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Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity☆

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A B S T R A C T

The Mediterranean Sea has been described as one of the most affected areas by marine litter in the world. Although effects on organisms from marine plastic litter ingestion have been investigated in several oceanic areas, there is still a lack of information from the Mediterranean Sea. The main objectives of this paper are to review current knowledge on the impact of marine litter on Mediterranean biodiversity, to define selection criteria for choosing marine organisms suitable for use as bioindicator species, and to propose a methodological approach to assessing the harm related to marine litter ingestion in several Mediterranean habitats and sub-regions. A new integrated monitoring tool that would provide the information necessary to design and implement future mitigation actions in the Mediterranean basin is proposed.

According to bibliographic research and statistical analysis on current knowledge of marine litter ingestion, the area of the Mediterranean most studied, in terms of number of species and papers in the Mediterranean Sea is the western sub-area as well as demersal (32.9%) and pelagic (27.7%) amongst habitats.

Applying ecological and biological criteria to the most threatened species obtained by statistical analysis, bioindicator species for different habitats and monitoring scale were selected. A threefold approach, simultaneously measuring the presence and effects of plastic, can provide the actual harm and sub-lethal effects to organisms caused by marine litter ingestion. The research revealed gaps in knowledge, and this paper suggests measures to close the gap. This and the selection of appropriate bioindicator species would represent a step forward for marine litter risk assessment, and the implementation of future actions and mitigation measures for specific Mediterranean areas, habitats and species affected by marine litter ingestion.

1. Rationale

Concern about the occurrence, quantity and effects of marine litter in the world's ocean and seas has grown rapidly in recent years, attracting interest from a wide range of stakeholders: governments, environmental Non-Governmental Organization

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(NGOs), the scientific community, the media and the general public. Mediterranean Sea, which is a crucial biodiversity hotspot and a critically polluted area, has been also described as one of the areas most affected by marine litter in the world (UNEP/MAP, 2015; Cózar et al., 2015). According to Suaria and Aliani (2014), 62 million macrolitter items were estimated to be floating on the surface of the Mediterranean basin. Marine litter has been detected on regional and local scales in the Mediterranean Sea: along the beaches, floating on the sea surface, in the water column and on the sea floor in (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). Debris that enters marine environments, spreads and accumulates in habitats and compartments, and interacting with marine organisms including the occurrence inside biota following ingestion (Kühn et al., 2015).

Marine litter impacts and interactions on Mediterranean marine organisms were reviewed by Deudero and Alomar (2015) reporting almost 134 species were affected by marine litter at basin scale. This research gives scientific evidence that marine litter is a threat to Mediterranean marine organisms, which are historically exposed to a plethora of other environmental pollutants and other man-driven changes. Marine litter issue requires a series of mitigations actions or solutions.

In addition to National Action Plans at country level, management of marine litter in the Mediterranean Sea falls within the framework of two main regional drivers: the Regional Plan on Marine Litter Management in the Mediterranean (UN Environment/Mediterranean Action Plan), which covers the whole region and the Marine Strategy Framework Directive (MSFD; 2008/56/EC, Descriptor 10) only for European marine waters. Actions are also supported by the Union for the Mediterranean (UfM), through the labelled Plastic Busters project (http://plasticbusters.unisi.it/), led by Sustainable Development Solutions Network (SDSN) Mediterranean Regional Centre and the University of Siena (Italy).

Although the effects of plastic litter on the marine environment and organisms have been recently investigated in several oceanic areas, more information is needed for the Mediterranean Sea. In particular, plastic and microplastic inputs, their spatial and temporal distribution, potential accumulation areas, transport dynamics, and interactions with biota and trophic web, all need further investigation.

Recent studies in the different subregions of the Mediterranean basin have suggested that some areas are affected by high concentrations of marine litter, including microplastics and plastic additives (phthalates), representing a potential risk for biodiversity (Darmon et al., 2017; Fossi et al., 2017) and for endangered species (baleen whales, sea turtles, filter feeding sharks) in particular.

In 2016, the UN Environment Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria (IMAP) adopted the Candidate Indicator 24 "*Trends in the amount of litter ingested by or entangling marine organisms focusing on selected mammals, marine birds, and marine turtles*" under Ecological Objective 10 (EO10) i.e. Marine Litter. Work is underway to define the most representative species to be used for this Indicator. Additionally, at a European level, the MSFD criteria D10C3 (Commission Decision 2017/848) states: "*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 criteria D10C4 "*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*." Specifically, the decision states that: "*Member States shall establish that list of species to be assessed through regional or subregional cooperation. 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 and invertebrates*".

For this reason, further research in this area which addresses multiple species with different ecological and biological importance is needed to decipher indicators species for marine litter ingestion. The quantification of marine litter in the environment (particularly floating macro- and micro litter) depends on several environmental factors (e.g. wind, currents, sea state, etc.) and may change according to multiple oceanographic features; and, in many cases, quantity alone cannot reflect the potential impact of litter on marine organisms and ecosystems. Information obtained from biondicator species would better integrate spatial and temporal presence of marine litter in the marine environment. The choice and identification of representative bioindicator species for marine litter in the Mediterranean can allow measuring of not only the occurrence of marine litter within species and their environment but also the threat posed to the organisms by the evaluation of contaminants (associated/sorbed to plastic litter) accumulation and any related biological effect (Rochman et al., 2013).

1.1. Harm caused by marine litter ingestion

Marine litter ingestion is one of the main threats to biodiversity in the Mediterranean. Ingestion has been reported in various organisms ranging from invertebrates to vertebrates, including endangered species (Deudero and Alomar, 2015; Kühn et al., 2015; Werner et al., 2016; Wright et al., 2013). Marine organisms may deliberately ingest litter items because of their resemblance to prey (Campani et al., 2013; Cole et al., 2011; Romeo et al., 2016; Wright et al., 2013) or accidentally ingest litter while they are 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 species, marine litter particles may be egested or accumulate in the gastrointestinal tract, and could 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 may cause pseudo-satiation resulting in reduced food intake (Kühn et al., 2015). In some cases when gastrointestinal tracts become blocked or severely damaged, marine litter ingestion may lead to mortality of the organism (Werner et al., 2016).

Marine litter, in particular microplastics (<5 mm), also represents a direct and indirect vector for the introduction of chemical substances into the food-web, although information on this issue is still debated (Koelmans et al., 2016). Given the high biodiversity (Coll et al., 2010) and the widespread distribution of marine litter in the Mediterranean basin, many species may be directly impacted by ingestion. The sub-lethal and the chronic effects of litter ingestion could compromise the species and consequently ecosystems having long term implications. This document will particularly focus on the bioindicator organisms for marine litter ingestion.

The main objective of this paper is to review the current knowledge on marine litter ingestion by Mediterranean species and to propose a methodological approach for the assessment of litter in the Sea, using marine organisms as bioindicator species and applying a new integrated approach to monitoring. Selection criteria for the choice of bioindicators are suggested and harmonization of the approach to the study of Mediterranean marine litter is discussed. In particular, this study: (i) reviews the current knowledge of the impact of litter on Mediterranean marine organisms, (ii) defines selection criteria for the choice of sentinel (bioindicator) species, (iii) proposes a threefold monitoring

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approaches to detect the presence and impact of marine litter in bioindicator species and, (iv) identifies gaps and proposes directions for further research.

