**Implementation of the MSFD to the
Deep Mediterranean Sea**
IDEM
Project Coordinator: Roberto Danovaro

*Review of literature on the implementation of the MSFD
to the deep Mediterranean Sea.*

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Leader: Ifremer (Marie-Claire Fabri)
Participants: CNR, CSIC, DFMR, ENEA, TAU, UB, UM, UNIVPM

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Implementation of the second cycle of the Marine Strategy Framework Directive: achieving coherent, coordinated and consistent updates of the determinations of good environmental status, initial assessments and environmental targets

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Table of Content

INTRODUCTION

The Marine Strategy Framework Directive (MSFD), adopted in June 2008, commits Member States to adopt an ecosystemic approach to manage the marine environment. By this directive, member states aim to achieve good environmental status (GES), described by 11 descriptors, of its marine waters by 2020.

The MSFD applies to the marine area over which a Member state exercises jurisdictional rights in accordance with the United Nations Convention On the Law of the Sea (UNCLOS). This includes deep-sea waters within the European Union Exclusive Economic Zone (EEZ) and embraces, as defined by the MSFD, the water column and the seabed and subsoil under the water column. Presently, the implementation of the MSFD fails in capturing such a huge dimension, and is mostly focused on coastal habitats and those reached by commercial fishing activities, like bottom trawling, a technique that is banned in the Mediterranean Sea below 1000 m and hopefully soon below 800 m after the EU agreed to the terms a ban in 2016. Monitoring, assessing its environmental status and managing the deep sea through the implementation of knowledge on ecosystems' biodiversity, functions and services is crucial actions to ensure the long-term sustainability of neighboring coastal zones and their resources, which rely on the functioning of deep-sea ecosystems. Indeed, recent studies have demonstrated the relevance for the Mediterranean Sea of mass and energy transfers from the shallow continental shelf to the deep basins through high-energy, episodic processes like dense shelf water cascading, open sea convection and severe coastal storms, a set also known as "the three tenors" (see descriptor 7). Such events carry huge amounts of sediments and organic matter to the deep but also anthropogenic CO_2, chemicals and litter. Bottom trawling on the continental shelf and upper slope also contributes to maintain a basin-wide layer of resuspended particles over the seabed, thus leading to a quasi-permanent "dust ocean" condition that is far from the natural one.. Therefore, targeting GES for only the continental shelves while ignoring the deeper Mediterranean environments does not satisfies a sound ecosystem-based approach to the management and protection of this European sea. It is to be noticed that the ecosystem-based management concept is fully considered in the MSFD, but also that there is currently a lack of standards and harmonized methodology for open sea deep waters and seabed.

The above-mentioned 11 descriptors of the MSFD framework cover from biological diversity and habitat quality (descriptor 1, D1) to underwater noise (D11), from introduced species (D2) to marine litter (D10), from status of fish stocks (D3) to contaminants in seafood (D9), from marine food webs (D4) to contaminants' pollution effects (D8), and from eutrophication (D5) to alteration of hydrographical conditions (D7) and seafloor integrity (D6). In practice, assessment of GES against these general indicators and, especially, the various levels of detail in each of them, is a tremendously complex exercise, plenty of data needs and questions that are often hard or impossible to answer from the current knowledge base. To minimise these difficulties and assist and give coherency to the monitoring and assessment process, the MSFD framework considers criteria, methodological standards, specifications and standardised methods, and threshold values. Two levels of **criteria** are distinguished: primary and secondary criteria. While **primary** criteria should be used to ensure consistency across the EU, flexibility should be granted with regard to **secondary** criteria. The use of a secondary criterion should be decided by Member States, where necessary, to complement a primary criterion or when, for a particular criterion, the marine environment is at risk of not achieving or not maintaining good

environmental status. Both primary and secondary criteria are addressed for each descriptor in the body of this document.

The main aim of the current document is to review the available literature, in the broadest sense, to evaluate the state of knowledge on the set of MSFD descriptors ultimately leading to the success or failure in the implementation of the directive with the focus in the deep Mediterranean Sea, i.e. beyond the continental shelf edge.

The internal organization within each chapter in this document is as follows:

The State of the art describes where and what is known on each descriptor. The structure of the section is adapted to each descriptor according to the prerequisites of the MSFD.

The section *Where is the knowledge* aims at identifying both the sources of information and the gaps, either geographically or thematically (when possible). This is addressed graphically by a compilation of the published studies.

The section *Tools used to address each descriptor* indicates the occurrence of monitoring activities, and the numerical tools or approaches used to qualify and/or quantify the existing knowledge. That section also identifies if current MSFD indicators are applicable or adaptable to the deep environments of the Mediterranean Sea.

The *Conclusion* section highlights gaps and provides recommendations when pertinent.

1 Descriptor 1: Biodiversity

Descriptor 1 of the Marine Strategy Framework Directive (MSFD) requires that biological diversity is maintained and that the quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions.

Marine biodiversity is an aggregation of highly inter-connected ecosystem components or features, encompassing all levels of biological organization from genes, species, populations to ecosystems, with the diversity of each level having structural and functional attributes (Cochrane et al., 2016). Structural biodiversity is often addressed using quantitative taxonomic datasets with typical indicators being species (taxon) richness, and population abundance and biomass within an area, between areas or over time (Cochrane et al., 2016). Knowledge of the structural taxonomic biodiversity of a particular area will help to preserve its endemic species especially because habitat destruction is a major driver of species extinctions, particularly those with a narrow distribution range (Cochrane et al., 2016). Sustainable management of deep-sea biodiversity requires science-based integrated knowledge on deep-sea ecosystem functioning, their resilience to anthropogenic stressors and the goods and services they provide (Thurber et al., 2014).

Current knowledge regarding important aspects of the deep-sea biota is insufficient. There is an urgent need to institute comprehensive data system and scientifically robust, standardized quantitative monitoring, inclusive of molecular tools. The resulting information is essential for documenting the full extent of deep sea biodiversity (Danovaro et al., 2010), and for providing information for the development of competent and pragmatic management plans and effective conservation policies.

1.1 State of the Art

For ease of description marine biodiversity in the MSFD has been divided into three compartments; i) the species groups, including birds, mammals, reptiles, fish and cephalopods, ii) the pelagic habitats, and iii) the benthic habitats. It has been decided in the last revision of the European Commission decision (EU 2017/848) that the assessment of the biodiversity for the species groups would not be conducted at the community level, but rather at the population levels.

The two latter compartments encompasses the whole water mass and the ocean floor, respectively. The benthos is further divided by size (i.e. megabenthos to nanobenthos), whether animals or plants (i.e. zoobenthos, phytobenthos) and position to the seabed (i.e. hyperbenthos to endobenthos). Life in the water column is similarly divided by size (i.e. megaplankton to ultranonplankton), whether animals or plants (i.e. phytoplankton and zooplankton), and their ability to maintain their position independent of the movement of water (Lincoln et al., 1998).

The deep Mediterranean Sea is unique, in the sense that there is a high homeothermy from 300–500 m to the bottom, where temperatures vary from 12.8°C–13.5°C in the western basin to 13.5°C–15.5°C in the eastern one; salinity is high and ranges between 36.5–39.5 psu at the surface while it is 38.4 psu and 38.7 psu in the deep water of the west and east basins respectively (Danovaro et al., 1999; Emig and Geistdoerfer, 2004; Edgcomb et al., 2016); oxygen and anthropogenic CO_2 concentrations are high even in deep water, there are limited freshwater inputs, an extreme oligotrophy, pronounced vertical and horizontal gradients, and rapid deep-water turnover (Danovaro et al., 1999; Hassoun et al., 2015).

General spatial patterns show a general decrease in biodiversity from northern to southeastern regions following a gradient of production, with some exceptions and caution due to the gaps in our knowledge of the biota along the southern and eastern coasts (Coll et al., 2010). Specific hydrological structures, such as fronts and the Alboran Sea gyres, locally lead to enhanced production. Also the areas

off the main river mouths (e.g. Ebro, Rhône, Po, Nile) show increased primary production that may extend significantly offshore, interact with mesoscale currents and be exported to the deep compartments. Short-lived events (i.e. weeks to a few months) directly influencing primary production and biodiversity, at least at local and sub-basin scales, play a major role in the dynamics of Mediterranean Sea. This is the case of large storms, cascading of dense shelf waters, offshore convection and the arrival of desert and volcanic dust playing a fertilizing role which impact reaches the deepest levels (Canals et al., 2006; Sanchez-Vidal et al., 2012; Durrieu de Madron et al., 2013; Pedrosa-Pamies et al., 2016). For instance, in some key areas for biodiversity such as submarine canyons and open slopes (see further down), the presence of cold-water corals (CWC), also known as "white corals" because of their color, has been directly linked to the occurrence of dense shelf water cascading (Orejas et al., 2009; Lo Iacono et al., 2012). Generally, biodiversity has been described to be higher in coastal and continental shelves and to decrease with depth (Coll et al., 2010). For many years, investigations on the deep sea suggested that the west–east gradient of decreasing surface water productivity of the Mediterranean Sea was reflected in a corresponding gradient of decreasing food availability in deep-sea sediments (Danovaro et al., 1999; Danovaro et al., 2008a). Such a gradient could be responsible for a significant decrease in the abundance and biomass of most benthic components, including meiofauna, macrofauna, and megafauna. Surprisingly however, recent investigations suggest that there is no corresponding gradient for most components of benthic biodiversity (e.g., number of species, Danovaro et al. (2010)). Only the diversity of foraminifera showed an apparent west-to-east decrease in species richness (Cita and Zocchi, 1978; De Rijk et al., 1999; De Rijk et al., 2000). However, species richness patterns may be more closely tied to research effort than to real species richness pattern (Goren and Galil, 2015).

1.1.1 Species groups of marine mammals, fish and cephalopods

Species groups considered in the deep-sea application of the MSFD cover essentially three groups belonging to marine mammals (i.e. deep-diving toothed cetaceans), deep-water fishes and deep-water cephalopods. Other important groups of species in that environment such as crustaceans, cnidarians, or other benthic species are included in the Commercial species(D3), Habitat Biodiversity (D1), or Sea-floor Integrity (D6) descriptors. In the present section, the focus is on fish and cephalopods. The main reasons for such focal point is the scarcity of data on marine mammals. However, as we will discuss later on, stomach contents of large predators or dead stranded marine mammals can sometime provide informative knowledge on the presence of fish and cephalopod species occurring in the deep-sea ecosystems (Spitz et al., 2011). The group of "deep-water fishes" is composed of fish species living on (benthic) or close (demersal) to the bottom beyond the continental shelf, *i.e.* from shelf edges to abyssal plains, and of species living in the water column (pelagic) at depths where no photosynthesis occurs, *i.e.* beyond the euphotic zone (mesopelagic, bathypelagic, and abyssopelagic zone). An example of deep-sea demersal fish is the blue ling *Molva dypterygia* recorded in MEDITS surveys throughout the Mediterranean sea at mainly 350-500 m depth on muddy bottoms (Fig. 1.1).
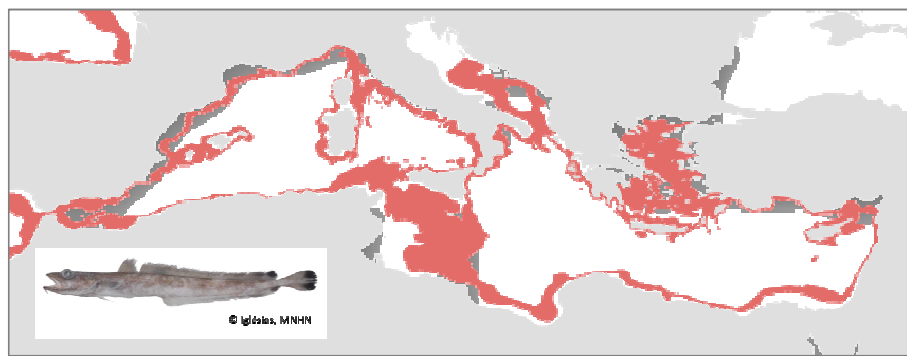
Figure 1.1: Suitable habitat map of Molva dypterygi, from FAO species catalogue (FAO, 1990)

This state of the art section addresses the knowledge we have on the diversity of deep-sea - benthic, demersal, meso and bathypelagic - fish and cephalopods. It first describes the taxonomic and functional diversity patterns of fishes and cephalopods in the deep Mediterranean Sea, notwithstanding the different habitats they might be occurring. Second, it outlines the temporal changes observed in the distributional patterns of the two groups of species. It then presents the studies interested in specific associations between highly structured deep-sea habitats and fish species. Then, the ecological role of the micronecton is described before ending with the anthropogenic pressures and the climate change effects on the deep-water fish and cephalopods.

A) Species taxonomic and functional diversity patterns: depth as a highly structuring factor

In a recent review on the deep-sea biodiversity patterns, Danovaro et al. (2010) indicated the absence of a longitudinal west-east gradient for most components of benthic biodiversity, including fish species. However, many other studies showed the presence of a decreasing deep-sea fish richness gradient from west to east (Cartes and Carrassón, 2004; Sarda et al., 2004; Tecchio, 2012). According to several authors, hydrological characteristics, food availability, sediment type, steepness of the slope, and historic environmental conditions (e.g. anoxic events) are the drivers best explaining fish and cephalopods diversity patterns (Cartes and Sarda, 1992; Cartes et al., 1994; D'Onghia et al., 2004; Bailey et al., 2009; Goren and Galil, 2015).

Throughout the continental slope, a strong zonation of benthic megafauna can be observed, associated with a constant reduction in abundance, biomass and diversity, accentuated under 1500 m (Peres and Picard, 1958; Company et al., 2004; D'Onghia et al., 2004). Below the 2600 and 2700 m isobaths, biomasses of megafauna are extremely low and population densities are reduced to minimum levels (Tecchio et al., 2011a). In parallel, Cartes et al. (2004) showed a general decrease of biomass with depth but they also underlined a second peak of biomass at 800 m corresponding to a shift in species composition (Fig. 1.2).
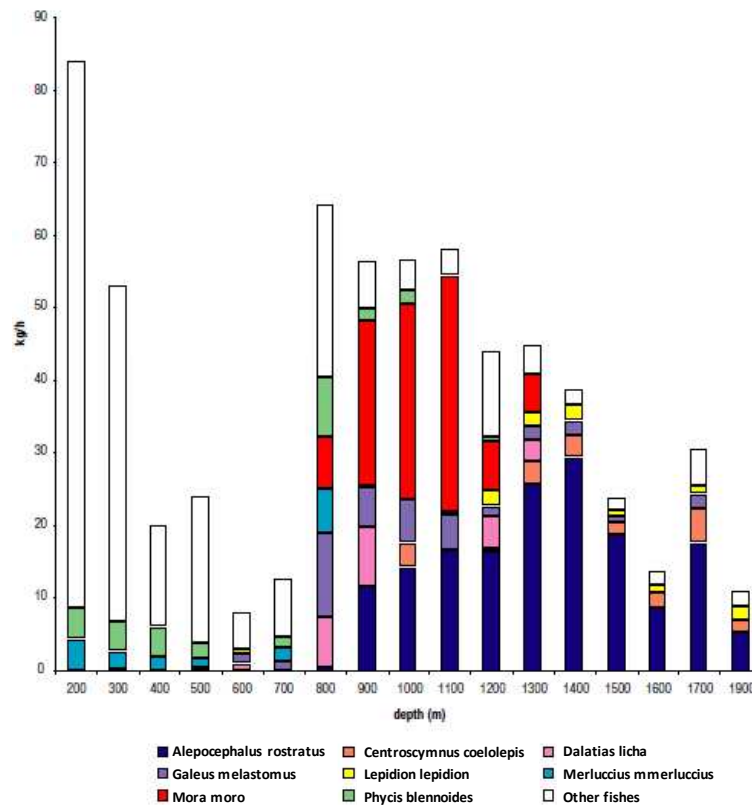
Figure 1.2: Comparison of biomass by depth intervals in the Western Mediterranean Basin, from Cartes et al. (2004).

