904. doi:10.1073/pnas.1502108112

Xue, J., Kannan, K., 2016. Novel Finding of Widespread Occurrence and Accumulation of Bisphenol A Diglycidyl Ethers (BADGEs) and Novolac Glycidyl Ethers (NOGEs) in Marine Mammals from the United States Coastal Waters. Environ. Sci. Technol. 50, 1703–1710. doi:10.1021/acs.est.5b04650

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Corresponding author: Cristina Panti, panti4@unisi.it

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