2. Bibliographic research and data analysis

In order to compile the most up-to-date information (February 2017) on marine litter ingestion by Mediterranean marine organisms, a complete search of peer-reviewed scientific literature, grey literature and reports was carried out. Search results were used to build an extensive database of studies on litter ingestion by Mediterranean marine vertebrates and invertebrates.

The bibliographic research used general search engines such as Google scholar and Google and computerised databases such as ISI Web of Knowledge and Scopus using the following keywords: marine litter ingestion, marine plastic ingestion, marine litter Mediterranean, marine debris Mediterranean, marine plastic Mediterranean, marine litter and vertebrates/marine mammals/seabirds/sea turtles, marine debris and vertebrates/marine mammals/seabirds/sea turtles, marine plastics and vertebrates/marine mammals/seabirds/sea turtles, 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 estimate the number of Mediterranean species affected by marine litter ingestion and the number of papers on litter ingestion in the Mediterranean subregions, the results of each bibliographic search were evaluated and, for the selected documents, the following information was extracted and recorded in the database (Table 1): scientific species name and taxonomic group (Annelids, Echinoderms Crustaceans, Molluscs, Fish, Turtles, Seabirds and Marine Mammals); area grouped into Mediterranean subregions (WMS= Western Mediterranean Sea; ISCMS= Ionian Sea and the Central Mediterranean Sea; AS = Adriatic Sea; ALS = Aegean-Levantine Sea); habitat; number of specimens investigated; evidence of marine litter ingestion; and, whenever possible, number of specimens with marine litter; frequency of occurrence (%); number and the weight of litter identified; reference to the source of literature.

It is worth noting that there is some bias in the dataset due the inaccuracy in reporting the absence of marine litter in organisms, especially in earlier studies. This lack of data may have led to a possible overestimation of ingestion for some species.

To date, 48 papers on the incidence of marine litter ingestion by marine organisms in the Mediterranean basin have been published. Fig. 1, shows the number of papers (P) on marine litter ingestion and number of affected species (S) by marine litter ingestion in Mediterranean sub-regions are reported. When a study covered two or more sub-regions, it was considered for both areas. Most studies were carried out in the Western Mediterranean Sea (WMS), while the Ionian Sea and the Central Mediterranean Sea (ISCMS), the Adriatic Sea (AS) and the Aegean Levantine Sea (ALS) were less investigated (Fig. 1). 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).

Marine litter ingestion in Mediterranean organisms has been reported since 1988, with a clear increase in the number of scientific papers in recent years (Fig. 2). First records of marine litter ingestion came from feeding ecology studies of Mediterranean species (Carrasón et al., 1992; Deudero, 1998; Madurell, 2003; Massutí et al., 1998), but in recent years, the detection and the occurrence of litter in gastrointestinal tracts have been the primary aim of most studies within the Mediterranean sub-regions.

In particular, marine litter ingestion by fish species has gained interest in the last decade (from 2010 to date), resulting in an increase in related studies. This may be due to concern about the impact of marine litter ingestion on fishery resources, and the potential risk human consumption.

Litter ingestion has been documented for 91 Mediterranean species, belonging to different taxonomic groups including invertebrates, fish, sea turtles, seabirds and marine mammals (Table 2).

While fish represent 65.9% of the affected species, belonging to 14 orders, 24% of reports on marine litter ingestion in Mediterranean species 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 litter ingestion. Most studies on these endangered species dealt with stranded individuals. Marine litter ingestion in seabirds is a well-documented phenomenon on a global scale, as reported by Laist (1997) and Kühn et al. (2015). However, in the Mediterranean basin only one paper, by Codina-García et al. (2013), reported on the presence of marine litter in several species belonging to Procellariiformes, Suliformes and Charadriiformes (Table 1). Cases of marine litter ingestion were also documented in marine invertebrates such as Annelids, Crustaceans, Echinoderms and Molluscs (Table 2) (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).

With particular regards to habitat, litter ingestion has also been reported in species from different habitats, with most studies conducted on demersal (32.9%), pelagic (27.7%) species, followed by benthic (14.7%), benthopelagic (16.5%), neritic (5.3%) and mesopelagic (2.9%) species.

In addition to the physical harm associated with marine litter, there is increased concern regarding the chemical harm related to marine litter ingestion. Some studies have examined the potential link between the chemical effects of plastic ingestion and the risk of bioaccumulation across the trophic web. Plastic litter could be a direct vector for plastic additives (e.g. leaching of PBDEs and phthalates) and an indirect vector of chemicals due to sorption and transport of persistent, bioaccumulating and toxic (PBT) substances that are adsorbed and transported by marine plastic litter (Hermabessiere et al., 2017).

The level of these toxic chemicals in bioindicator species has been proposed as a possible as a proxy indicator of plastic exposure.

Higher brominated congeners of PBDEs (e.g. BDE#s 183–209), added to plastics as flame-retardants, have been found at significantly larger concentrations in tissue of myctophids and other fish species (Gassel et al., 2013; Rochman et al., 2014) and seabirds (Tanaka et al., 2013, 2015) that had ingested plastics.

Similarly, phthalate concentrations in the uropygial gland of live and dead birds have been correlated with numbers of pieces of plastic ingested by birds (Hardesty et al., 2015).

In the Mediterranean area, 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* suggesting a possible exposure to plastics of these species which mostly live in pelagic areas.

If these chemicals become bioavailable, they can penetrate cells and chemically interact with biologically important molecules, and may cause adverse effects at different levels of biological

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Table 1
Mediterranean species studied for marine litter ingestion with details on area of study, habitat and references.

	Taxa	Species	MED sub-regions	Habitat	References
INVERTEBRATES	**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
	Annelida				
	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	MEE 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., 2015b); 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., 2015b)
		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

Table 1 (*continued*)

	Taxa	Species	MED sub-regions	Habitat	References
		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	MEE 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., 2015b; 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 and 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	MEE 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

(*continued on next page*)

Table 1 (*continued*)

	Taxa	Species	MED sub-regions	Habitat	References
		Upeneus pori Ben-Tuvia and 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., 2015a,b
		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
	Taxa	**Species**	**MEE sub-regions**	**Habitat**	**References**
VERTEBRATES	**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 and Schneider, 1801)	ISCMS	demersal	Anastasopoulou et al., 2013
		Centroscymnus coelolepis Barbosa du Bocage and 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., 2015a,b; 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
	Taxa	**Species**	**MEE sub-regions**	**Habitat**	**References**
VERTEBRATES	**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
			WMS	neritc	Codina-García et al., 2013

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Table 1 (*continued*)

Taxa	Species	MED sub-regions	Habitat	References
	Rissa tridactyla (Linnaeus, 1758)			
	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 coeruleoalba (Meyen, 1833)	WMS/AS	pelagic	Baini et al., 2017; Baulch and Perry, 2014

organization, from molecular level to tissue level, including alterations of gene expression (Karami et al., 2017; Sleight et al., 2017), genotoxic effect (Avio et al., 2015a), endocrine disruption (Rochman et al., 2014; Teuten et al., 2009) liver toxicity (Avio et al., 2015b; Rochman et al., 2013), and histological alterations (Avio et al., 2015a, 2015b; Pedà et al., 2016). Although, most of these effects have been shown in laboratory studies, very few are available from field studies. Currently, there are limited studies from the Mediterranean (Avio et al., 2015a).