Peristeraki et al. (2017) looked at fine-scale diversity patterns in relation to depth for major megafaunal groups, including fish and cephalopods, in three subareas of the eastern Mediterranean (Crete, Cyclades and Dodecanese islands). The analyses suggested that the importance of depth-related factors in structuring communities was higher for cephalopods and less important for fish, and that Crete showed a distinct diversity-depth relationship, a fact that can be attributed to its specific geographical and oceanographic characteristics.

Keller et al. (2016) investigated the spatial distribution of cephalopods diversity and found no longitudinal nor latitudinal gradient at the whole Mediterranean Basin scale. They identified hump-shaped cephalopods diversity curves with depths across six locations situated on the western and middle Mediterranean regions (Iberian-Lion, Tyrrhenian, Ionian, Adriatic, Aegean, Strait of Sicily). In all of the Mediterranean bioregions, cephalopod biodiversity peaks at depths between 200 and 400 m (Fig. 1.3). However, it is to be noticed that the maximum depth of this study was 700 m only.
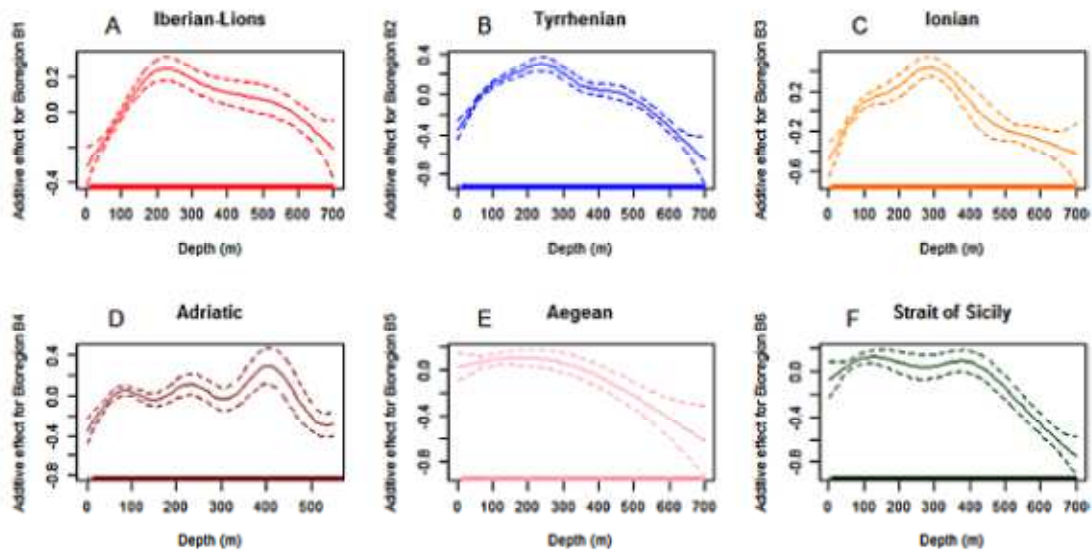
Figure 1.3: GAM outputs on the effect of depth on cephalopods diversity at six bioregions of the Mediterranean sea (Keller et al., 2016).

Recent studies on fish species have gone beyond the description of deep-sea diversity by computing the number of species, abundance and biomass. They looked at the functional characteristics of species, which are key factors for defining the role of each species within communities (Petchey and Gaston, 2006; Mouillot et al., 2014; Brind'Amour et al., 2016). The functional characteristics are quite relevant in understanding the role of (deep-sea) habitats for fish as these characteristics are directly related to foraging and diet strategies, trophic level in food webs, size, locomotion, mobility, lifestyle, activity or distribution within habitat. Hence, functional diversity has been recently used as a tool to i) assess how complementary, and thus more complete and efficient, is the use of the available deep-sea resources, and ii) suggest selective processes, such as resource limitation, that promote species with particular functional traits that perform best under extreme ecosystem conditions. For instance, using morphological traits of fish species collected in the Balearic Islands (Western Mediterranean Sea), Farré et al. (2016) underlined lower richness of body forms and a proliferation of more extreme body traits as depth increases, suggesting lower morphological redundancy. In addition, a trend toward the elongation of body shape was also observed with depth. Functional diversity increased with bathymetry down to 1400 m, where it sharply decreased downwards. The authors showed lower redundancy in deeper waters suggesting an adaptation to environmental conditions and a sharp specialization of species in the deep-sea.

B) Fish and cephalopods association with deep-sea benthic habitats

Submarine canyons seem to act as hotspots for local faunal diversity and biomass, providing strong habitat heterogeneity, increased food availability, and refuge areas for motile species (De Leo et al., 2010; McClain and Barry, 2010). These canyons are known to host diverse fauna (Stefanescu et al., 1994; Ramirez-Llodra et al., 2010b) and influence, along its life cycle, the movements of species of commercial interest, such as the red shrimp *Aristeus antennatus* (Sarda et al., 2009). The same assumptions seem to be valid for other canyons in the Mediterranean Sea, although data in this sense are still scant (Danovaro et al., 2010).

Several studies suggested differences in fish and decapods biodiversity metrics (species richness, species evenness, species composition) among various types of deep-sea habitats (Ramirez-Llodra et al., 2010a; Danovaro et al., 2015). For instance, Ramirez-Llodra et al. (2010b) underlined differences in megafauna community (*i.e.* fish) structure between the open margin *vs.* the canyon heads and walls. In the scientific progress report of the EU project CoralFISH, D'Onghia at al. (2011) noticed that many observations from the literature showed co-occurrence between CWC habitats and fish and cephalopods. However, the association is mostly supposed or unknown and none of the species reviewed in their report displayed specific (or unique) distribution in CWC habitats. Furthermore, the fauna distributed in the various CWC habitats is not taxonomically different from surrounding or regional fauna. CWC and other deep-water habitats are supposed to act as refuges from surrounding fishing grounds ("refuge from fishing") and for juveniles ("nurseries").

C) Seasonal and long-term changes in fish and cephalopods diversity and communities

The deep-sea was thought in the past to be a stable and invariable environment. However, variations of biological processes have been documented over both large time scales (i.e. decadal community shifts; Billett et al. (2010)) and at the 24-h frequency (i.e. day-night migrations; Aguzzi and Sarda (2008)). According to Childress (1995), photoperiodic adaptations of metabolism seem to contribute in maintaining a seasonal pattern in biological activities. For instance, some deep-sea benthic species apparently synchronize their reproductive behavior with the periodic food input from the surface strata caused by phytoplankton blooms, eventually involving high-energy transfer processes, to increase larval survival and hence the fitness of species (Canals et al., 2006). Such processes may also involve the temporary demise of some species locally (Company et al., 2008). Also, seasonal fluctuations of species abundance and certain trophic groups such as bacteria and protozoa, have been documented especially for the upper and middle continental slope (Sarda et al., 1994; Kallianiotis et al., 2000; Ramirez-Llodra et al., 2008). The knowledge on biodiversity seasonal patterns of fish and cephalopods in the deep sea of the Eastern Mediterranean is still very scarce. Although, Madurell et al. (Madurell et al., 2004) recenlty revealed a maximum dominance of pelagic feeders (e.g. *H. mediterraneus* and *C. agassizi*) on the upper slope (473–603 m) in the eastern Ionian Sea (Greece) in August, coinciding with a higher biomass of benthopelagic zooplankton taxa preyed by fish, with significant correlations between both trophic levels.

In addition to the seasonal variability of the deep-sea diversity, Sarda et al (Sarda et al., 2004) also noted the importance of cataclysmic events in structuring momentarily the biological productivity of the deep eastern Mediterranean. These most likely "climate-driven events" create temporary deep oasis that may affect through cascading effect the diversity of large bodied organisms, including fish species.

Biomass, diversity and composition of deep-living fish respond to hydroclimatic changes and fishing pressure in the last 25 years. In a recent study, Cartes et al. (2015) found a generalized deepening of

middle-slope communities (950–1250 m), which was suggested to be a response to the long-term increase in salinity of the Levantine Intermediate Waters (LIW) . They also underlined the shallowing of all of the lower slope species (1600–2250 m), accompanied by a significant decrease of biomass in the last two decades.

D) Environmental drivers of changes

Food supply is an essential factor shaping deep-waters biodiversity (Tecchio, 2012). The rationale underlying that conclusion is that most of the deep-sea is considered to be food-limited, excluding chemosynthetically driven ecosystems or other particular conditions such as areas beneath upwelling systems (Danovaro et al., 2003; Gage, 2003). Bathyal and abyssal ecosystem composition and structure are strongly modulated by the quantity and quality of food sinking or being advected from the surface and edges, respectively, of the ocean or basin (Smith et al., 2008a). Thus, the overall trophic status of deep margin sediments would be controlled mostly by the primary productivity of the overlying waters rather than by the local topography (Pusceddu et al., 2010). In addition to the food quantity limitations, Tecchio (2012) also suggest limitation in food quality compared with the outer Atlantic Ocean due to the enhanced degradation of the organic matter sinking in the water column (Danovaro et al., 1999). The quantity and nutritional quality of sediment organic matter in canyons and adjacent open slopes is not consistent depth-related pattern (Pusceddu et al., 2010).

The deep-sea ecosystems are increasingly submitted to human activities and the most commonly accepted being fishing pressure. Fishing and the effect of fishing is dealt with as a pressure descriptor (D3) in the MSFD. It is also briefly addressed in here as it also influences non-targeted fish species either directly, by resource extraction or by-catch, or indirectly, through food-web interactions. Fishing effort generally declines with depth as it becomes more expensive and the abundances and behavior of fish or other targeted species, such as *Aristeus antennatus*, appears to be related to depth structure of the bottom. Although, fish species inhabiting below 1000 m may also be exposed to fishing even though fishing activity is banned at these depths. Indeed, deep-sea species may undergo bathymetric migration rendering them accessible to fisheries at lower depths (Bailey et al., 2009). According to Sarda et al. (2009), ripe females of certain fish species concentrate in the deepest portions of their distribution range. Vulnerability of deep-sea species is also highlighted by the biology of deep fish species. Hence, several deep-sea fish species are characterized by high life expectancy (*e.g.* the orange roughy, absent in Mediterranean waters but present in Atlantic can live up to 120 years), lower metabolism, slower growth rates, and delayed reproduction, which make them sensitive to fishing exploitation (Sarda et al., 2009).

E) Mesopelagic and bathypelagic fishes

Pelagic zones of the deep-sea represent the widest habitat of the marine realm in the Mediterranean. This compartment shelters a rich biodiversity still largely unknown, partly because of its low economic potential. The oceanic micronekton communities (Fig. 1.4), being defined as organisms swimming actively and classified according to their size (in here between 2 to 10 cm), trophic level or catchability, are potentially of prime importance in the functioning of this marine ecosystem. They play an essential role in the biogeochemical cycles of these ecosystems by their involvement in the maintenance of the biological pump. Indeed, most species of micronekton carry out nychthemeral migrations moving towards the shallow layers to feed at night. These migrations contribute to the vertical transport of carbon, nitrogen, phosphor, mesopelagic organic matter, or even pollutants from their feeding layers towards deeper zones where these intakes are then metabolized (Robinson et al., 2010). The mesopelagic micronekton, including fish, cephalopods, and crustaceans, also plays an important trophic role in supplying marine mammals in the oceanic zone (Spitz, 2014). However, in spite

of their multiple ecological roles in the deep ecosystem, the species composition of the micronekton fish communities is generally badly known (Brodeur et al., 2005). The difficulty in sampling the deep pelagic zones using classical approaches such as pelagic trawling limits existing data and even the prospect of new data.
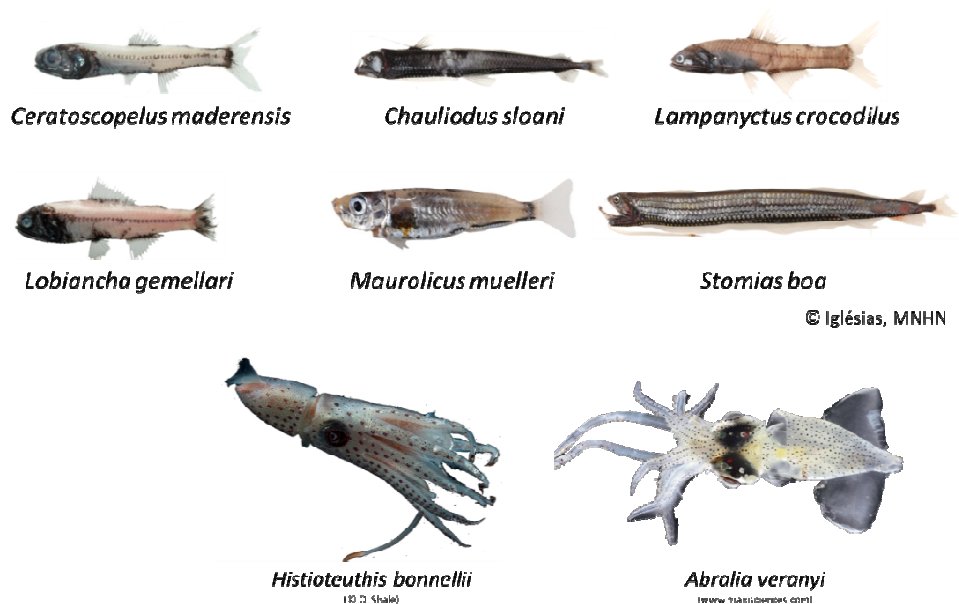
Figure 1.4: Pictures of deep-sea micronekton (fish and cephalopods) in the western Mediterranean (Brind'Amour and Delaunay (2017), adapted from Spitz (2014)).

1.1.2 Pelagic habitats

The diversity of life in the complete pelagic province is difficult to describe, in both qualitative and quantitative terms, because of the variability of the environment and our limited capacity to perform efficient sampling (because of small size plankton or fast-swimming animals). Studying the feeding ecology of pelagic predators can help us to investigate the abundance of a set of species, from macroplankton to fast-swimming nekton (Würtz, 2010) (see descriptor 4).

According to the ICES Working Group on Marine Habitat Mapping, a habitat is: *"A recognizable space that can be distinguished by its abiotic characteristics and associated biological assemblage, operating at particular spatial and temporal scales"* (ICES, 2005). Mediterranean Sea circulation is complex (see descriptor 7) and its interaction with biological processes defines a variety of marine pelagic habitats, from the surface to the deeper waters. Offshore waters are typically considered as oligotrophic, or nutrient-poor. Nevertheless the enrichment of surface layers is assured by upwelling and water mixing, by the concentration and retention of nutrients by eddies and front action. All these oceanographic features determine favorable conditions both for primary production and for the autotrophic and heterotrophic microbial processes. Microbe food webs exceed a thousand times over the production of the "classic food web" (phytoplankton zooplankton-fishes) which "can now be considered as a variable phenomenon in a sea of microbes" (Pomeroy, 2001; Würtz, 2010), thus enhancing the ecosystem's carrying capacity.

Furthermore, reduced continental shelves, steep slopes, canyons and seamounts accelerate through space and time the energy flow and the turnover from the sea bottom to the surface, as well as from coastal to pelagic waters (and *vice versa*). The presence and abundance of top predators in relation to specific topographic and oceanographic structures seem to confirm this new scenario of pelagic productivity (Würtz, 2010). Studies have suggested that odontocetes, for example sperm whales, feeding mainly on deep-squids, are commonly associated with topographic structures such as canyons and submarine mountains (David, 2000), while mysticetes, e.g. fin whales feeding on plankton, aggregate on thermal fronts or convergent structures rich in zooplankton (Druon et al., 2012).