Despite the increase in the number of studies in recent years, information on the interaction between Mediterranean biota and marine litter is currently poor and inconsistent. As already suggested by Deudero and Alomar (2015), this is in part due to the lack of standardized methods and protocols for monitoring. Moreover, most studies mainly look at the occurrence of macro- and meso-litter in marine organisms, and that can lead to underestimation of the impact of micro- and nano-litter.

2.1. Statistical analysis

A preliminary analysis was performed on the data available from the literature, ranking each species with recorded marine litter ingestion. If a species was analyzed in different papers, all the data available were combined together to calculate the percentage of ingestion on the total data. Given the wide range of species collected from the literature, the resulting data (Table SM1) were used to carry out a statistical analysis, to elaborate an impact index. Not all the papers reported the percentage of occurrence, especially for invertebrates, thus reducing the number of observations from 167 to 137. Additionally, only the species with more than six specimens per species analyzed for marine litter ingestion were included in the analysis.

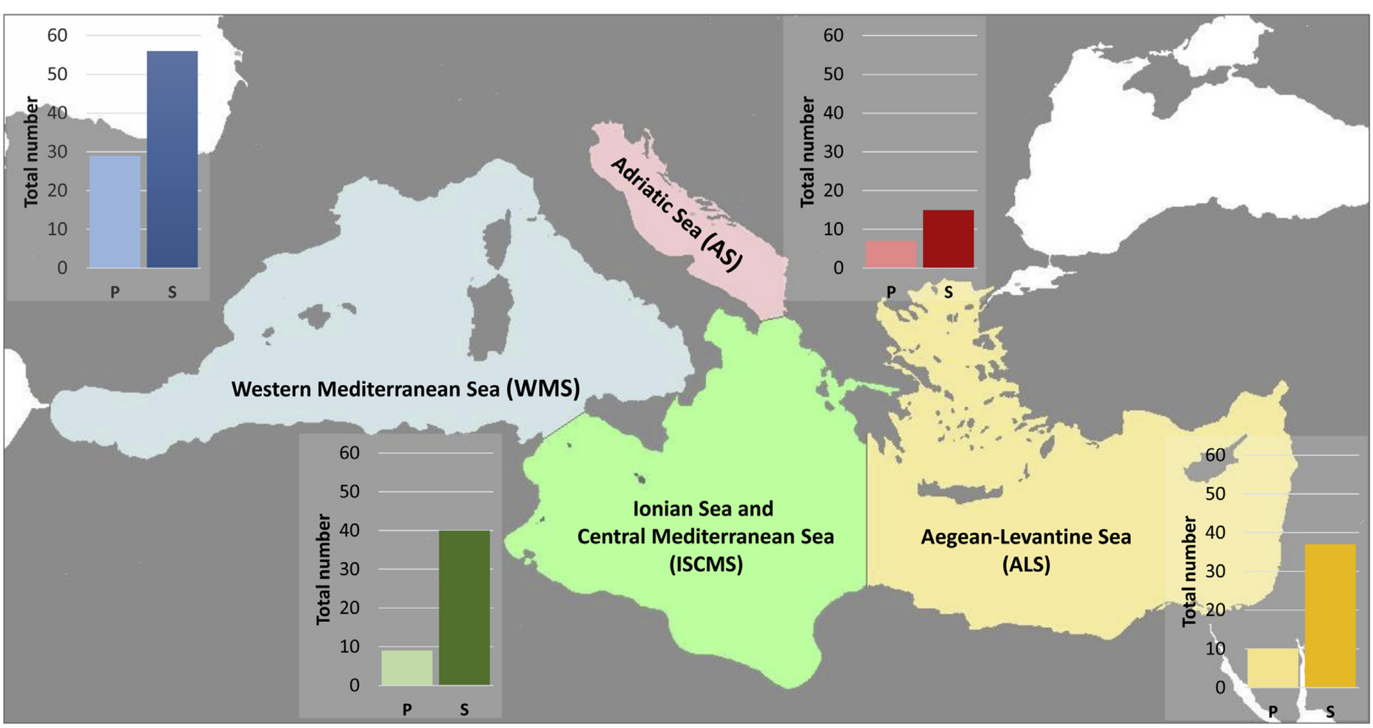

Fig. 1. Number of papers (P) and number of species (S) reporting marine litter ingestion in Mediterranean UN Environment/MAP sub-regions (WMS = Western Mediterranean Sea; ISCMS = Ionian Sea and the Central Mediterranean Sea; AS = Adriatic Sea; ALS = Aegean-Levantine Sea).

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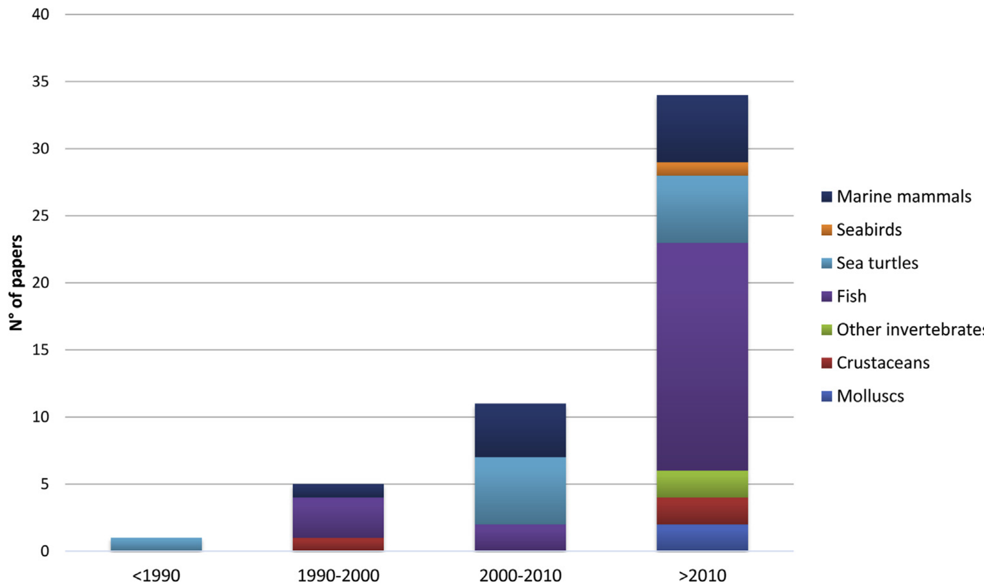

Fig. 2. Number of published papers on marine litter ingestion by Mediterranean species per decade for the taxonomic groups.

Table 2
Number of Mediterranean species with documented records of marine litter ingestion. In brackets number of species per taxonomic group.