Upper–trophic level predators (top predators) feeding and breeding grounds frequently represent biodiversity hotspots, associated with topographic and oceanographic features. Therefore using the upper–trophic level predators (top predators) as indicators of the ecosystem status and performances may be considered from the point of view of cost-benefit ratio. Their distribution and aggregation may be effectively used in pelagic habitat mapping, even if a whole-system approach could be the proper strategy and the top predators approach could be part of a set of insights and interpretations used for management measures (Boyd et al., 2006).

Three types of pelagic hotspots have been identified (Hyrenbach et al., 2000): **ephemeral habitats**, including wind - current driven upwelling, eddies and water filaments; **persistent hydrographic systems**, like currents, gyres and thermal fronts, and **static systems**, such as seamounts, canyons and other continental slope features. Moreover, among these hotspots some migratory corridors could exist and persist both in location and in time.

Upwelling and deep convections can create small-scale fronts and convergence zones (see descriptor 7). Although the ecological significance of these ephemeral fronts is poorly understood, they appear to constitute important nurseries and foraging habitats for many pelagic species (Hyrenbach et al., 2000). However our knowledge about the biological component of the pelagic system is still poor, both for drawing descriptive mapping and for implementing species environment interaction models. It is clear that most of the future research effort should be devoted to filling this gap.

1.1.3   Benthic habitats

The MSFD benthic broad habitat types in the deep sea, including their associated biological communities, listed in the European Commission Decision 848/2017 are:

> Upper bathyal rocks and biogenic reefs
> Upper bathyal sediment
> Lower bathyal rock and biogenic reef
> Lower bathyal sediment
> Abyssal

It is also stated that Member States may select, through regional or subregional cooperation, additional habitat types. A single set of habitat types shall serve the purpose of assessments of those benthic habitats under descriptor 1 and sea-floor integrity under descriptor 6.

As no extensive exploration has been made, our knowledge on the benthic deep-sea biodiversity is incomplete (Coll et al., 2010). The bathymetric divisions between upper and lower bathyal domains, commonly used in the Atlantic Ocean are hard to follow in the Mediterranean Sea for the purpose of this document. The EUNIS classification (http://eunis.eea.europa.eu/habitats.jsp) for deep sea habitat mapping does not allow enough discrimination for, and is not adapted to the Mediterranean Sea, i.e. Communities of deep-sea corals (A6.61) are only mentioned as Deep-sea *Lophelia pertusa* reefs (A6.611). In the Mediterranean Sea, communities of deep-sea corals are not only based on *L. pertusa* reefs, but also on *Madrepora oculata* and many other species (see hereafter).

In addition, the CoCoNet project (towards COast to COast NETworks of marine protected areas -from the shore to the high and deep sea; http://cordis.europa.eu/result/rcn/189828_en.html) evaluated existing habitats classification schemes and proposed the following main categories for the deep-sea domain (deeper than 200 m) :

> Bathyal rocks and other hard substrata
> > Bathyal rocks
> > Bathyal bioconstructions
> > Anthropic substrates
> Bathyal sediments
> > Bathyal muds
> > Bathyal sands
> > Bathyal coarse sediments
> Bathyal chemosynthetic habitats
> > Hard substrates
> > Sediments
> Abyssal rocky bottoms
> Abyssal sedimentary bottoms

Therefore in this document, benthic habitats are addressed according to the requirements of the MSFD, and according to the specificities of the Mediterranean Sea, with respect to what is currently known. They are addressed as follows:

A- BATHYAL ROCKS AND BIOGENIC REEFS
     a- Head of canyons (rocky canyons)
     b- Large cnidarians on outcropping hard rocks
          Structure forming white corals (*Lophelia pertusa* and *Madrepora oculata)*
          The alcyonacean *Corallium rubrum* (precious red coral)
          The antipatharian *Leiopathes glaberrima* and other black corals
          Forests of *Callogorgia verticillata*
     c- Bivalves on outcropping hard rocks
          Large deep-sea oyster *Neopycnodonte zobrowii* banks
B- BATHYAL SEDIMENTS
     a- Head of canyons (sedimented canyons)
     b- Large cnidarians on open slopes
          Meadows of *Funiculina quadrangularis* and meadows of *Isidella elongata*
     c- Bathyal muds and sands
C-BATHYAL CHEMOSYNTHETIC HABITATS
     a- Soft substrata (Mud Volcanoes)
     b- Hard substrata (Hydrothermal Vents)
D-SEAMOUNTS AND OTHER TOPOGRAPHIC ELEVATIONS (BATHYAL AND ABYSSAL DOMAIN)
E- ABYSSAL

A- BATHYAL ROCKS AND BIOGENIC REEFS

a- Head of canyons (rocky canyons)

Submarine canyons are outstanding features of continental margins from a geological, biological and oceanographic perspectives (See Fig. 1.6). They are focal points for fisheries and are critical habitats for threatened ecosystems. The Mediterranean Sea is estimated to contain more than 800 canyons that are quite distinct from those occurring in the rest of the world: in particular they are steeper, more closely spaced, and are amongst the most dendritic (Harris and Whiteway, 2011; Würtz, 2012).

"Rocky" canyons are indenting steep continental slopes and along them hard rock outcrops are often exposed. Those canyons are mainly distributed on the Riviera and Liguria margins, Western Corsica, western Sardinia, Calabria and Algeria (Migeon et al., 2012). Rocky canyons are also found in the eastern Mediterranean along the southern Aegean domain and off Lebanon (Elias, 2007). Shelf-incising canyons are characterized by steep terrains, containing vertical to overhanging bedrock exposures, which create important habitats for benthic ecosystems where biologically diverse communities may settle and develop (Migeon et al., 2012).

Substrate heterogeneity is a key factor contributing to highly diverse faunal assemblages present in a submarine canyon. Some benthic species, restricted to hard substrata (scleractinians, antipatharians, gorgonians, most sponges) also depend on hydrodynamic processes such as dense shelf water cascading and other gravity currents and internal waves, or on the trapping effect of the canyon topography for their food transport (Canals et al., 2006; Fernandez-Arcaya et al., 2017).

Exploration of rocky substrates and fragmented ecosystem is a real challenge in these rough environments and depends on advanced marine technologies, such as manned submersibles and Remotely-Operated Vehicles (ROVs) (Fernandez-Arcaya et al., 2017).

b- Large cnidarians on outcroping hard rocks

Rocky bottoms associated with strong currents and high turbidity provide ideal environmental conditions for suspension-feeders. Large benthic cnidarians can largely contribute to deep-sea diversity thanks to their habitat forming capabilities resulting in complex three-dimensional habitats that become biodiversity hotspots (Mastrototaro et al., 2010). This type of biocenosis is dominated by the structure forming white corals (*Lophelia pertusa* and *Madrepora oculata*) sometimes associated with the red coral *(Corallium rubrum)*, the black corals (*Leiopathes glaberrima* and *Antipathes fragilis*), the alcyonacean *(Callogorgia verticillata),* the gorgonians (*Viminella flagellum*, *Eunicella* spp., *Acanthogorgia* sp., *Plexauridae* and others), the yellow scleractinian *Dendrophyllia cornigera*, large-sized sponges, and many bryozoans, brachiopods, polychaetes and echinoderms. Decapods of commercial interest like *Palinurus elephas* and *P. mauritanicus* are also common in these rocks.

Cold-water corals (CWC) are one of the main contributors to deep-sea habitat complexity. This term encompasses scleractinians (stony corals), but also antipatarians (black corals), zoantharians, octocoralians and Stylasteridae (Mytilineou et al., 2014). CWC are especially fragile and vulnerable as they are characterized by low productivity, low fecundity, older age at first maturity and high longevity. In addition, they are subject to numerous threats, largely reported in the literature, such as bottom trawling and longlines, seafloor dredging, pollution and ocean acidification (Freiwald et al., 2004; Guinotte et al., 2006; McCulloch et al., 2012; Fabri et al., 2014; Roberts and Cairns, 2014; Lastras et al., 2016).

The Food and Agriculture Organization (FAO) of the United Nations has formulated management guidelines and criteria for defining Vulnerable Marine Ecosystems (VME) among which uniqueness and rarity of species or habitat, functional significance, fragility and structural complexity, and life history limit the probability of recovery (FAO, 2009b). CWC have been identified as sensitive habitats by the General Fisheries Commission for the Mediterranean Sea (GFCM, 2009a), but GFCM has not defined VMEs within its management regulations, and there are no formally declared and adopted VMEs within the Mediterranean Sea. Instead, and through its ecosystem approach, the GFCM has adopted Fisheries Restricted Areas (FRAs) as a multi-purpose spatial-management tool used to restrict fishing activities in order to protect deep-sea sensitive habitats, such as VMEs, and essential fish habitats. And GFCM has protected areas that are known to host organisms that would satisfy the criteria for VMEs according to the FAO Deep-sea Fisheries Guidelines. These areas have been closed to fishing with bottom contact gears, in a manner that is similar to VME closures in other regions (FAO, 2016b).

- Structure-forming cold-water coral ecosystems (*Lophelia pertusa*, *Madrepora oculata*)

Scleractinian species, *Lophelia pertusa* and *Madrepora oculata*, make up the dominant structure-forming corals providing further hard substrate and a three dimensionally complex structure that supports other sessile (e.g. sponges) (Longo et al., 2005; Gocke et al., 2016) and epibiontic organisms (e.g. thoracican cirripeds, mollusls, crustaceans) (Mastrototaro et al., 2010; Angeletti et al., 2011), and offers shelter to mobile species (D'Onghia et al., 2016).

Whereas the true extent of the deep-water CWC community in the Mediterranean Sea is getting progressively known, the occurrence of living *L. pertusa* and *M. oculata* exhibits a scattered distribution pattern. The habitat-forming CWC in have been described to live mostly on vertical walls in the Mediterranean Sea (Freiwald et al., 2009). The present distribution of living deep-water CWC is currently considered to be mainly located in the LIW, an intermediate depth water mass formed in the northern portion of the Eastern Mediterranean Basin that flows westward all across the basin (Freiwald et al., 2009; Taviani et al., 2017). These oceanographic factors coupled with proper substrata, such as steep (canyon heads, seamount walls) or rugged (slumped blocks) topographies, determine the CWC

distribution (Taviani et al., 2011; Lastras et al., 2016; Fabri et al., 2017). However, there are also places, such as submarine canyons in the Northwestern Mediterranean Sea where CWC occur in the uppermost levels of the LIW and in shallower waters above the LIW (Orejas et al., 2009; Lo Iacono et al., 2012; Lastras et al., 2016; Fabri et al., 2017). Although the inventory of CWC sites is still incomplete, there are evident differences amongst bioregions. In particular live CWCs are rather common in the Western, Central and Adriatic seas whereas they are much sparser in the Eastern Mediterranean Basin with no known CWC sites in the Aegean-Levantine area.

More precisely, structure-forming CWC are in the following western Mediterranean Sea locations:
Strait of Gibraltar (Alvarez-Perez et al., 2005; De Mol et al., 2012); Melilla margin in the Alboran Sea (Fink et al., 2013); La Fonera (Lastras et al., 2016), Cap de Creus (Orejas et al., 2009; Gori et al., 2013), Lacaze-Duthiers Canyon (Zibrowius, 2003; Gori et al., 2013; Fabri et al., 2014), Cassidaigne (Bourcier and Zibrowius, 1973; Fabri et al., 2014; Fabri et al., 2017) canyons in the northwestern Mediterranean Sea; Levante canyon in the Ligurian Sea (Fanelli et al., 2017); Nora canyon off Sardinia (Taviani et al., 2017).
And also in the following Central Mediterranean Sea locations: the Urania Bank and the Linosa trough and other places of Sicily and Malta (Schembri et al., 2007; Freiwald et al., 2009; Evans et al., 2016), off Gallipoli and Santa Maria di Leuca, in the northern Ionian Sea besides Otranto Strait, where there is an extended CWC province (Tursi et al., 2004; Freiwald et al., 2009; Mastrototaro et al., 2010; Vertino et al., 2010; D'Onghia et al., 2011; D'Onghia et al., 2012; Negri and Corselli, 2016; Bargain et al., 2017; D'Onghia et al., 2017).
And in the Adriatic Sea: Bari Canyon (Bo et al., 2012; Sanfilippo et al., 2013; Angeletti et al., 2014; D'Onghia et al., 2015a) and Gondola Slide (Angeletti et al., 2014; Taviani et al., 2016)
And finally in the Eastern Mediterranean Sea off Thassos (Vafidis et al., 1997) (Fig. 1.5).

Recently, live aggregations of another CWC species, *Desmophyllum dianthus*, have been found deeper than 1,400 m in the La Fonera Canyon, which is a new depth record for this species in the Mediterranean Sea (Aymà et al., 2018). This finding proves how much is still to be discovered about CWC and other groups' distribution in the deep Mediterranean Sea.

CWC communities of the Mediterranean Sea are never as large as those found in the Atlantic Ocean, where the prevailing conditions are more favorable, with water temperatures around 4°C and salinities around 35 psu whereas Mediterranean Sea waters are much warmer and saltier (see section 1.1). According to current knowledge on the temperature limits for CWC, the Mediterranean Sea is close to the upper tolerance limit of the species occurring in its bathyal domain (i.e. 14.7°C for *L. pertusa* in the Atlantic Ocean but 25.5°C for *M. oculata* in the Indian Ocean) (Keller and Os'kina, 2008).
In addition to their sparser distribution and to the generally smaller size of their colonies and aggregations, CWC of the Mediterranean Sea, are often damaged by bottom long lines and other fishing gear (Lastras et al., 2016; D'Onghia et al., 2017; Fanelli et al., 2017). In Maltese waters, they have been recorded entangled with ropes used to anchor fish aggregating devices (LIFE BaHAR for N2K project, unpublished data).
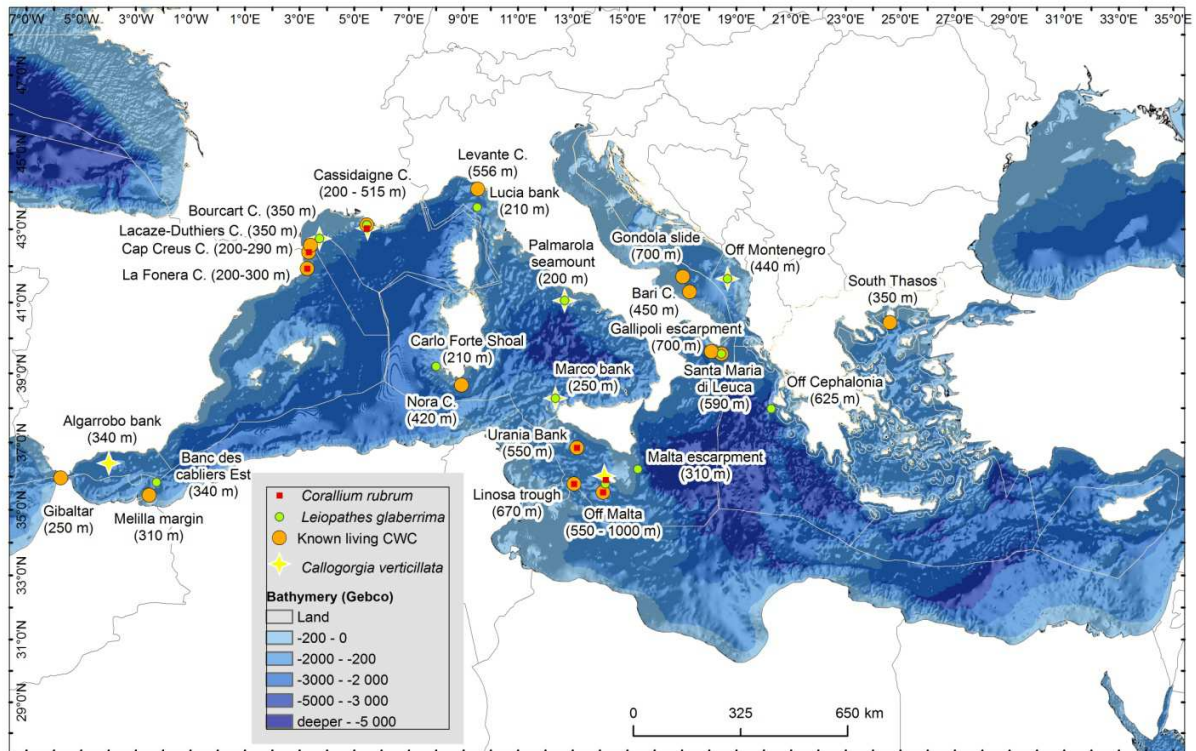
*Figure 1.5: Distribution of several species of cnidarians living on rock outcrops in the deep (>200 m) Mediterranean Sea: Cold-Water Coral (*Lophelia pertusa *and/or* Madrepora oculata*) as yellow dots, Callogorgia verticillata as orange-white stars, Leiopathes glaberrima as green dots, Corallium rubrum as red squares, updated from Freiwald et al. (2009) and Taviani et al. (2016).*

- The alcyonacean *Corallium rubrum (*precious red coral)

The precious red coral *Corallium rubrum* has also been recorded in several places in the deep Mediterranean Sea where it is found in association with CWC (Rossi et al., 2008; Costantini et al., 2010; Lastras et al., 2016) (Fig. 1.5), such as off Malta at 458 m depth and in the Linosa Throught at 673 m depth (Freiwald et al., 2009; Costantini et al., 2010). Few colonies were also found in La Fonera canyon (down to 290 m depth; Lastras et al., 2016), Cap the Creus (down to 230 m depth; Rossi et al., 2008) and Cassidaigne canyon (down to 210 m depth; Fabri et al., 2014) together with *M. oculata* colonies.

Recent ROV investigations in Maltese waters have detected the occurrence of *C. rubrum* down to a maximum depth of 1,016 m, where it formed part of a cnidarian-dominated megabenthic community on deep-water hard substrata characterized by a mixture of habitat-forming scleractinians, gorgonians and antipatharians (e.g. in order of abundance: *C. verticillata, M. oculata, Placogorgia massiliensis, Muriceides lepida, Isozoanthus primnoidus, Pachastrella monilifera, L. pertusa, Acanthogorgia hirsuta, D. dianthus* and *L. glaberrima*). Habitats where red coral colonies were found included rocky outcrops and slopes, vertical escarpments, overhangs, and in several instances, dead coral frameworks (Knittweis, 2016).

- The antipatharian *Leiopathes glaberrima* and other black corals

Antipatharians commonly known as black corals, have been recognized in the last decade to be among the most important anthozoan components of Mediterranean deep coral gardens (del Mar Otero et al., 2017). Four species are commonly reported from the Mediterranean Sea: *L. glaberrima, Antipathella subpinnata, Antipathes dichotoma* and *Parantipathes Larix.*

Leiopathes glaberrima is a tall arborescent black coral (antipatharian) species structuring facies of the deep-sea rocky bottoms of the Mediterranean Sea. Their patchy distribution and limited larval dispersal ability (Deidun et al., 2015) , together with their slow growth rates emphasize their fragility. They are commonly found together with CWC (*L. pertusa* and *M. oculata*) but they can also be associated with *C. verticillata*, and occur alone (Fig. 1.5). Antipatharians, which known bathymetric range is from 60 to 450 m depth, are severely damaged by fishing activities. When appearing in large abundances they form the so-called "antipatharian forests", as seen in the Alboran Sea on the Cabliers Bank (Pardo et al., 2011); in the Bourcart and Cassidaigne canyons in the Gulf of Lion (Fabri et al., 2014); on Carlo Forte shoal off south western Sardinia (Bo et al., 2015); in the Tyrrhenian Sea on Santa Lucia Bank (Bo et al., 2014a); Palmarola Seamount (Ingrassia et al., 2016) and Marco Banks (Bo et al., 2014b); in the Adriatic Sea off Montenegro (Angeletti et al., 2014) and in Santa Maria di Leuca Province (Mastrototaro et al., 2010); in the Ionian Sea off Cephalonia (Vafidis et al., 2006; Mytilineou et al., 2014); and on Malta escarpment (Angeletti et al., 2015) and off southern Malta (Deidun et al., 2015; Evans et al., 2016). Interestingly, in Maltese waters the main habitat-forming taxa are vertically stratified*: L. glaberrima* is dominant at depths of 200-400 m, *M. oculata* dominates in deeper waters with peak abundances at depths of 500-700 m, and patches where *C. verticillata* is dominant occur at depths of 800-1000 m (Evans et al., 2016).

The longevity of *L. glaberrima* has been investigated using radiocarbon techniques. This arborescent species may live thousands of years. A colony from south-western Sardinia was 1973 years old when it was collected as a fishery by-catch (Bo et al., 2015).

- Forests of *Callogorgia verticillata*

The fragile and poorly known alcyonacean *C. verticillata* may be highly abundant on rocky substrates at depths of ca. 350-1000m, with its highest densities known at depths of 800-1000 m (LIFE BaHAR for N2K project, unpublished data). Mastrototaro et al. (2010) also recorded it at depths of 425-910m. It can grow up to a height of 1 or 2m. It is a suspension feeder forming large fans (1 m wide) oriented in the direction of the predominant current. This species is often seen associated with species of epifauna not observed in other communities, making this association unique (Nudibranchia Tritoniidae, zoanthid *Isozoanthus primnoidus*). This species is often seen associated with epifaunal species not observed in other communities, making this association unique (e.g. Nudibranchia Tritoniidae, zoanthid *Isozoanthus primnoidus*). This species appears accompanied by Porifera, Scleractinia (*D. cornigera*, *D. dianthus* and *M. oculata*), Antipatharia (*L. glaberrima* and *Antipathes* sp.) and also other alcyonaceans including *Acanthogorgia hirsuta, Swiftia pallida, Bebryce mollis, Paramuricea macrospina, Muriceides lepida, Villogorgia bebrycoides* and *Placogorgia* spp.(Angeletti et al., 2014; Bo et al., 2014b; Evans et al., 2016), Echinodermata (*Antedon* sp., *Cidaris cidaris*, *Echinus melo* and *Echinus* sp.), Crustacea (lobster *Palinurus mauritanicus*, Caridea *Plesionika* sp. and Galatheoidea), Mollusca (Octopodidae), Actinopterygii (*Phycis blennoides*, *Phycis phycis*, *Lophius piscatorius*, *Helicolenus dactylopterus*, *Scorpaena scrofa, Conger conger*, *Benthocometes robustus* and Trichiuridae), and Holocephali Chimaeridae (*Chimaera monstrosa*) (Fabri et al., 2014).

In the Western Mediterranean Basin (Fig. 1.5), large colonies of *C. verticillata* have been observed in the Alboran Sea on the Algarrobo Bank (Pardo et al., 2011), in the Gulf of Lion in the Bourcart and Cassidaigne canyons Fabri et al., 2014; Fabri, unpublished data), and in the Tyrrhenian Sea on the

Palmarola Seamount (Ingrassia et al., 2016). Meadows of *C. verticillata* have been also reported from the Adriatic Sea off Montenegro (Angeletti et al., 2014) and off Malta (Evans et al., 2016). Few *C. verticillata* colonies are encountered on the open slope of the Gulf of Lion, always settling on outcrops rocks (Fabri, unpublished data).

This structure-forming cnidarian sheltering high diversity is often seen entangled in longlines and nets (Bo et al., 2014b). Hard bottoms in the upper bathyal zone of the Mediterranean Sea should be further investigated to census occurrences of this cnidarian in order to better assess its distribution and vulnerability.

c- Bivalves on outcropping hard rocks

- Large deep-sea oyster *Neopycnodonte zibrowii* banks

The large deep-sea oyster *N. zibrowii* can reach a length of 26 cm (Wisshak et al., 2009). Live *N. zibrowii* in the Mediterranean Sea were observed between 300 and 900 m deep in the Western and Central basins (Beuck et al., 2016). *N. zibrowii* grows on hard substrates in current-exposed zones, such as rocky overhangs and (sub-) vertical rock exposures on seamounts, escarpments and canyons. The lifespan of the thick-shelled *N. zibrowii* can exceed 500 years, thus belonging to the longest-living non-colonial animals known (Wisshak et al., 2009).

Live *N. zibrowii* and their post-mortem remains serve as substrate for their own larvae, generating a built-up over several generations forming chaotic clusters or stacks. Also, they provide a suitable space for a diverse associated community of attached/excavating sclerobionts, mobile organisms and demersal benthopelagic fishes and, therefore, configure a well-defined deep-sea habitat. *N. zibrowii* often co-occurs with scleractinians, such as *L. pertusa*, *M. oculata* and *D. dianthus* (Beuck et al., 2016).

The assessment of their global distribution in the Atlantic and in the Mediterranean Sea is in progress (Beuck et al., 2016).

B- BATHYAL SEDIMENTS

a- Head of canyons (sedimented canyons)

"Sedimented canyons" are partly fed and covered by recent soft sediments. These features cut across wide and thick sedimented continental shelves and slopes and supply sediment to large clastic cones known as "deep-sea fans"; most of these canyons off old and modern river mouths, and it is known they were more active during sea level Quaternary low stands. The Northwestern Mediterranean Sea including the Catalan, Gulf of Lion and Ligurian margins is characterized by a dense network of submarine canyons actually forming a complex submarine drainage network for sediment and dense shelf waters (see section 1.1) (Migeon et al., 2012; Canals et al., 2013). Other margins with abundant submarine canyons are those of Corsica and Sardinia, the southern tip of mainland Italy and Sicily, amongst other. Canyon density off eastern Lybia and western Egypt is also high (Fig. 1.6). To fully understand the role of submarine canyons with respect to biodiversity it is convenient to understand how canyon systems form, develop and behave, and the nature of their interactions with water column and near-bottom physical processes, organisms and human activities.

Canyons play a fundamental role in enhancing the abundance and diversity of marine organisms through the transport of organic matter and other food resources and seascape heterogeneity at various scales. Canyons are characterized by steep and complex topography that influences current patterns and provides a heterogeneous set of habitats, from rocky wall and outcrops to soft sediment (Fernandez-Arcaya et al., 2017). The complexity of canyon systems may benefit benthic fauna in several

ways, allowing the occurrence of a high functional diversity from sessile to highly motile species, including suspension feeders, detritivore sediment-dwelling feeders, planktivores and scavengers (Shepard et al., 1974; Macquart-Moulin and Patriti, 1996; Vetter and Dayton, 1999; Vetter et al., 2010). Because they concentrate sediments rich in phytodetritus and other sources of organic matter, canyons show frequently enhanced abundances and/or biomasses of benthic, benthopelagic and pelagic fauna (Albaina and Irigoien, 2007; McClain and Barry, 2010; Cunha et al., 2011).

Some epi-benthic species live or find refuge on the flanks of canyons (e.g. most pennatulids and some scleractinians, gorgonians and sponges) where enhanced levels of primary production delivered by strong currents may support high densities (Bo et al., 2012; Fernandez-Arcaya et al., 2017).

Patches of organic detritus have been described as hotspots of food sources in canyons. These patches not only support locally high numbers of detritus-feeders, but also a variety of crustaceans associated with downwelling. Sediment instabilities, turbidity flows, dense water cascades and other gravity-driven fluxes give rise to disturbance regimes in canyons that can affect the dynamics of some benthic and benthopelagic populations and communities. For example, the benthopelagic deep-sea shrimp *A. antennatus* may experience periodic cycles of disturbance, recolonisation and eventual recovery of communities due to the occurrence of dense shelf water cascading events (Company et al., 2008). Episodic disturbance events contribute to the instability of sediments, making conditions temporarily unfavorable to many infaunal species (e.g. nematodes and foraminifera) leading to the dominance of opportunistic species inside canyon systems (Hess et al., 2005; Gambi and Danovaro, 2016).
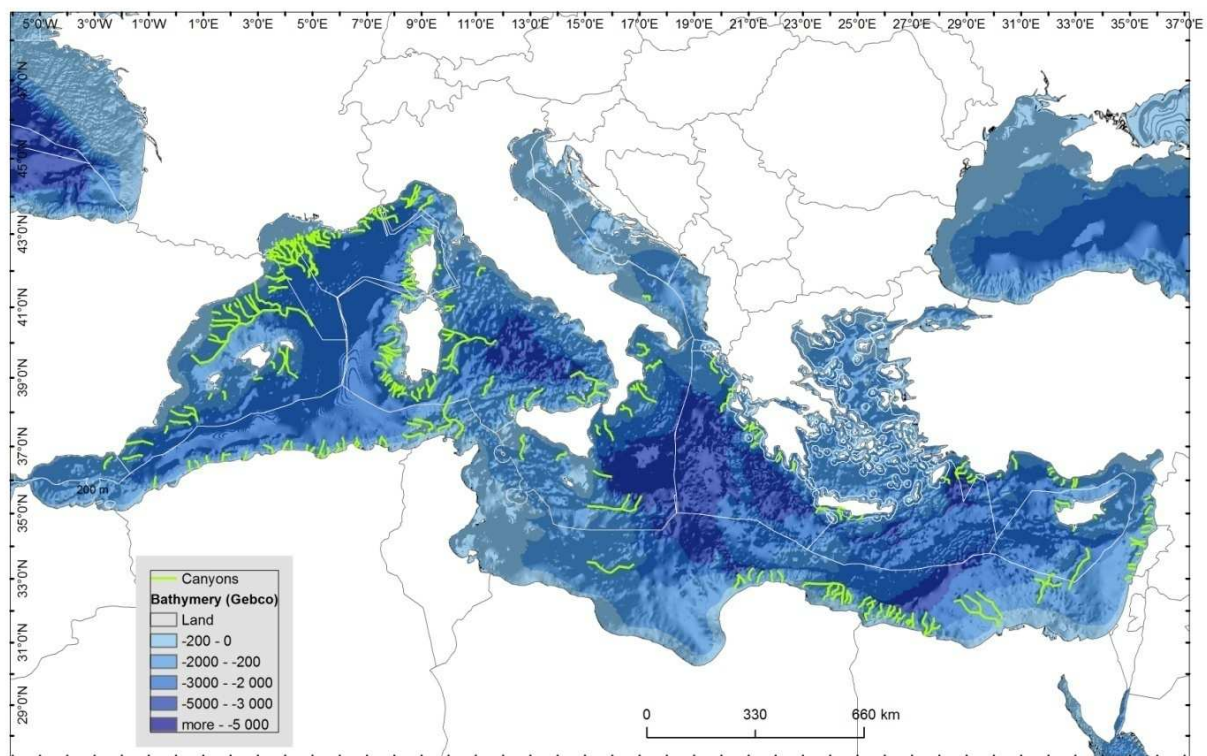
Figure 1.6: Distribution of the main canyons of the Mediterranean Sea, adapted from Migeon et al. (2012)

Soft bottoms infaunal communities are historically considered with regard to their size. As mentioned above (cf. Section 1.1), three size classes are usually considered: meiofauna (20 to 250 μm), macrofauna (250 μm to 2 cm) and megafauna (> 2 cm) (Ritt et al., 2012; Lampadariou et al., 2013). However, the limits between size classes may change according to the method used by every research team (Kroncke et al., 2003; Baldrighi and Manini, 2015; Lubinevsky et al., 2017). Recently, bacteria, viruses and prokaryotes have been also investigated (Danovaro et al., 2015).