Taxonomic group		Number of species
Invertebrates		**14**
Annelids	*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**

The impact index was designed and assigned to each observation (study-species) for habitat type and species groups. For the different taxa groups and habitat types, a boxplot of the % occurrence of all observations was compiled (Fig. 3) and the impact index was assigned dependent on the boxplot quartiles using the following three categories: low (minimum to first quartile), medium (first quartile to third quartile) and high (third quartile to maximum). To determine which species are of high concern for marine litter ingestion, those whose percentages fell within the third quartile (above red line in Fig. 3) to the maximum for both their habitat were selected to be representative of sentinel species (Table 3). All of the species included in the high impact index were selected giving an indication of 30 species spread across 6 taxa groups and 6 habitat types as bioindicator species for monitoring for marine litter ingestion in the Mediterranean Sea.

During the analysis, each species was included in the category habitat (benthic, benthopelagic, demersal, mesopelagic, neritic, and pelagic) according to the classification made in Table 1.

For each of the habitat types, the high impact index ranged from 5.71% in the benthic species to 100% in pelagic species. Although there is a wide range between the species, it is important to note that the number of study-species observations and the taxa represented in the literature provide key information on the availability of the species as a bioindicator for marine litter. It is important that the species selected are representative across taxa to fully monitor different habitats.

However, not all of the taxa were represented in the high impact index for each of the habitat types, the taxa group for elasmobranchs was omitted. An additional analysis following the same methodology used for habitat was also used to determine which species within each taxa group fell within the high impact index.

3. Bioindicator selection strategy

It is crucial that appropriate sentinel species are used to monitor the impact of marine litter on Mediterranean fauna, so as to be able to assess the threat posed by litter and to establish a regional marine litter management policy. The selection of sentinel species should be based on specific criteria and should allow different marine habitats (from coastal areas to offshore, from benthic environments to pelagic waters) to be monitored at different spatial scales (Schwacke et al., 2013).

With this main aim of the paper a series of selection criteria for species and habitat have been analyzed and critically evaluated in

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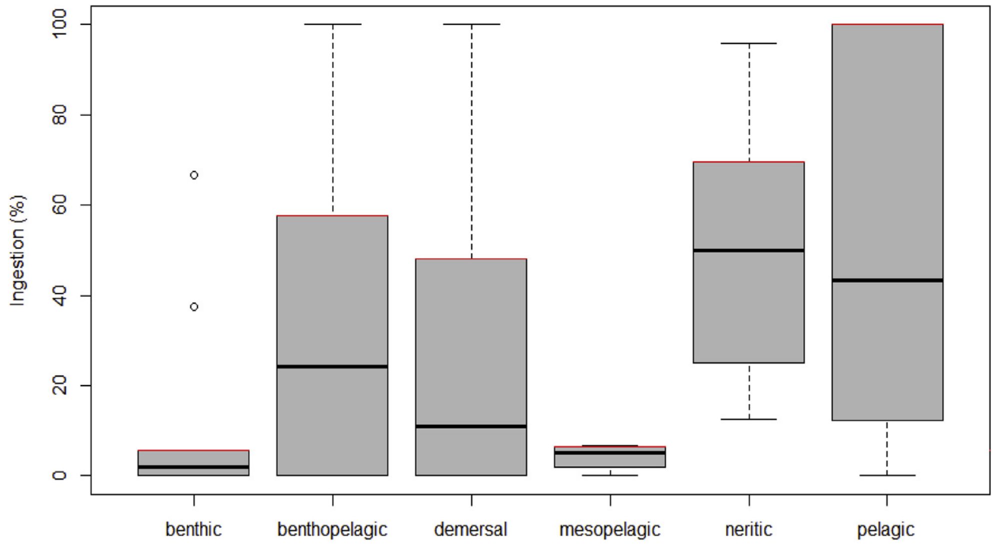

Fig. 3. Results from boxplot analysis for ingestion (%) for each habitat types: benthic, benthopelagic, demersal, mesopelagic, neritic, and pelagic. Red line indicates the cutoff for high impact index (third quartile). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Species whose impact index for ingestion for each habitat type was high and their taxa type. The threshold percentage is in parenthesis.

Habitat (high impact threshold %)	Taxa	Species
benthic (5.71)	teleosts	*Trigla lucerna, Chelidonichthys lucerna*
	echinodermata	*Holothuria forskali*
benthopelagic (57.71)	teleosts	*Argyrosomus regius, Boops boops, Pagellus acarne*
	reptiles	*Caretta caretta, Dermochelys coriacea*
demersal (48.15)	teleosts	*Siganus luridus, Serranus cabrilla, Sciaena umbra, Saurida undosquamis, Diplodus annularis, Mullus barbatus, Mullus surmuletus, Nemipterus randalli, Pagellus erythrinus, Pagrus pagrus, Pelates quadrilineatus, Pomadasys incisus*
mesopelagic (6.48)	teleosts	*Hygophum benoiti*
neritic (69.57)	seabirds	*Calonectris diomedea, Puffinus yelkouan, Puffinus mauretanicus*
pelagic (43.24)	mammals	*Balaenoptera physalus, Physeter macrocephalus*
	teleosts	*Engraulis encrasicolus, Caranx crysos*

this paper, and relevant sentinel species are identified. However, every monitoring program will have its own objectives, in terms of spatio-temporal scale and areas investigated. The experimental design is tailored according to these objectives and relevant sentinel species should be selected according to the criteria proposed. These aspects of the management model, although, is not be covered in the present paper.

3.1. Ecological and biological selection criteria

In the present paper, we identify a range of selection criteria for the choice of sentinel species, meeting some general categories:

(1) *background information*: this category includes the biological and ecological characteristics of the species. The taxonomic identification of a bioindicator should be clearly addressed. Moreover, it is important to have enough information about the ecology and biology of the selected species, to be able to relate the monitored phenomenon to specific ecological issues;
(2) *habitat information*: habitat and home range of the species are essential information in the selection of sentinel species, as they allow monitoring at different spatial scales. Some species are sessile (e.g., mussels) and can give accurate information on restricted areas and on certain areas where marine litter accumulates. Others may travel over wide horizontal areas (large pelagic predators) or migrate vertically (e.g. micronekton) to large depth displacements in the water column, and can represent a wide integrator of marine litter over that spatial scale;
(3) *trophic information and feeding behavior*: the feeding mechanism (e.g. filter feeding) and feeding behavior (e.g. feeding on schooling, opportunism, feeding on pleuston, benthivorous feeders, etc.) strongly influence marine litter ingestion by marine organisms. Filter feeders, such as baleen whales (Fossi et al., 2012, 2014, 2016), basking shark (Fossi et al., 2014) anchovies (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 plastic have been also found in stomachs of opportunistic feeders preying on shoals (i.e. bluefin tuna, albacore) or near surface (*Trachinotus ovatus*) (Battaglia et al., 2016; Romeo et al., 2015) within the Mediterranean sub-regions. Species which feed on the seafloor (e.g. red mullets, shrimps, worms) can be also exposed to the risk of litter ingestion. Trophic level is an important issue, since species at higher levels (e.g. large pelagic predators) maybe subject to chemical bioaccumulation. Therefore, sentinel species used to monitor marine litter should also include key species of the marine

trophic web, as these have a crucial ecological role in maintaining the structure and integrity of the marine communities;

(4) *spatial distribution*: monitoring the impact of litter on Mediterranean marine biota implies selection of sentinel species which allow an appropriate spatial coverage. The extension of sampling to the Mediterranean scale requires the selection of widely distributed species that are within the Mediterranean basin and its subregions.