The decrease in the diversity and biomass of the meiofauna and macrofauna with an increasing depth, and from the Western to the Eastern Basin, has been shown since long and is explained by the limited food inputs reaching the deep seabed (Soetaert et al., 1991; Tselepides et al., 2000; Riaux-Gobin et al., 2004; Tselepides et al., 2004; Danovaro et al., 2008b; Soetaert et al., 2009; Lubinevsky et al., 2017). However, a global survey demonstrated that macrofauna abundance does not show any decrease, but a change in the community composition and in the trophic structure from the Western to the Eastern Basin (Baldrighi et al., 2014). Macrofauna in the Western Mediterranean Sea was studied locally in the Gulf of Lion (Reyss, 1970; Bourcier et al., 1993; Gerino et al., 1995; Stora et al., 1999; Mamouridis et al., 2011), north of Sicily (Romano et al., 2016) and in the Adriatic Sea (D'Onghia et al., 2015a). Episodic events involving the sudden arrival of large amounts of edible matter may trigger a fast response by the deep-water pelagic and benthic communities, as shown both in the Western and the Eastern Basin (Tamburini et al., 2013; Pedrosa-Pamies et al., 2016).

Temporal change in meiofaunal diversity has been also evidenced through a study conducted during 3 years at the DYFAMED station on the side of median fan valley of Var Canyon at about 2350 m depth in the Ligurian Sea. Results showed a high seasonal and interannual fluctuation due to a recurring food inputs from the surface and physical disturbances that generated profound modification in the community structure (Guidi-Guilvard and Dallot, 2014).

b- Large cnidarians on open slopes

The GFCM has issued a list of criteria for the identification of sensitive habitats of relevance for the management of priority species in the Mediterranean Sea (GFCM, 2009a). These habitats are potentially vulnerable as fisheries target them.

- Meadows of *Funiculina quadrangularis* and meadows of *Isidella elongata*

Soft mud facies with *F quadrangularis* forms an essential habitat for some crustacean species (e.g. *Parapenaeus longirostris* and *Nephrops norvegicus*) and compact mud facies with *I. elongata* are relevant habitats for red shrimps (*A. antennatus* and *Aristaeomorpha foliacea)*. These habitats were historically abundant in the Western Mediterranean Sea, but today they seem to have been swept away by persistent trawling (Maynou and Cartes, 2012; Cartes et al., 2013; Fabri et al., 2014; Pierdomenico et al., 2016; Mastrototaro et al., 2017).

Isidella elongata is listed as critically endangered species in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (http://www.iucnredlist.org/details/50012256/3). The declining trend of the population is inferred to be around 80% over 100 years, and probably is less than three generations. It seems that surviving meadows thrive only in certain areas appearing less suitable for fishing activities such as shallow enclaves protected by hardgrounds or deeper than 1,000 m where trawling is forbidden (Mytilineou et al., 2014; Bo et al., 2015; Evans et al., 2016; Mastrototaro et al., 2017). Whereas the maximum depth range published in the literature for *I. elongata* is 1800 m in the Mediterranean Sea (Fabri et al., 2014), this species is mainly described between 500 and 700 m depth

(Maynou and Cartes, 2012; Cartes et al., 2013; Fabri et al., 2014; Pierdomenico et al., 2016; Mastrototaro et al., 2017). The recovery ability of this species is very low, due to extremely slow growth rates, low dispersal ability, and a very long life span.

The destruction of these coral gardens (*I. elongata)* involves a great ecosystemic and biodiversity loss as all pelagic and demersal species gravitating around these habitats are affected. The Mediterranean population probably represents the large majority of the global population, as there are very few Atlantic records, such that *I. elongata* is considered nearly-endemic (http://www.iucnredlist.org/details/50012256/3).

c- Bathyal muds and sands

Benthic invertebrates in bathyal sediments are diverse and abundant, but often patchily distributed, depending on the background fluxes of particles and on event-driven pulses of organic matter. Species may adapt to this scarceness by applying a wide variety of responses, such as feeding specialization, niche width variation, and reduction of metabolic rates (Tecchio et al., 2013b). Small invertebrates are functionally important because they burrow deeply into sediments and accelerate nutrient cycling (Pape et al., 2013). Benthic macrofauna (i.e. bivalves, polychaetes, amphipods, gastropods) mix the sediments, aerate the deeper layers and increase rates of nutrient recycling by bioturbation and fecal production (Covich et al., 1999).

Man-made pressures like oil and gas extraction, fishing as disposal of industrial waste can affect sediment composition and properties and, therefore, infaunal diversity.

C-BATHYAL CHEMOSYNTHETIC HABITATS

a-Soft substrata (Mud Volcanoes)

Mud volcanoes are geological structures where mud, water and gas are seeping through the seafloor. This may involve releasing large quantities of methane to the seafloor and deep waters. In the Mediterranean Sea they occur both in active and passive settings, such as the Mediterranean Ridge and the Nile deep-sea fan, respectively. Data on the distribution and characteristics of Mediterranean mud volcanoes and other significant fluid or gas venting features (i.e. mud cones, mud pies, mud-brine pools, mud carbonate cones, gas chimneys and pockmark fields) have been compiled by Mascle et al (2014) (Fig. 1.7). Large quantities of methane are often released at the surface of the mud volcanoes.
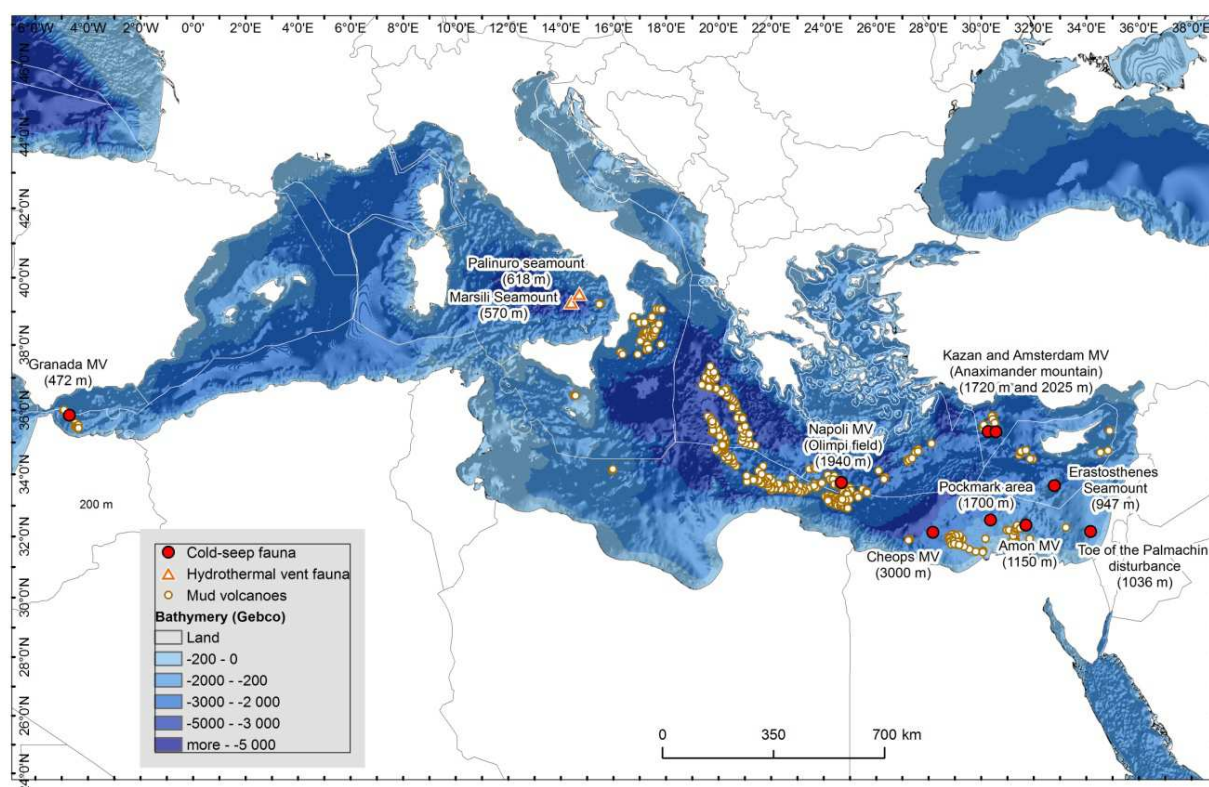
Figure 1.7: Distribution of mud volcanoes (white-brown dots) and known chemosynthetic fauna on hydrothermal vents (white-red triangles) or cold-seeps (red dots) in the Mediterranean Sea, adapted from Mascle et al. (2014).

It is known that several mud volcanoes are colonized by various chemosynthesis-based communities (Ritt et al., 2012). Archaea and bacterial consortia use the methane to produce sulfides in the sediment by chemoautotrophic processes whereas symbiotic bacteria use methane or sulfide to sustain high biomasses in large size invertebrates. In the Alboran Sea, chemosynthetic polychaetes were described in 2011 on Granada mud volcano (Hilario et al., 2011). The biological communities of the Olimpi mud field of the Napoli Volcano on the Mediterranean Ridge and Kazan and Amsterdam mud volcanoes on the accretionary prism along the Cyprus Arc south of Turkey have been extensively studied (Olu-Le Roy et al., 2004; Werne et al., 2004; Ritt et al., 2011; Ritt et al., 2012). On the contrary, the Anaximander Mountains off southern Turlkey, with Amon, Osiris, Isis and North Alex volcanoes, as well as fluid release

structures on the Nile deep-sea fan, such as Menes Caldera, Cheops Volcano and pockmark fields, have been studied for the geological viewpoint but not the biological one (Dupre et al., 2007; Bayon et al., 2009; Huguen et al., 2009). In 2010, chemosynthetic fauna was discovered off Israel on the Toe of the Palmachim Disturbance (Dwight et al., 2012) and on the Eratosthenes Seamount (Rubin-Blum et al., 2014) with the NOAA ship *Okeanos Explorer* (Bell et al., 2012).

Small bivalves from families Mytilidae, Vesicomyidae, Lucinidae and Thyasiridae dominate the chemosynthetic communities of Mediterranean mud volcanoes. Siboglinid polychaetes as well as heterotrophic mega- and macro- faunal species have also been observed. While some mud volcanoes are more active (i.e. those in the Cyprus Arc) than others (i.e. the Olimpi mud field), high methane and low oxygen concentration are typical of these environments, which constitute a rather constraining environment for faunal colonization subsequently inducing higher faunal densities but lower taxonomic richness (Ritt et al., 2012). However, at the micro-habitat scale mud volcanoes display high heterogeneity, which seems to control the structure of seep communities rather than geographical location.

Species composition and biomass of nematode communities have been shown to vary significantly between two investigated seeps Amsterdam and Napoli mud volcanoes, both located in the Eastern Mediterranean Basin. Nematode assemblages in the mud volcanoes are highly dominated by non-selective deposit feeders (i.e. direct swallowing), whereas their periphery shows assemblages typical of the deep-sea with an almost uniform distribution of feeding strategies indicative of non-specialized nematodes (Lampadariou et al., 2013; Kalogeropoulou et al., 2015).

b-Hard substrata (Hydrothermal Vents)

Chemosynthetic fauna associated to hydrothermal vents has been studied on rocky outcrops of Palinuro and Marsili volcanic seamounts in the Tyrrhenian Sea (Carey et al., 2012; Rubin-Blum et al., 2014; Zimmermann et al., 2014) (Fig. 1.7).

Palinuro Seamount is a complex feature, consisting of five coalescing volcanic edifices lying along an east-west trending fault system that extend seaward off the northern limit of Calabria (Carey et al., 2012). Massive sulfide fragments had been recovered from the western portion of the seamount at approximately 600 m of water depth by gravity coring, dredging, and video-guided grab sampling (Monecke et al., 2009). Exploration by ROV revealed fluid venting at temperatures up to 54°C. Large colonies of living tubeworms (Siboglinidae) draped by cotton- and cobweb-like bacteria surrounded the venting sites (Carey et al., 2012).

Marsili is a diffuse hydrothermal vent site at a depth of approximately 550 m on Marsili Seamount in the Tyrrhenian Sea (Fig. 1.7). Annelids (Siboglinidae) observed and sampled were *Lamellibrachia anaximandri* (Zimmermann et al., 2014). Single tubeworms, small groups and large bushes of several hundreds of them were observed. They grew on steep basalt cliffs and in crevasses. Marsili Seamount is known to be an active venting site. However, at the time of sampling, physicochemical conditions at Marsili Seamount more closely resembled those from cold seeps than those typically found at hydrothermal vents (Zimmermann et al., 2014).

D - SEAMOUNTS AND OTHER TOPOGRAPHIC ELEVATIONS (BATHYAL AND ABYSSAL)

Seamounts feature all the world oceans and may be defined as hot spots of biodiversity, greatly affecting the productivity of offshore ecosystems (Würtz and Rovere, 2015). There is no generally accepted definition for a seamount. Geologists will call seamount a conical or flat-topped (also named "guyot") elevation taller than 1000 m above the surrounding seafloor. However, biologists and ecologists feel comfortable with a broader and simpler definition of the term seamount, as they are interested in seamount effects on both the pelagic and benthic communities. Seamounts include all elevations rising more than 100 m -from the surrounding seafloor, but excluding those elevation rising within the continental shelf (Staudigel et al., 2010). Seamounts interact with currents and create flow complexities, which depend upon current speed, water mass stratification and seamount morphology. Seamount effects and interactions display a great variability depending on overall size and shape, water depth and location, seabed type and summit character (i.e. conical, uneven or flat), and appearance either as single features, alignments or clusters. Current knowledge on seamounts is far less comprehensive than for many other marine ecosystems (Staudigel et al., 2010) and available information is marked by large gaps, mainly on the Eastern Mediterranean seamounts (Morato et al., 2013), but also by an asymmetry between the amount of geological studies and the biological ones. Seamounts in the Mediterranean Sea concentrate in specific sub-basins and regions such as the Alboran Sea, around the Balearic Promontory, the deep Algero-Balearic Basin, the Tyrrhenian Sea, the Sicily Channel and the Aegean Sea (Fig. 1.8).
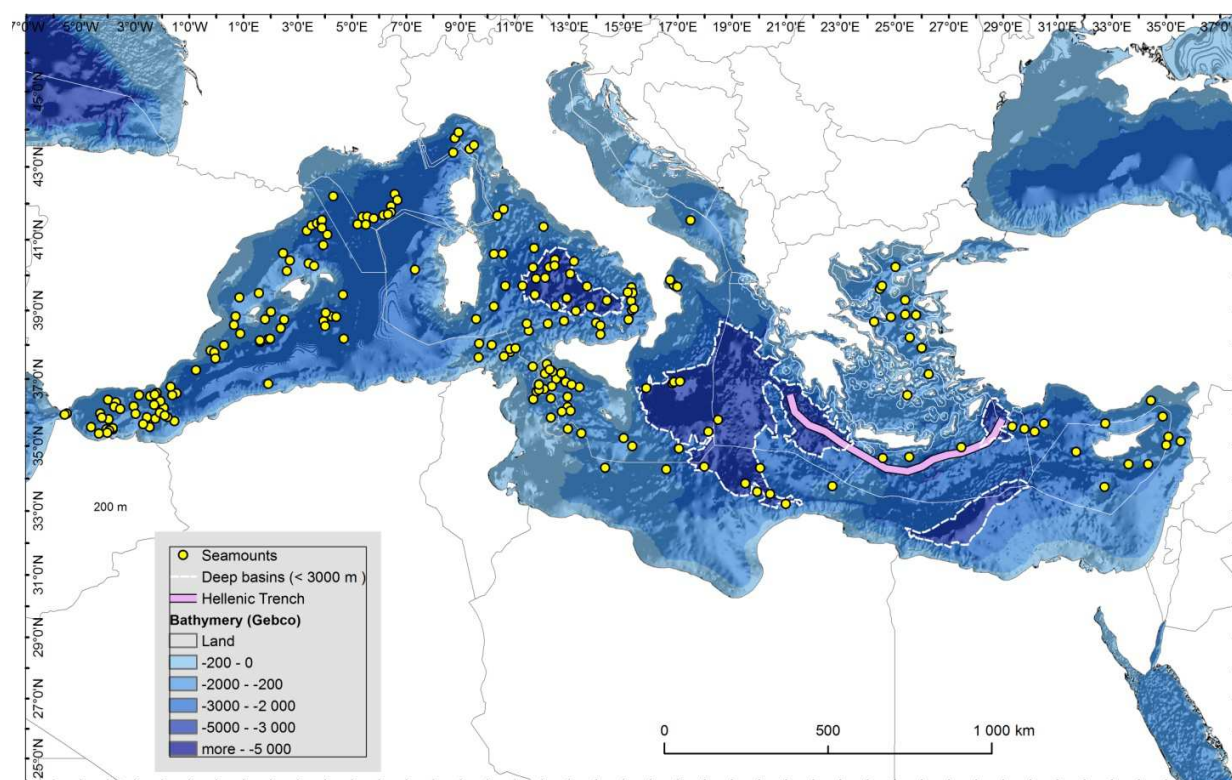
Figure 1.8: Distribution of seamounts and other topographic elevations of the deep Mediterranean Sea, from Würtz and Rovere (2015). Deep basins and Hellenic trench are highlighted as dashed grey lines and pink line respectively.

Peculiar biological communities often characterize seamounts. They can host species assemblages similar to those found in adjacent deep-sea habitats, but can show different structure in terms of abundance or frequency of species.

Suspension-feeders, particularly deep-sea corals and sponges, usually dominate the hard-bottom habitats of seamounts where they can form "submarine forests". The most important habitat-forming coral taxa usually found on Mediterranean seamounts is alcyonaceans (as sea fans and soft corals and, at least for soft bottoms, sea pens), antipatharians (forming large forests down to 500 m depth) and scleractinians (such as *D. cornigera* or the well-known CWCs *M. oculata*, *L. pertusa* and *D. dianthus* thriving at bathyal depths) (Würtz and Rovere, 2015). These coral forests account for some of the most longevous species known in nature, such as the isidid alcyonacean *I. elongata* or the black coral *L. glaberrima*, characterized by slow growth rates and a centennial or millennial life span, representing unique biological archives of paleoceanographic data (Robinson et al., 2014). A seamount, the Stony Sponge Seamount, located in the Valencia Gulf, Western Mediterranean Sea, has also been reported where the main habitat forming species is the lithistid demosponge *Leiodermatium pfeifferae* (Maldonado et al., 2015; Würtz and Rovere, 2015). And the first live *Caryophyllia calveri* and *D. dianthus* in the Levantine Basin were recorded on Eratosthenes Seamount (Galil and Zibrowius, 1998).

E - ABYSSAL

Three deep basins exceeding 3000 m occur in the Mediterranean Sea, which are the Tyrrhenian, Ionian and Levantine basins, with the arched, deeper Hellenic Trench extending along the northern edges of the Ionian and Levantine basins (Amblas et al., 2004; MediMap Group, 2007) (Fig. 1.8).

Those basins are scarcely explored and only few studies are reported, which are dedicated to macrofauna and meiofauna. From the Ionian Sea to the Levantine Basin, benthic biomass, abundance and diversity of macrofauna have been shown to decrease with depth, depth being a proxy for food availability (Kroncke et al., 2003; Lubinevsky et al., 2017). A noticeable higher abundance in the Western Basin than in the Eastern Basin has been shown based on nematodes, which account for more than 90% of meiofauna (Danovaro et al., 2008a). These observations are in agreement with the quantity and nutritive quality of organic matter, which decrease from west to east along the Mediterranean Basin (Pusceddu et al., 2010).

1.2 Where is the knowledge

The Mediterranean Sea is divided into the four subregions for MSFD purposes, namely: (i) the Western Mediterranean Sea; (ii) the Adriatic Sea; (iii) the Ionian Sea and the Central Mediterranean Sea; and (iv) the Aegean-Levantine Sea.

For many years, investigations on the deep Mediterranean Sea suggested that the west–east gradient of decreasing surface water productivity was reflected in a corresponding gradient of decreasing food availability in deep-sea environments (Danovaro et al., 1999; Danovaro et al., 2008b). Such a gradient could be responsible for a significant decrease in the abundance and biomass of most benthic components, including meiofauna, macrofauna, and megafauna. Surprisingly however, recent investigations indicate that there is no corresponding gradient for most components of benthic biodiversity (e.g., number of species, (Danovaro et al., 2010)). Only the diversity of Foraminifera showed an apparent east-to-west increase in species richness (Cita and Zocchi, 1978; De Rijk et al., 2000).

The occurrence of living CWCs in the Mediterranean Sea does not match with the occurrence of subfossil CWCs, particularly in the Eastern Mediterranean Basin (Taviani et al., 2011). Although the inventory of CWC sites is still incomplete, there are evident regional differences. In particular, records of live CWCs from the Eastern Mediterranean are much sparser than in the Western, Central and Adriatic seas, with no known CWC provinces in the Aegean-Levantine, contrary to the other three subregions, which all house at least one such provinces.

The last two decades represented an extraordinary turning point for the investigation of the deeper habitats and species, completely changing our perspective on deep-sea Mediterranean biodiversity. ROV footage contributed to give a visual identity to the assemblages that were listed and partially characterized for the first time through the classic syntheses of Pérès and Picard (1964). Despite the huge amount of work carried out, a lot still needs to be done and information is still discontinuous and unevenly available within the Mediterranean Sea.

However, as rightly stated by Emig and Geistdoerfer (2004) and advocated by Goren and Galil (2015), knowledge on the species diversity and richness is often correlated with the level of research (albeit sampling) effort. Several countries located on the eastern and southern Mediterranean basins are not EU members and consequently have little access to EU financial support. This funding-limited situation may sometimes explain disparity in the general knowledge of fish and cephalopods biodiversity. Furthermore, as stated before the MEDITS surveys offer a unique and standardized platform for investigating temporal and spatial changes in mobile species diversity. It is unsurprisingly that most of the recent investigations of climate change and fisheries impact have been conducted in the western and central regions of the Mediterranean Basin.

1.3   How is diversity measured

1.3.1   Methodological sampling aspects

Species groups of marine mammals, fish and cephalopods

At species level, monitoring data on deep-sea fishes and deep-sea cephalopods (cf. Section 1.1.1) are available through MEDITS surveys, for both target and non-target species (Bertrand et al., 2002; MEDITS Group, 2016) (Fig. 1.9), and through fishery statistics, although for commercial species only. There are also several one-time dedicated surveys, in particular on specific deep-sea ecosystems such as CWCs and canyons (D'Onghia et al., 2012; 2015b), and bathyal environments deeper than 1000 m (Sarda et al., 2009), which also provide useful data. Dedicated surveys commonly used either baited landers or longlines as fishing gears. Spitz et al. (2011) have also published useful information after analyzing the stomach contents of dead stranded marine mammals. This kind of opportunistic sampling faces the limitation of the number of specimens available, which in the Mediterranean Sea is lower than in the Atlantic Ocean, where it has been more extensively applied.
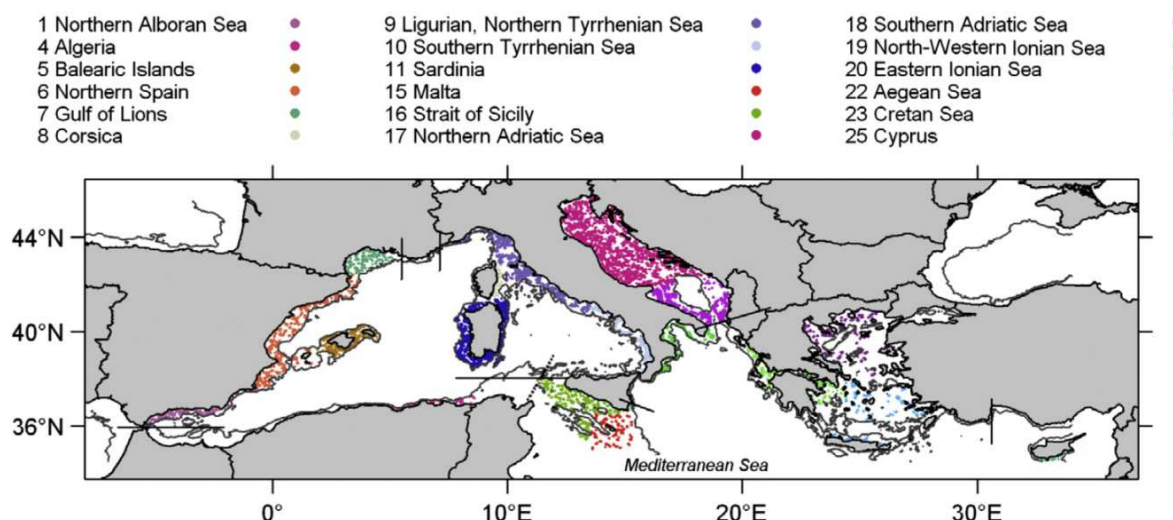
Figure 1.9: Distribution of trawling stations in the Mediterranean Sea conducted by MEDITS surveys, from Granger et al. (2015).

Benthic habitats

Monitoring ecosystems' biodiversity imply measuring and quantifying. Statistical tools previously developed to monitor coastal areas are based on multiple sampling and on the presence or absence of species known for a particular ecological trait reflecting an adaptation to a particular unbalanced environment. However, significant water depth and remoteness, locally complex and steep topography often associated to enhanced currents, occasional down-canyon flushing events and occasional hard substrates found in the deep-sea make sampling, surveying and monitoring difficult and did limit early studies and monitoring.

Indexes based on biological variables are commonly based on the absence of sensitive species or the presence of tolerant species and were developed to follow ecosystem response to a gradient of disturbance (e.g. enrichment in organic matter).

Recent advances in seafloor technology (e.g. the development of autonomous and robotic vehicles, new *in situ* measurements techniques) create new opportunities for seafloor observation in the challenging deep-sea environments. They significantly increase the accessibility of deep sea floors, and provide tools to achieve a new break-through in deep-sea communities monitoring.

Soft substrate bottoms

The large list of existing indexes is mainly focused on the monitoring of infauna communities living inside the sediment and requires sediment sampling, sorting and taxonomic classification (Diaz et al., 2004). Generally species are grouped according to their ecological traits (tolerant vs sensitive, filter vs deposit feeders). It is noteworthy that the evolution through time went from the development of indexes based on extensive sampling in which exhaustive community identification was carried out towards indexes based on a reduced number of species known to be tracers of disturbance. In the deep-sea extensive sampling is not realistic because of time and technical reasons, and most of the species are not described yet (Bouchet, 2006). Consequently an indirect measurement of a disturbance using a limited number of pertinent taxonomic groups (all organisms are not equally sensitive to all types of anthropogenic disturbances) used as proxys has become a common way to monitor infauna (i.e. Dauvin and Ruellet (2007) focused on annelids and arthropoda; Fontanier et al. (2012) focused on foraminifera).

The different amount of data collected in different benthic habitats and the heterogeneous focus on different taxa in each system makes it difficult to compare abundance and distribution of deep-sea benthic species in the Mediterranean Sea. Each kind of ecosystem has to be considered for local biodiversity, and comparison between ecosystems gives information on regional biodiversity. As sampling effort is different from one place to another, the Expected Species Number for 100 Individuals (standardized using the rarefaction curves) can be used to compare benthic ecosystems of the deep Mediterranean Sea at basin scale (Danovaro et al., 2009).

Soft substrate bottoms compose the majority of the deep Mediterranean seafloor. Nevertheless, even if some topographic features may enhance the heterogeneity of continental slopes like submarine canyons or mud volcanoes, a comprehensive and continued monitoring is not realistic in the vast deep-sea expanses. Areas known for their anthropogenic pressure need to be targeted for an efficient monitoring. Vessel Monitoring System (VMS) data can be used in order to highlight areas of heavy fishing pressure (see descriptor 6).

A monitoring objective would be to launch an underwater vehicle in autonomous mode to cover a large area on soft substrate where meadows of erect megafauna species have been described to live on in the past in order to measure the extent of the habitat (ecosystem) or to detect anthropogenic impact. Increased resolution is the key to discriminate habitats using either bathymetric or backscatter data.

Facies classification based on backscatter data from high-resolution multibeam echo-sounding (MBES) is a technique commonly used for seabed mapping and is increasingly being used for biological habitats (Harris and Baker, 2012). Automatic segmentation, opposed to expert interpretation, of backscatter data together with ground-truth sampling or video data could help to the development of fast and repeatable methods of seabed classification for an efficient monitoring with regard to environmental and anthropogenic impact assessments (Stephens and Diesing, 2014).

When seafloor cannot be differentiated on the basis of acoustic backscatter, a textural analysis from side-scan sonar may be useful to differentiate bottom types. Side-scan sonar has also been demonstrated to allow observation of trawl damage (i.e. Darwin Mounds, NE Atlantic) at almost 1000 m water depth as well as the decrease in coral abundance (Roberts et al., 2006; Fanelli et al., 2017).

The use of high-resolution side-scan sonar data could be a way to map and measure the extent of erected megafauna species meadows (e.g. *F. quadrangularis* and *I. elongata)* as they should induce a difference in the sediment texture compared to flat sediment areas. In a monitoring purpose the advantage would be to benefit from the automatic segmentation that could be used on a large area (as opposed to a manually drawn classification from a video mosaic). A temporal evolution of the spatial extent of large scale ecosystems could be monitoring based on high resolution signals.

Hard substrate bottoms

The study of fragile erect epifauna communities living on hard bottoms (e.g. sponges and cnidarians) requires using non-destructive optical techniques. Indexes based on optical imagy have been developed, taking advantage of the availability of high-resolution underwater cameras. However, very few indexes exist to measure community status from optical images, and they were mainly developed in the coastal and photic zone (0-60 m), such as CARLITT used for macroalgae on rocky-shores (Ballesteros et al., 2007); FAST a Fish Assemblage Survey Technique index (Seytre and Francour, 2008); Marine Landscape Indice for anthropogenic impact and biodiversity at scuba diving sites (Creocean, 2009); CAI for Coralligenous Assemblage Index (Deter et al., 2012); INDEX-COR to evaluate the conservation state of Coralligenous Assemblages (Sartoretto et al., 2017). The use of non destructive methods to monitor deep-sea ecosystems such as engineer species providing a structural habitat, a refuge and food to other species and recently exposed to anthropogenic pressure is a new challenge for research.

Species or ecosystems' spatial distribution assessment from high quality optical images is nowadays available in the deep sea since state-of-the-art technologies allow scientists to use underwater vehicles, mostly unmanned, as observation platforms, including precise georeferencing by means of advanced underwater positioning systems. Video and photograph sampling provide a non-destructive alternative to physical sampling but are subject to a number of challenges among which the identification of organisms without physical specimens available. High-resolution imagery is a requirement for species recognition and identification on video records and photographs, thus minimizing the risk of misidentification (Howell et al., 2014). High definition video survey data collected along transect lines for ecological data analysis may be gathered into georeferenced two-dimensional mosaics so as to ease mapping of ecosystems'spatial distribution, commonly using a vertical camera orthogonal to an assumed flat horizontal bottom. A mosaic created at regular intervals enables the temporal monitoring of the spatial extent of an ecosystem on flat bottoms (Olu-Le Roy et al., 2007; Marcon et al., 2014). Most 2D algorithms suppose that the seafloor does not present strong variations and that the scene is seen from the top.