(5) *commercial importance and conservation status*: monitoring activity should also include species with commercial importance and have good availability through fishing markets or landing places; this attribute could also allow to estimate the potential transfer of plastics and associated contaminants from sea food to humans. Among selected sentinel species, the presence of protected, threatened or endangered species is desirable to understand at what extent marine litter can affect the species conservation. An emblematic example is the case of the loggerhead sea turtle (*Caretta caretta*), a species of ecological and conservation interest, whose Mediterranean population is strongly affected by the ingestion of litter, which has, in several cases, caused the death of analyzed specimens (Campani et al., 2013; Lazar and Gračan, 2011; Tomás et al., 2002).

(6) *Documented ingestion of marine litter*: statistics and data availability on marine litter ingestion.

Not all sentinel species will fit all criteria but each taxon, selected as part of a complementary set, will satisfy multiple attributes. A full set of sentinel species (as reported below) will allow a comprehensive plan for monitoring marine litter in the Mediterranean region to be established.

Finally, the selection of sentinel species and the planning of monitoring activities should take into account some difficulties linked to the behavior of the organisms and/or the inadequacy of sampling methods. Indeed, several authors (Duhem et al., 2003, 2008; Ramos et al., 2009) have observed that opportunistic feeding habits of the seabird *Larus michahellis* are strongly influenced by urbanization and, as a consequence, this species modifies its feeding behavior, moving from marine feeding grounds to the litter dump sites on-land, where it easily finds food without any effort. This would lead to an overestimation of the amount of litter ingested should this species be considered among 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" (Davison and Asch, 2011).

3.2. Habitat and home range selection criteria

Based on the data available on the interaction of marine litter (including microplastics) with Mediterranean marine organisms and the biological and ecological criteria for the choice of sentinel species, different bioindicator species are proposed as sensitive indicators of the presence and effects of marine litter 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: small-scale (FAO Geographical subareas, GSAs), Medium-Scale (Mediterranean UN Environment/MAP sub-regions) and large scale (whole Mediterranean Basin), so as to serve as sentinels on the Mediterranean environment at different geographical scales. A short description of the selected species per habitat, home range and type of litter ingested are discussed below and summarized in Table 4.

3.3. Small-scale bioindicators of microplastics along the coast line and in beach sediment

In general, most invertebrates can be considered as local scale indicators of the presence and impact of microlitter (mainly microplastics) in specific areas of Mediterranean coastal shores. The shore plays an important role in the fragmentation of plastics in the marine environment, facilitating the formation of microplastics and nanoplastics. Therefore, studies on the impact of these fragmentation processes in coastal environments and the identification and selection of suitable bioindicators are essential in the monitoring and future governance of the marine environment. The usefulness of bivalve molluscs, and in particular mussels (*Mytilus* spp.), as sentinel organisms for monitoring pollution in coastal environments has been established in several laboratory and field studies (Beyer et al., 2017). These intertidal filter-feeding invertebrates are known to accumulate high levels of contaminants (e.g. heavy metals and POPs) as well as microplastics (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, low position in the food web, easy accessibility, the possibility to carry out in-cage studies as well as its well understood biology, the mussel (*Mytilus galloprovincialis*) is proposed as a bioindicator of microplastics in Mediterranean coastal shores. Being a sessile suspension feeder, the mussel effectively reflects spot environmental contamination. It is therefore an internationally accepted sentinel "early warning" species for monitoring marine pollution used both the U.S. Mussel Watch and for the assessment and Control of Pollution in the Mediterranean region (MED POL). In addition, it is proposed to investigate the uptake of microplastics by marine (epi)benthic organisms selecting *Arenicola marina* (lugworm) as the bioindicator organism, because *A. marina* is a robust and quantitatively important deposit feeder at the base of the Mediterranean benthic 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). *Carcinus* spp. is also proposed as a suitable bioindicator species of the Mediterranean coastline. *Carcinus* spp. (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 organisms and litter present in the coastal environment. To support this hypothesis, *Carcinus* spp. has been demonstrated to uptake and retain microplastics in laboratory studies (Watts et al., 2014, 2015).

3.4. Small-scale bioindicators of microplastics on the seafloor

Demersal fish live in close connection with sediments on the sea bed 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. are fish species living on muddy and sandy bottoms, feeding mainly on benthic species. Red mullet was extensively used in MED-POL for monitoring chemical pollution monitoring (Burgeot et al., 1996). They inhabit the whole Mediterranean Sea and because of their close association with the sea bed they are strongly exposed to the ingestion of microlitter (Avio et al., 2015b; Bellas et al., 2016; Digka et al., 2016; Güven et al., 2017; Vlachogianni et al., 2017). Microplastic ingestion has been reported in red mullet from different Mediterranean sub-regions and contiguous areas (Avio et al., 2015b; Bellas et al., 2016) as well as in the sole (Neves et al., 2015). In addition, other sentinel species suitable for monitoring the presence of microlitter on the seafloor are: (i) the selachian *Galeus melastomus* in whose stomachs

Table 4
Bioindicator species for marine litter ingestion selected in relation to habitat and home range. In **blue**: biondicator for macrolitter, in **red**: bioindicator for microplastic.

	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				Holoturians	*Mytilus galloprovincialis Arenicola marina* Decapods *(e.g. Carcinus sp.)*

plastic litter has already been found (Alomar and Deudero, 2017; Carrasón et al., 1992; Cartes et al., 2016) which provides an ecological component to ecosystems; and (ii) the demersal fish *Merluccius merluccius*, for its commercial importance worldwide and its trophic links between pelagic and demersal habitats (Bellas et al., 2016).

3.5. Small-scale bioindicators of microplastics in coastal waters

A high percentage of plastics has been already found in the stomach contents of *Boops boops* (67.7%; Nadal et al., 2016; Neves et al., 2015) and *Trachinotus ovatus* (24.3%; Battaglia et al., 2016) and these species can be proposed as good sentinel species at small-scale level. The two species do not carry out large movements and they are exploited by artisanal fisheries. 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 determined in part by their predation on gregarious prey. Moreover, the hunting behavior of *T. ovatus* just below the surface (suggested by the predation on insects and some neustonic organisms such as *Porpita porpita*) may make *T. ovatus* more vulnerable to the ingestion of floating plastic debris (Battaglia et al., 2016; Santillo et al., 2017).

3.6. Small-scale bioindicators of microplastics in open waters

Due to their trophic level and habitat, mesopelagic fish can represent indicators of the presence and impact of microplastics in the Mediterranean pelagic environment at small-scales (GSAs). A high level of microplastics was found in the stomachs of mesopelagic fish species from the Atlantic Ocean (Boerger et al., 2010; Davison and Asch, 2011; Lusher et al., 2016), and recently plastic ingestion in some Mediterranean myctophids (*Electrona risso, Diaphus metopoclampus, Hygophum benoiti, Myctophum punctatum*) was recently documented by Romeo et al. (2016). *Maurolicus muelleri* (Sternoptychidae), *Hygophum benoiti* and *Electrona risso* (Myctophidae) are small, mid-water predators foraging mainly on zooplankton and micronekton community (e.g. Battaglia et al.,

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2016; Scotto di Carlo et al., 1982). As other mesopelagic fishes, they usually carry out diel vertical migration and 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; Lusher et al., 2016). The results of the trophic model ECOPATH (Fig. 4) indicate that mesopelagic fish are among the most important prey species of pelagic predators, playing a key role in the pelagic trophic web of the Tyrrhenian Sea (central Mediterranean).

The ingestion of plastic is potential cause of death for vertically migratory fish as lantern fish are one example due to the buoyancy of this debris (Romeo et al., 2016) and because the larger plastic (mesoplastics) may be retained in digestive tracts, lading to malnutrition and possibly even 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 be impacted by microplastics in the water column (Collard et al., 2017). They are also among the most important commercial fishing resources of the Mediterranean Sea and the represent main prey of several pelagic predators.

3.7. *Medium-scale bioindicators of microplastics in open waters*

At medium scale, large pelagic predators *Thunnus alalunga* and *Coryphaena hippurus* can represent a more suitable sentinel species for monitoring microplastic in the trophic web, because of the wide range of their migration within the Mediterranean basin. These important fishing resources are widely distributed in Mediterranean subregions and have been already been identified as species impacted by plastic ingestion (Deudero and Alomar, 2015; Romeo et al., 2015).

3.8. *Basin-scale bioindicators of microplastics in open waters*

Large filter feeding marine organisms, such as baleen whales and sharks, which can ingest microplastic during their filtrating feeding activity, are here 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, feed primarily on planktonic euphausiid species. The basking shark (*Cetorhinus maximus*) is a large and filter-feeding pelagic species (Sims, 2008), having migratory behavior and being widely distributed in Mediterranean

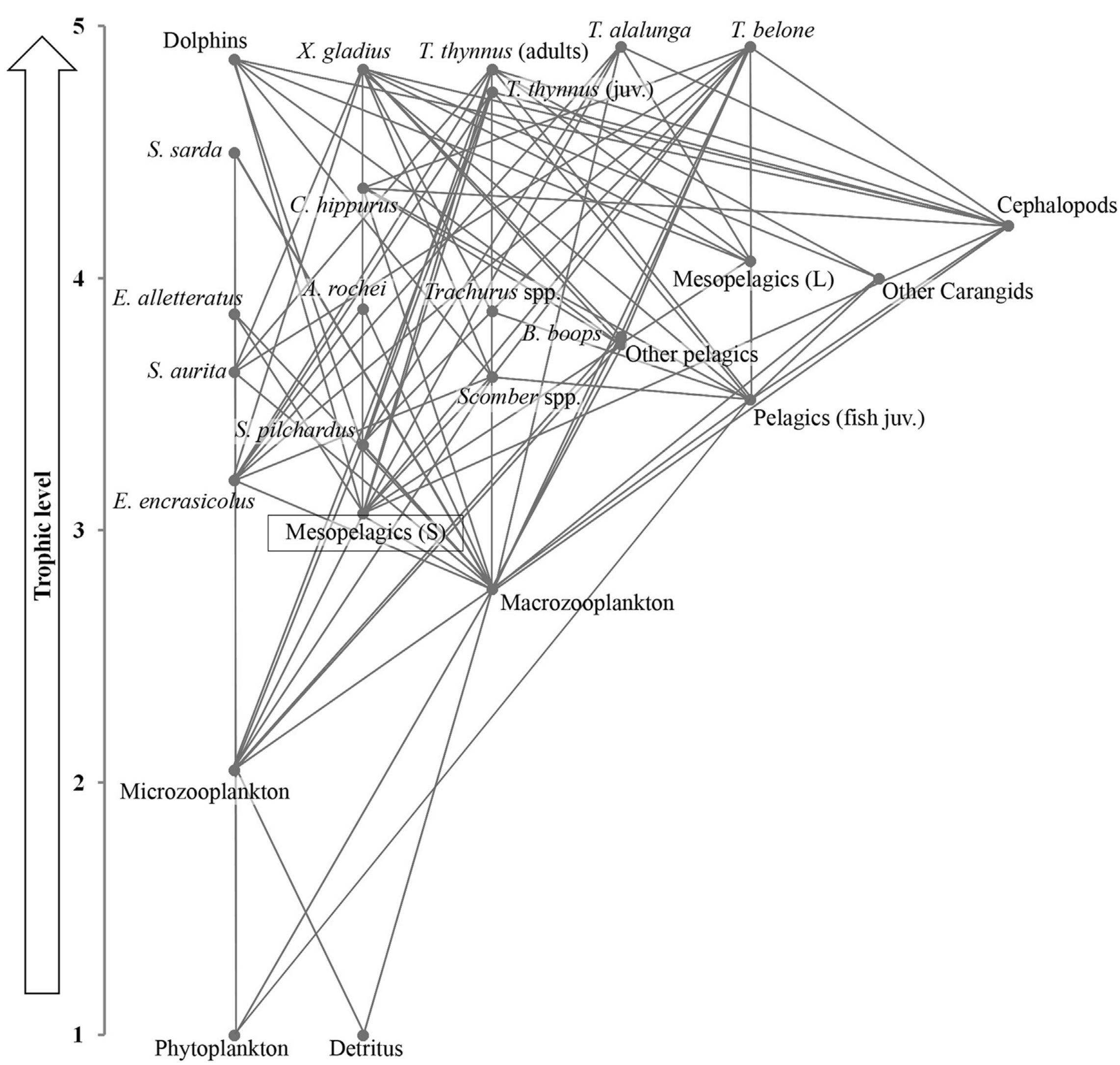

Fig. 4. Structure of the pelagic trophic web of the Tyrrhenian Sea, obtained by the ECOPATH model (ICRAM, 2007). Other carangids = *Caranx crysos, Trachinotus ovatus, Naucrates ductor*; Other pelagic = *Belone belone, Scomberesox saurus*, Exocoetidae.

waters. Basking sharks feed upon zooplankton by forward swimming with an open mouth, causing a passive water flow across the gill-raker apparatus. Both species are at from the ingestion and degradation of microplastics.

Also the large pelagic predators *Thunnus thynnus* and *Xiphias gladius* have also been included as wide scale sentinel species, based on to recent findings by Romeo et al. (2015). They are migratory fish widely distributed in the Mediterranean and top predators with an important role in the pelagic trophic web. Their high commercial value and importance for food consumption is an additional reason for the choice of these species in the monitoring strategy. However, for *T. thynnus* it is important to take into consideration the migration between Atlantic Ocean and Mediterranean Sea in the evaluation of the plastic impact.