Vertical cliffs and complex morphologies having both horizontal and vertical surfaces are particularly challenging for image mosaicking. The first issue is the acquisition process itself. Then, following appropriate acquisition, images are processed through a 3D model of the scene. For that purpose, video films or still images need to be recorded in repeated transects allowing several views of the objects (e.g. north, south, east and west views). This will enable to reconstruct 3D textured georeferenced scenes through non-destructive measurements.

Hard bottom substrates often display complex structures for which 3D mosaics can bring supplementary information on the species layout and arrangement with regard to predators, currents or silting exposure for instance.

The European Commission produced a set of detailed criteria and methodological standards to help Member States implement the MSFD (cf. Section 1). These were revised in 2017 (EC, 2017) leading to the new Commission Decision on Good Environmental Status and are presented in the following paragraphs.

The MSFD aims at characterizing the status of **species groups diversity** by the mean of the five following criteria.

1.3.2 The mortality rate per species from incidental by-catch (D1C1) - Primary

The MSFD requires that the mortality rate per species from incidental by-catch is below levels which threaten the species, such that its long-term viability is ensured. Member States shall establish the threshold values for the mortality rate from incidental by-catch per species, through regional or subregional cooperation.

Data shall be provided per species per fishing metier for each ICES area or GFCM Geographical Sub-Area or FAO fishing areas for the Macaronesian biogeographic region, to enable its aggregation to the relevant scale for the species concerned, and to identify the particular fisheries and fishing gear most contributing to incidental catches for each species.

Fish mortality (*F*) is a parameter used in fisheries population dynamics to account for the loss of fish in a fish stock through death. It corresponds to the proportion of fish in a specific age group captured by the fisheries in one year. This indicator, is associated to the fishing effort (*E*) and is characterized by the coefficient of catchability, noted *q* (the catchability being the probability for a fish to be taken at random in a group of species; Laurec and Guen, 1981):

$$F = q*E$$

The computation of this indicator depends on the availability of data on fishing activity and the knowledge on the population dynamics of the concerned species. For the exploited species, fishing mortality is actually known for a very limited number of stocks. This is mainly due to the lack of data for its computation. Furthermore, the MSFD requires the mortality rate from incidental by-catch, which represents only a part of the fishing mortality, itself being difficult to assess. Some Member States have developed an at-sea observers program to get information onboard fishing vessels. However, this represents only a small proportion of fishing vessels, i.e. 12% in 2016 in France (Cornou et al., 2016).

The majority of the available "impoverished" information is for exploited species inhabiting the continental shelf. Therefore, there is an enormous lack of information for deep-sea species targeted (or not) by the different fisheries. For the time being, **D1C1 criterion is impossible to compute for any fish and cephalopod species in the deep- Mediterranean Sea**.

1.3.3 The population abundance of the species (D1C2) - Secondary

The MSFD requires that the population abundance of the species is not adversely affected due to anthropogenic pressures, such that its long-term viability is ensured. Threshold values shall be established for each species through regional or subregional cooperation, taking account of natural variation in population size and the mortality rates derived from D1C1, D8C4 and D10C4 and other relevant pressures. For species covered by Directive 92/43/EEC, these values shall be consistent with the Favourable Reference Population values established by the relevant Member States under Directive 92/43/EEC.

Unit of abundance shall be (number of individuals or biomass in tonnes (t)) per species.

This criterion includes two types of assessments. The MSFD has prescribed the use of the Spawning Stock Biomass (SSB), that is the D3C2 for the targeted species. For other species, indices of abundance or biomass are to be computed to inform the D1C2 criteria. Computation of D1C2 requires standardized time series of abundance and/or biomass by species. Actually, the only sources of data available to compute such indicators are the MEDITS surveys for the demersal species and PELMED and MEDIAS for small pelagic species. The actual approach used in the MSFD and recent studies suggested to analyze the groups of species according to a selection of life history traits sensitive to fishing (Tsagarakis et al., 2013): traits defining long-lived and/or low growth rate species (e.g., Chondrichthyes as a group) and of some short-lived species. Nevertheless, **current computation of the D1C2 would only give an incomplete assessment of the deep-sea environment as a large proportion of the deep-sea is uncovered by these surveys**.

1.3.4 The population demographic characteristics of the species (D1C3) - Secondary

The MSFD requires that the population demographic characteristics (e.g. body size or age class structure, sex ratio, fecundity, and survival rates) of the species are indicative of a healthy population which is not adversely affected due to anthropogenic pressures. Member States shall establish threshold values for specified characteristics of each species through regional or subregional cooperation, taking account of adverse effects on their health derived from D8C2, D8C4 and other relevant pressures.

D1C3 mirrors D3C3 for exploited species, which assumes that the good status of a stock is characterized by a large proportion of old and large-sized individuals. Therefore, indicators used in the assessment of the D3C3 are also applicable for D1C3. According to Tsagarakis et al. (2013), size-based indicator such as maximum length of deep-water fish species is one of the most informative metrics related to fishing impact. There is a set of size-based indicators that already exists in the literature (see review by Shin (2005)) but there are no agreed thresholds. Hence, ICES has been appointed by the European Commission to supply a guide on the development of operational methods to assess D3C3. For the 2018 MSFD assessment, ICES advised against computing D3C3 indicators until operational reference points have been developed and scientifically accepted (ICES, 2016b). Given the large amount of available data on continental shelf species but given also the opposition by ICES to compute D1C3 (and equally D3C3) indicators, **it is very unlikely that this criterion, will be ready for an assessment of deep-sea fish and cephalopod species in the near future. This underlines the need to gather information on the size of sampled species in the deep-sea. Just as in D1C2, the only data available to eventually compute size-based indicators come from the MEDITS and MEDIAS surveys**.

1.3.5   The species distributional range (D1C4) - Secondary

Primary for species covered by Annexes II, IV or V to Directive 92/43/EEC and secondary for other species.

The MSFD requires that the species distributional range and, where relevant, pattern is in line with prevailing physiographic, geographic and climatic conditions. Member States shall establish threshold values for each species through regional or subregional cooperation. For species covered by Directive 92/43/EEC, these shall be consistent with the Favourable Reference Range values established by the relevant Member States under Directive 92/43/EEC.

The species distributional range is defined as the spatial limits in which a species is naturally present (excluding erratic occurrences). This area is not static and can vary in time. It can be described by the geographic limits where a species occurs, whereas the species distributional pattern is the way the species patches are distributed within this area. It can be more or less discontinuous and reflect various patterns of occupation.

The distributional range of deep-sea species can be highly variable in space and time, as it is often driven by biological and environmental variables, interactions among life history traits and anthropogenic pressures, and climate forcing. Changes in the distributional range of species can be resumed into three categories:

- Parallel shift: the distribution stays the same (values and shape), but it is shifted in a specific direction;
- Contraction / expansion;
- Fragmentation / fusion.

Series of potential indicators addressing these three categories already exist (ICES, 2016b). It is strongly advised, when characterizing D1C4, to use multiple indicators, including (at least) metrics on the geographical extension and the aggregation of species. As for the other criteria, the limiting factor in their computation is the availability of data, and this is more so in the deep-sea environment. Indeed, **spatial mismatch between the area covered by scientific surveys and the natural range of a species is most likely to occur in the deep-sea environment**.

1.3.6   The habitat extent and condition (D1C5) - Secondary

Primary for species covered by Annexes II, IV or V to Directive 92/43/EEC and secondary for other species.

The MSFD requires that the habitat for the species has the necessary extent and condition to support the different stages in the life history of the species.

Habitat is defined as the physical, chemical and biological properties required by species for growth, reproduction, survival and for completing its life cycle. It can be described either qualitatively (e.g. available food, pollutants,...) or quantitatively. In either case, a significant amount of information is needed to understand, identify, and predict the habitat of a species. Practically, this could be done by developing suitable habitat models (Lauria et al., 2017), given that sufficient data would be available. **There are two major drawbacks to the computation of the D1C5 criteria. First, the lack of knowledge of the habitat conditions required by deep-sea species to complete their life cycle, and second, the absence of a (minimum) threshold the habitat of a species should cover without threatening that species**.

The MSFD aims at characterizing the status of **pelagic habitats** by the mean of the following criterion.

1.3.7   The condition of each habitat type (D1C6) - Primary

The MSFD requires that the condition of the habitat type, including its biotic and abiotic structure and its functions (e.g. its typical species composition and their relative abundance, absence of particularly sensitive or fragile species or species providing a key function, size structure of species), is not adversely affected due to anthropogenic pressures.

Member States shall establish threshold values for the condition of each habitat type, ensuring compatibility with related values set under Descriptors 2, 5 and 8, through regional or subregional cooperation.

The extent to which good environmental status has been achieved shall be expressed for each area assessed as:

(a) an estimate of the proportion and extent of each habitat type assessed that has achieved the threshold value set;

(b) a list of broad habitat types in the assessment area that were not assessed.

The marine pelagic ecosystem includes tremendously large and diverse habitats. The deepest part of those habitats are less studied and to our knowledge, no available nor operational indicators have been developed in the deep Mediterranean sea.

The MSFD aims at characterizing the **benthic habitats** biodiversity and seafloor integrity by means of the two following criteria:

These two primary criteria should be used to ensure consistency across the European Union. A single set of habitat types shall serve the purpose of assessments of both benthic habitats under descriptor 1 and sea-floor integrity under descriptor 6.

1.3.8   The extent of loss of the habitat type (D6C4) - Primary

The MSFD requires that the extent of loss of the habitat type, resulting from anthropogenic pressures, does not exceed a specified proportion of the natural extent of the habitat type in the assessment area.

The maximum allowable extent of habitat loss shall be established as a proportion of the total natural extent of the habitat type, through cooperation at Union level, taking into account regional or subregional specificities.

In order to measure the extent of loss of the habitat type, the extent of the natural area has to be known. This is not always the case in the deep sea because few decennia ago access to the deep seafloor was extremely rare. Data may have been collected punctually, giving indication on the presence of some habitats, but the surface covered by the whole ecosystem could have not been measured.

Quantifying the extent of each habitat type implies the routine use of a large variety of tools that so far have been used only for research purposes (see descriptor 6). High resolution maps of benthic substrata and habitats are in increasing demand both to underpin environmental and socioeconomic impact assessments and to help in the development of effective management measures (Kenny et al., 2003; Brown et al., 2011; Stephens and Diesing, 2014; Holler et al., 2017). Over the last decades, developments in acoustic remote sensing techniques, coupled with precise *in situ* sampling, have enabled extensive seafloor mapping and groundtruthing interpretations, and we are now able to map the seabed at high spatial resolution and accuracy (Micallef et al., 2012; Puig et al., 2012; Lastras et al., 2016). The increasing use of modern robotic technology, such as ROVs, will make deep-sea observations

and *in situ* experimentation easier and more frequent. ROVs are used extensively by the offshore drilling industry to support deepwater activities, and are now being increasingly used by the international scientific community for enhanced deep-sea observation, fine scale mapping and sampling (Fabri et al., 2014; Lastras et al., 2016). Optical imagery can also be used for local mapping, especially on hard substrata that are very often highly uneven for MBES and on which Vulnerable Marine Ecosystems (VME) may have established. Autonomous Underwater Vehicles (AUVs) have also demonstrated their capabilities to acoustically map and take optical images of the deep seabed at high resolution, with its full potential still to be developed. Underwater observatories (UOs) and networks of observatories have been developed by a number of countries for a variety of purposes (i.e. the NEPTUNE network in Canada or the ESONET network in Europe). Although UOs occupy fixed locations and, therefore, do not have a large spatial coverage, they can provide both direct (e.g. video records and photographs) and indirect (e.g. through bioluminescence measurements) evidence on pelagic, benthopelagic and benthic organisms otherwise difficult to observe (Tamburini et al., 2013; Doya et al., 2014). The main value of UOs for biodiversity observational purposes relies on their long-term permanence at fixed locations. Huge amounts of imagery obtained by UOs are waiting to be scientifically exploited.

Habitat suitability modeling is useful to predict the distribution of species that are tightly correlated with environmental parameters (e.g. slope gradient, substrate type, temperature, etc.). This technique is useful to evaluate the theoretical distribution of the ecosystem and compare it to the actual extent in order to measure the extent of loss. Examples include CWCs (Lastras et al., 2016; Bargain et al., 2017; Fabri et al., 2017) as well as other soft and hard framework building corals (Giusti et al., 2014; Lauria et al., 2016; Giusti et al., 2017; Lauria et al., 2017). Recent developments have been achieved in predictive models of seafloor habitats and benthic biotopes at the scale of large areas (Martin et al., 2014a). These habitat or biotope models seem highly suitable to monitor long-term variations of seafloor attributes, and make increasing use of MBES backscatter imagery (Brown and Blondel, 2009).

Sampling adds crucial information to data from remote techniques, either acoustic or optic. It allows comparison of the community and the population structures and the determination of the limits of impacted areas. An example off Malta shows how to determine the effects of fishing on communities of fishing grounds (Dimech et al., 2012). This technique could be used to delimit the extent of the disturbance by looking at the community structure. Sampling is the only way to measure in a tangible way adverse impacts on fauna living in deep-sea environments. A long list of indexes have been used in shallow water in the framework on the Marine Water Directive (2000/0/EC), but a major effort of adaption is required for those or equivalent indices to fulfill the MSFD objectives and to evaluate the ecological status of deep sedimentary bottoms instead of just the quality of the water (Diaz et al., 2004; Borja et al., 2009; Pinto et al., 2009; Martinez-Crego et al., 2010).

1.3.9   The extent of adverse effects on the condition of the habitat type (D6C5) - Primary

The MSFD requires that the extent of adverse effects from anthropogenic pressures on the condition of the habitat type, including alteration to its biotic and abiotic structure and its functions (e.g. its typical species composition and their relative abundance, absence of particularly sensitive or fragile species or species providing a key function, size structure of species), does not exceed a specified proportion of the natural extent of the habitat type in the assessment area.

The maximum allowable extent of those adverse effects shall established as a proportion of the total natural extent of the habitat type, through cooperation at Union level, taking into account regional or subregional specificities.

Threshold values for adverse effects on the condition of each habitat type, shall be established ensuring compatibility with related values set under Descriptors 2, 5, 6, 7 and 8, through cooperation at Union level, taking into account regional or subregional specificities.

In order to define a threshold (Good Ecological Status boundary) that separate a level of impact that is acceptable from one that is not acceptable, a collection of additional information is needed, especially on known impacted ecosystems. Studies focused on the assessment of recoverability of seafloor integrity are mandatory in order to determine the threshold.

1.4   Knowledge on Threshold

Based on the poor existing knowledge on the deep-sea groups of species and benthic habitats, it is quite difficult and most likely irrelevant to develop thresholds on any indicator. Indeed, major research efforts should be dedicated to understanding the connectivity and the ecological roles of the various deep-sea habitats on fishes and cephalopods. Statistical approaches to develop targets and thresholds, at least for fish and cephalopods, are under development on the shelf and coastal habitats (Probst and Stelzenmuller, 2015; Rossberg et al., 2017). Would these methodological tools be suitable for the deep-sea? This remains to be assessed and proved.