3.9. Basin-scale bioindicator of macrolitter in the water column

The loggerhead sea turtle (*Caretta caretta*), which ingests macro litter during 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. Several studies show the high occurrence of ingestion of marine litter in *C. caretta* worldwide (Nicolau et al., 2016; Pham et al., 2017; Schuyler et al., 2014). Depending on its age and on food availability, this species feeds in different ecological marine compartments from the surface to the bottom. Due to its wide distribution and propensity to ingest marine litter, the loggerhead turtle has been proposed as a target indicator species within the MSFD to evaluate the impact of litter in the Mediterranean sea (D10 C3 indicator). The sea turtle has also been selected as a candidate indicator within OSPAR in 2016 (Claro, 2016) and has also 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 litter ingestion is recommended at the Mediterranean scale within MedPol Marine Litter Action Plan. Being carnivorous to omnivorous, loggerhead sea turtle can ingest a high amount of debris that may be mistaken for gelatinous prey or encrusted by food, causing in the worst case the death of the animal by occlusion of the gastro-intestinal tract. Within the Mediterranean basin, before 2013, the recorded occurrence of ingestion varied from 35% in the Adriatic Sea to nearly 80% in Mediterranean Spain (Galgani, 2017); recent results suggest that the occurrence may have increased over time. In France for example, the occurrence of ingestion increased from 35% to 76% between 2003 and 2008 (Darmon and Miaud, 2016). However, several biological constraints and sources of bias have been identified (Casale et al., 2016; Claro et al., 2014) and further tests are necessary in order to define the conditions in which the indicator should be implemented. Stranded/dead or hospitalized loggerhead turtles (in rescue centres) can be used to monitor plastic ingestion, by analyzing: (i) gastro-intestinal tract contents of dead animals or the faeces excreted by live animals in tanks, (ii) accumulation of contaminants in the tissue and (iii) responses of a set of biomarkers. TMarine litter ingestion is already being monitored in several Mediterranean European countries using the standardized protocols included in the European monitoring guidance (MSFD-TSGML, (2013)), and training sessions are being organized (CORMON, MedPol Action Plan).

As well as loggerhead sea turtles, the sperm whale is one of the most affected species among cetaceans. High occurrence of marine litter ingestion has been reported in stranded organisms along the Mediterranean coasts (de Stephanis et al., 2013; Mazzariol et al., 2011). However, a harmonized and standardized protocol for the analysis of marine litter for large marine mammals needs to be further validated.

3.10. Basin-scale bioindicators of macrolitter on the sea surface and in coastal waters

Birds are the most studied species in regards to litter ingestion. In some 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 for monitoring microplastics and mesoplastics (of between 5 and 25 mm in size). However, in the Mediterranean Sea, marine litter ingestion by seabirds is currently poorly investigated in the Mediterranean Sea (Steen et al., 2016) and only Codina-García et al. (2013) have described the relevance of seabirds for the monitoring of plastic ingestion and impacts on Mediterranean marine fauna. Indeed, these authors quantified and measured plastics accumulated in the stomach of 9 species of seabirds accidentally caught by longliners in the western Mediterranean from 2003 to 2010 (Codina-García et al., 2013). The shearwaters *Calonectris diomedea*, *Puffinus yelkouan* and *P. mauretanicus* presented the highest occurrence of litter ingestion (70–94% of individuals according to species) and the greatest number of tiny particles of plastic per affected bird. Yet these species have a restricted distribution in the Mediterranean. The other species, i.e. the Audouin's gull (*Larus audouinii*) and the yellow legged gull (*Larus michahellis*), great skua (*Catharacta skua*), and northern gannets (*Morus bassanus*) are less affected (10–33%). The kittiwake (*Rissa tridactylus*), with an ingestion rate of nearly 50%, represents a locally interesting target species but its distribution in the Mediterranean remains fairly restricted.

4. The threefold monitoring approach to detecting marine litter presence ingestion and the related impact in bioindicator organisms

Understanding the degree to which biota ingest marine litter (including microplastics) is essential to monitor and defining the levels that harm organisms, populations and, ultimately, species that are exposed to marine litter and plastic pollution, altering its ecological functioning, and therefore the community structure. Robust diagnostic methodologies are needed in order to define threshold levels of adverse negative effects. This approach is reliant on toxicological studies with ecological relevance.

The impact of ingested marine litter on marine organisms should be assessed using a threefold monitoring approach described below. This combines an accurate measure of marine litter and microplastic loads in organisms, the evaluation of plastic additives and POPs levels in tissues and the related toxicological effects.

The monitoring approach should rely on the following three types of data (Fig. 5):

i) analysis of gastro-intestinal content to evaluate the marine litter ingested by the organisms, with a particular focus on plastics and microplastics. The results of this analysis must focus on assessing the occurrence (%), abundance (n°), weight (g), colour, polymer type of the marine litter and microplastics ingested by the different species;
ii) quantitative and qualitative analysis of plastic additives (e.g. phthalates and PBDEs) and PBT compounds used as plastic tracers in the tissues of bioindicators;
iii) analysis of the effects of litter ingestion by biomarker responses at different levels of biological organization (from gene/protein expression variations to histological alterations).

Each of the investigation tools of this threefold approach, can be applied independently or simultaneously to the bioindicator

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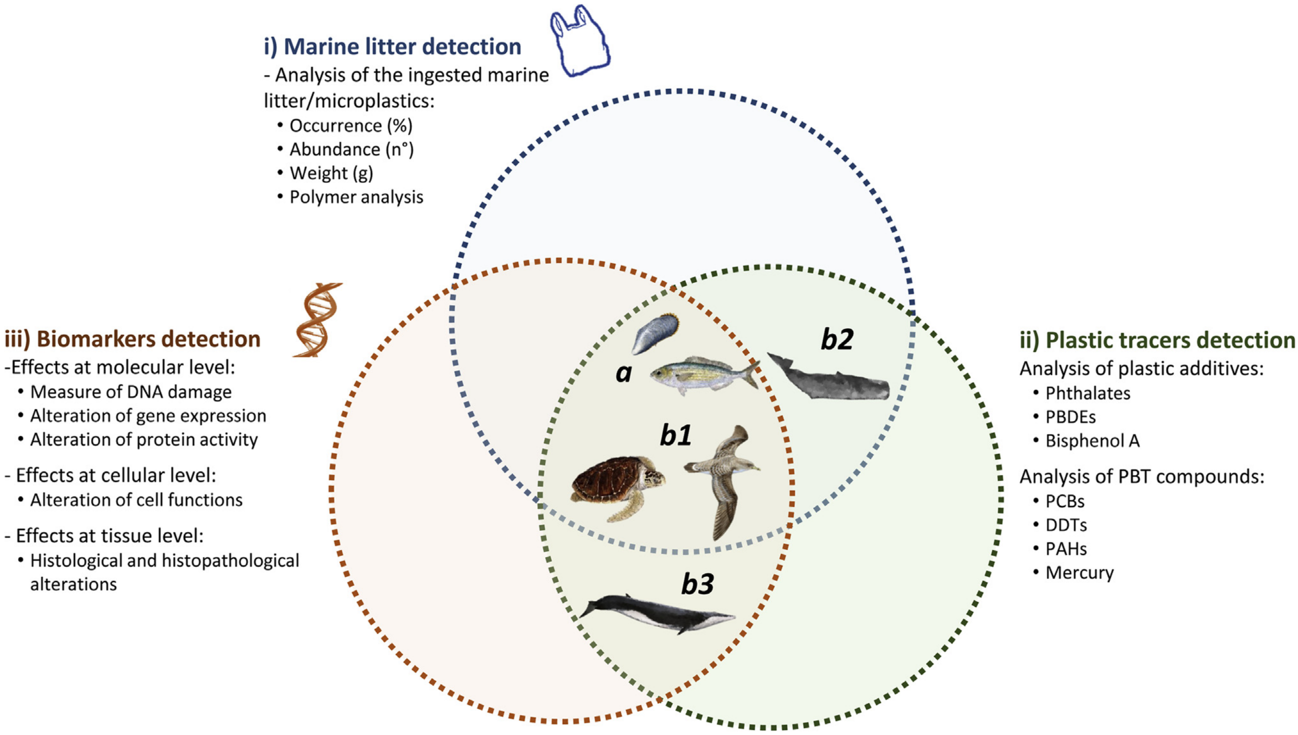

Fig. 5. The threefold monitoring approach to detect marine litter presence and impact in bioindicator organisms.

organisms in relation to the following criteria (see Fig. 5):

a) *For commercial species*, for instance mussels and fish, it is possible to detect the occurrence and rate of marine litter ingestion, and to quantify the potential contaminants accumulation and their relative biological effects (eg. genotoxicity biomarkers, lysosomal stability, lipid peroxidation);
b) *For protected species* (e.g. sharks, sea turtles, seabirds or marine mammals) the approach will depend on whether the organisms have been found dead (e.g. stranded or bycatch) or if a free-ranging organism has been sampled non-invasively:
b1) in hospitalized organisms and stranded organisms (2–3 h after death), it is possible to detect the occurrence of marine litter ingestion, and to quantify the accumulation of possible contaminants and their biological effects (biomarker responses) (Camedda et al., 2014);
b2) in stranded organisms (not in a good state of conservation), analysis of contaminants and gastro-intestinal content (with a particular focus on plastics and microplastics) can be carried out (Matiddi et al., 2017);
b3) an indirect approach can be used for free-ranging animals: the levels of plastic additives, PBT compounds and biological effects can be measured to evaluate the exposure to marine litter, for example using a skin biopsy taken from free-ranging cetaceans (Fossi et al., 2016).

The harm caused to marine organisms by marine litter ingestion is evident, since a large number of marine species ingest plastic and it can cause lethal effects. However, the extent of harm can be underestimated, because of the difficulties in obtaining samples and performing necropsies.

It is more difficult is to determine the level of chemical harm related to plastic ingestion and to ascertain the related sub-lethal impacts. The application of the threefold approach can elucidate not only the occurrence of litter ingestion in the different bio-indicators, but also the multiple sub-lethal stresses caused by marine litter ingestion in the short and long term.

5. 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, to which compartment of the environment, where and when (Werner et al., 2016). Conceptually, risk assessment provides a structured process to inform judgements of the risks posed by an activity or various activities and their significance. It involves four stages including: (1) assessing the potential consequences after exposure at a particular level (hazard identification/characterization); (2) assessment of the exposure (probability that a hazard will occur); (3) the characterization of the risk, combining hazard and exposure; and (4) the evaluation of uncertainties (Werner et al., 2016).

In the case of 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 et al. (2014) 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. Ecological threats to marine biota exist at a population level are often unclear, as is the geographical extent impacts are. To address this knowledge gap, Hardesty et al. (2015) identified a broad 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) ingest litter in nearly all the regions studied, and models of global marine plastic distributions combined with habitat maps and species distribution have enabled to predict 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 field observations 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 to map the probabilities of encounters of sea turtles encountering floating litter to be mapped, and the areas at risk to be defined.

In a recent paper Fossi et al. (2017) investigated the possible overlap between microplastic, mesoplastic and macrolitter accumulation areas and the fin whale feeding grounds in the Pelagos Sanctuary (North Western Mediterranean Sea). 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 marine litter (micro-, meso-, and macrolitter) and on the presence cetaceans were collected simultaneously. The resulting data were compared, as a multi-layer, with the simulated distribution of plastic concentration and the whale habitat model. The simulated microplastic distribution was confirmed by field observations. The fin whale feeding habitat model and the microplastic hot spots overlapped, contributing to the risk assessment of fin whale exposure to microplastics.

The approaches, used in these two papers, predicted where species were most affected, enabling sensitive areas for species-specific ingestion to be defined, and providing 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 used to predict areas where the risk of ingestion occurs, with possible consequences for fish quality and associated risk, including after human consumption.

6. Gaps and future developments

As reported recently by UNEP/MAP (Galgani, 2017), monitoring the impacts of marine litter on marine biota depends on the availability of indicator species in which to measure the prevalence and effects of litter ingestion. It was also underlined that monitoring of effects should 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, necto-benthic, pelagic, aerial) and feeding habits (detritus-eaters, suspension-eaters, omnivores, carnivores) of the species that interact with it. While this paper can contribute to the implementation this strategy, several gaps have been identified, and further work is needed.

6.1. 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 detection methods for 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, with the exception of litter ingestion by Northern Fulmars used to monitor litter 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 used to detect 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. Subsequent polymer identification again uses 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 developed and previously applied to monitoring of chemical contamination (Hylland et al., 2017). Standardized protocols and harmonized monitoring methods are needed to allow spatial and temporal comparisons and to enable assessment of the presence of plastic and its effects in marine biota at subregional and regional level. Inter-calibration exercises can help validate and harmonize methods used across different research teams and laboratories.

6.2. Food chain and human health

Microplastic ingestion by aquatic organisms has been confirmed in laboratory and field work, including in commercially important species. 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 litter in the stomachs of Mediterranean fish presents a potential risk to human health, due to ecotoxicological aspects related to the potential effects of the transfer of contaminants from litter to edible fish tissue. Although the presence of these persistent pollutants in several fish species has already been studied, the origin and pathways of that introduce pollutants into the trophic web has not been yet 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 tissues and then to final consumers (top predators, humans).

6.3. Future developments

Future research is strongly recommended. The transfer of chemicals associated with plastic to 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, this is due to the difficulty in separating the contaminant load due to consumption of natural prey items with 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. Chemicals that are added to plastics during manufacturing, e.g. phthalates and PBDEs, and are found in the marine food chain, particularly in fish consumed by humans should be evaluated. The future investigation of specific “plastic tracers” it is highly recommended.

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 proposed threefold monitoring approach to detect marine litter presence and its

impact in bioindicator organisms is highly recommended. The simultaneous analysis in several bioindicator species of (i) gastrointestinal content, to evaluate the marine litter ingested by the organisms; (ii) tissue, for plastic additives and PBT compounds used as plastic tracers; and (iii) the effects identified by biomarkers responses at different levels of biological organization, will allow a more complete assessment of the real impact.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.11.019.

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