1.5   CONCLUSIONS

Understanding diversity in the deep sea is a major challenge. The Mediterranean Sea has been long investigated because of its attractiveness and the goods and services it supplies to human population. Nevertheless, there are still enormous gaps in scientific knowledge of its deep basins and ecosystems for which in-depth knowledge is urgently required. The last two decades represented an extraordinary turning point for the investigation of the deeper Mediterranean Sea habitats and species, which resulted in a complete change of previous perspectives on deep-sea biodiversity. ROV footage has given a visual identity to the assemblages that were listed and partially characterized for the first time through the classic syntheses of Pérès and Picard (1964). Despite the huge amount of work carried out, a lot still needs to be done and information is still discontinuous and unevenly available within the Mediterranean Sea (Fanelli et al., 2017). The present synthesis highlighted the following components:

- Investigations on the deep sea general patterns of biodiversity underlined a west–east gradient corresponding to a decreasing surface water productivity of the Mediterranean Sea and thus a gradient of decreasing food availability in deep-sea sediments. Recent investigations challenged the existence of such longitudinal gradient
- Depth is a major driver of the fish and cephalopods diversity distributional patterns
- Deep-sea benthic habitats, such as submarine canyons or cold water corals, seem to act as hotspots for local faunal diversity, providing heterogeneous habitats, increasing food availability, and refuge areas for motile species
- Benthic habitats have been separated into four categories, each of them contributing in their own way to the overall diversity of benthic marine organisms through food web interactions
- Pelagic habitats are setting the scene for complex trophic interactions by allowing access nutrients coming from the enrichment of surface layers
- The deep-sea ecosystems are increasingly submitted to human activities and the most commonly accepted being fishing pressure. Several deep-sea species are characterized by high life expectancy (*e.g.* the orange roughy, absent in Mediterranean waters but present in Atlantic can live up to 120 years), slower growth rates, and delayed reproduction, which make them sensitive to fishing exploitation.

1.5.1   Gaps

Pursuing the work started and developing knowledge are essential steps to understanding the processes regulating the functioning of those exceptional ecosystems. How such oligotrophic ecosystems have the capability to sustain large populations of benthic suspension-feeders, or host momentary top predators like tunas, swordfish, whales, dolphins and sharks? How pelagic-benthic coupling mechanisms influence deep-sea assemblages? Which kind of connectivity exists among the deep benthic populations of the Mediterranean Sea and those from the Atlantic Ocean?. How atmosphere-driven high-energy processes occurring in the Mediterranean Sea, and mesoscale circulation and turbulences determine the distribution and composition of seamount assemblages? How are the communities structured over steep bathymetric gradients?

Answers to these questions are mandatory for the development of robust indicators in relation to anthropogenic pressures.

1.5.2 Recommendations

Understanding the functioning of deep-sea ecosystems requires a sufficient amount of data. Increasing knowledge on such ecosystems will necessarily involve new observational cruises or adaptations of actual surveys to supply biological materials and data, and also key data on environmental parameters such as substrate type, near-bottom currents, food supply events and the like. It also requires food web studies in order to assess the functional roles and the relative contribution of the different deep-sea habitats (i.e. canyons, soft bathyal bottoms, coral gardens, ...) on mobile fauna. The MEDITS surveys represent a good opportunity to obtain supplementary data on mobile fauna. Expanding MEDITS surveys with additional hauls over 200 m depth (whenever possible) learning from the current sampling protocol could be suggested as well as expanding them to the data-deficient Levantine Basin.

Future efforts should be devoted to the functional analysis of the deep-sea ecosystems, in particular those potentially serving as essential habitats for mobile species such as deep-sea spawning grounds, feeding habitats and deep-sea nurseries for megafauna. Identifying such essential habitats should be done in parallel with efforts to tackle the D4 food web descriptor. Understanding the ecological processes underlying the diversity patterns is a mandatory step towards the modeling, prediction and sound management of deep-sea diversity under the on-going global climate change, which will increasingly affect the deepest Mediterranean Sea (Rohling and Bryden, 1992; Schroeder et al., 2008; Schroeder et al., 2010; Vargas-Yanez et al., 2010; Zunino et al., 2012; Somot et al., 2016).

Spatial zonation of deep-sea fish assemblages in the Mediterranean Sea associated with a selection of environmental factors, such as those mentioned above has been the topic of a number of Western Mediterranean studies (see review in Cartes and Carrasson, (2004). Expanding such studies to the eastern and southern Mediterranean Sea is crucial to understand the connectivity among the different sub-basins. Distinguishing the effect of depth among other structuring habitat variables has been and still is a major challenge.

With the expansion of human activities in the high seas, and the increased interest of mining industries in deep sea mineral resources, more activity conflicts are to be expected in the future (e.g. between fishing and mining interests). Marine spatial planning can help avoiding or minimizing such conflicts, and enable consideration of cumulative impacts on deep ecosystems. Thus, addressing the sustainability of deep sea ecosystems strongly requires addressing some general fisheries and ocean governance issues.

The FAO recently developed the International Guidelines for the Management of Deep-sea Fisheries in the High Seas (Deep-sea Fisheries Guidelines), which were adopted in 2008. These guidelines provide recommendations on governance frameworks and management of deep-sea fisheries with the aim to ensure long-term conservation and sustainable use of marine living resources in the deep-seas, and to prevent significant adverse impacts on benthic habitats.

2   Descriptor 2 : Non-Indigenous Species

The Marine Strategy Framework Directive (MSFD) defines **Non-indigenous species** (NIS; synonyms: alien, exotic, non-native, allochthonous) as species, subspecies or lower taxa introduced outside of their natural range (past or present) and outside of their natural dispersal potential. This includes any part, gamete or propagule of such species that might survive and subsequently reproduce. Their presence in the given region is due to intentional or unintentional introduction resulting from human activities. Natural shifts in distribution ranges (e.g. due to climate change or dispersal by ocean currents) do not qualify a species as a NIS. However, secondary introductions of NIS from the area(s) of their first arrival could occur without human involvement due to spread by natural means." Natural shifts in distribution ranges (e.g. due to climate change or dispersal by ocean currents) do not qualify a species as a NIS.

Marine NIS are acknowledged as one of the rapidly increasing global environmental problems because of the adverse impacts which NIS may have on native biodiversity, ecosystem functioning, seabed habitats as well as commercially valuable marine resources and services. The problems are greatly exacerbated in the Mediterranean Sea (Galil et al., 2014; Galil et al., 2016).

The MSFD aims to adopt integrated ecosystem-based management approaches to achieve or maintain GES for marine waters, habitats and associated resources, including the goal that **"non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystem"**. Descriptor 2 is closely related to several other MSFD descriptors of Good Environmental Status (GES) (e.g.: D1: biodiversity change, community structure shift, decline and displacement of native species populations; D3: Competition, displacement and replacement of commercially valuable native species, interference/disruption of fisheries/culture activities; D4: food web alteration; D5: eutrophication risks reducing native diversity and increasing invader dominance; D6: Alien species change physical structure of seafloor by biodeposition, bioturbation). The most relevant to the deep sea environment is descriptor 1.

2.1   State Of The Art

The introduction and spread of non-indigenous species (NIS) is an aspect of global change and is considered to be a major driver of biodiversity loss, causing ecological and evolutionary change and economic damage. The negative effects of NIS are exacerbated by climate forcing, pollution, habitat loss and human-induced disturbance. The most harmful NIS displace native species, alter community structure and food webs, and change ecosystem functioning. NIS have transformed marine coastal habitats and their impact has been increasing inexorably (Millenium Ecosystem Assessment, 2005).

Complex and fundamental alterations to the Mediterranean Sea, including increases in NIS, have affected the structure and functioning of the sea and the consequent provision of goods and services (Micheli et al., 2013; European Environment Agency, 2015; Galil et al., 2016). The number of recorded introductions into the Mediterranean Sea is far higher than in other European seas (Galil et al., 2014). Their number more than doubled between 1980 and 2016 and is substantially greater in the Levant than in the central and western Mediterranean: the Levantine countries in particular saw an increase in the number of introductions. Their occurrence often resulted in dramatic restructuring of biotic communities and shifts in ecosystem function with impacts on the availability of marine resources and

ecosystem services (Galil, 2007; Katsanevakis et al., 2014; Fanelli et al., 2015; Galil et al., 2016; Goren et al., 2016; Katsanevakis et al., 2016).

Vectors determine which taxa are introduced, their geographical origin, propagule pressure and depth range. The Mediterranean Sea is unique in that the Suez Canal serves as a long-term, large volume, ceaseless corridor for thermophilic biota. This singular pathway has resulted in the establishment of numerous invasive NIS: about two thirds of the multicellular NIS recorded in the Mediterranean Sea are considered Erythraean NIS – species considered to have been introduced through the Suez Canal – the balance were primarily introduced by shipping and mariculture (Galil et al., 2017). A comparative analysis among the NIS recorded across the Mediterranean regions shows that vectors determine their geographical origin and the taxonomy: in countries where the Suez Canal is the main vector (Levant), most NIS are of tropical/subtropical Indo-Pacific origin and comprise molluscs, fish and crustaceans, i.e. taxa actively spreading as adults or passively transported as larvae. In countries where vessels and aquaculture are the prevailing vectors (Italy, France, Spain), the taxonomic composition and native ranges of alien species are more diverse and depend on shipping routes and mariculture trade. In France, where aquaculture is the main vector, a large number of species are of subtropical/temperate Pacific origin, and are mainly represented by macroalgae often associated with imported shellfish (Galil et al., 2014; Galil et al., 2016).

The implications of the successive enlargements of the Suez Canal combined with higher through-current velocities on propagule pressure are all too clear – increasing the delivery of multiple species and in particular deeper living ones. Since 1979 the Levantine surface waters (LSW) and Levantine intermediate waters (LIW) masses in the SE Mediterranean displayed positive long-term trends in salinity of $+0.008 \pm 0.006$ and $+0.005 \pm 0.003$ year^{-1}, respectively, and temperature of $+0.12 \pm 0.07$ and $+0.03 \pm 0.02°C$ year^{-1}, respectively (Ozer et al., 2017). Similar trends were noted in the Balearic Basin, NW Mediterranean (Fanelli et al., 2016). **As the sea is warming, the expansion of the bathymetric range of Erythraean NIS may be accelerating. In fact, the recently observed "descent" of Erythraean NIS from the upper to lower continental shelf and upper slope may be a harbinger of temperature-dependent range expansion, both horizontal and vertical.**

2.1.1 Pressures

Since the main bioinvasion vectors into the Mediterranean Sea – Suez Canal, shipping and mariculture – have been introducing biota into the shallow shelf, it was assumed that the populations of the established NIS will be restricted to that region. Indeed, until the millennium introduced biota were largely limited to coastal habitats in the Mediterranean Sea.

Recent findings of NIS populations established in deeper waters have upended this paradigm – a growing number of NIS have been collected on the deeper shelf, even beyond the shelf break and well into the upper slope.

The Erythraean portunid crab *Charybdis longicollis* (Leene, 1938), recorded in the Mediterranean since 1954, and its sacculinid parasite, *Heterosaccus dollfusi* (Boschma, 1960), were collected off Israel as deep as 250 m (Innocenti et al., 2017). Off the Mediterranean coast of Israel in 1960-62 *C. longicollis* was abundant at 36 m depth, but at a depth of 82 m "only single specimens were taken" (see figure 4 in Gilat (1964)). In the late 1970s 3.5 times as many individuals were collected in 35 m as in 50 m, and only two specimens were collected in 80 m (Galil and Lewinsohn, 1981). However, in recent surveys (2008-2012) the highest numbers of specimens were collected between 40 and 80 m, but specimens were collected also at 100, 120 and 250 m depth (Innocenti et al., 2017).

The lethally poisonous silver-cheeked toadfish, *Lagocephalus sceleratus* (Gmelin, 1789), was collected off the Mediterranean coast of Turkey as deep as 150 m (Özgür Özbek et al., 2017). Its carnivorous habits have already noticeable impacts on Levantine fisheries.

The Erythraean mantis shrimp *Erugosquilla massavensis* (Kossmann, 1880), recorded in the Mediterranean since 1933, was collected off Turkey at depths between 150 and 200 m (Özcan et al., 2008). The species is widespread across the Levant and was recently collected off Tunisia and Sicily (Ounifi-Ben Amor et al., 2016; Corsini-Foka et al., 2017).

The Erythraean portunid crab, *Gonioinfradens paucidentatus* (A. Milne Edwards, 1861) was collected off Rhodes at depth of 200 m (Corsini-Foka et al., 2010).

The goldband goatfish *Upeneus moluccensis* (Bleeker, 1855) and the Eryhtraean lizardfish *Saurida lessepsianus* (Russell et al., 2015), first recorded in the Mediterranean in 1935 and 1952 respectively, constitute a significant component of the local trawl fisheries and have been common in trawls hauled from depths of 100 and 120 m (Levitt, 2012). *C. longicollis*, *U. moluccensis* and *S. lessepsianus* are widespread and abundant throughout the eastern Mediterranean Sea, and the latter has spread westwards to Albania and Tunisia (Rakaj, 1995; Ben Souissi et al., 2005).

In 2004 the bathydemersal spiny blaasop *Tylerius spinosissimus* (Regan, 1908) was collected off Rhodes at 90 m (Corsini et al., 2005), and in 2010 off Israel at 120-140 m (Golani et al., 2011). In 2010, specimens of the spotfin cardinal *Apogon queketti* Gilchrist, 1903 were collected at depths of 140-150 m in the Gulf of Antalya, Turkey (Gökoğlu et al., 2011).

In 2010 and 2011, large numbers of Randall's threadfin bream *Nemipterus randalli* Russell 1986 were collected at depths of 100 and 120 m off the Israeli coastline (see figure 5a in Stern et al. (2014)).

Though the Levantine populations of the Erythraean invasive lionfish, *Pterois miles* (Bennett, 1828), and recent records in the Central Mediterranean were confined to the upper shelf (Suppl. mat. in (Azzurro et al., 2017)), this may be due to survey effort bias. Invasive lionfish, linked to declines in reef health, were regularly sighted in the western Atlantic at upper-bathyal zones (to 304 m off Bermuda) (Gress et al., 2017). Their presence at 250 m (water temperature 15°C) off Roatan island, Honduras, call for efforts to establish the maximum depth distribution of lionfish in the Mediterranean Sea.

IDEM is concerned specifically with the deep sea (sites deeper than 200 m). The recently recorded presence of NIS beyond the shelf break in the Levant may be indicative of their potential to spread into deeper waters.

2.1.2 Possible Impacts

Many of the Erythraean NIS are benthivorous predators. Some of the species listed above have been documented as parties to sudden changes, where populations of native Mediterranean species appear to have been wholly outcompeted or partially displaced from their habitat space (Galil, 2007). Local population losses and niche contraction of native species may not induce immediate extirpation, but they foreshadow niche limitation, displacement and eventually loss of function, habitat structure and local extinction. Though no deep living NIS were studied, it is expected that as more NIS extend their bathymetric range to encompass the deep sea similar impacts to those discussed will be observed.

Goren et al. (2016) investigated the impacts of the non-indigenous fish on the structure of the food web along the Israeli Mediterranean upper shelf as expressed by their biomass, relative abundance, and by comparing the mean and distribution patterns of the trophic levels among the aliens and the local native species. NIS comprised 54% of individuals and on average 56% of total biomass. The authors found a significant modification of the local food web, with mean weighted trophic level of the non-indigenous fish 3.74, while that of native fish 3.39. Lesser and Slattery (2011) suggested a shift to algal dominated communities in Bahamian mesophotic reefs is associated with the invasion of the Indo-

Pacific lionfish. *Pterois miles* has been spreading rapidly also in the Mediterranean Sea (Ounifi-Ben Amor et al., 2016). The trophic ecology of the eastern Mediterranean mesophotic coral communities is unknown, so that NIS impacts and risk analysis are uncertain. Mediterranean cold water corals are already threatened by human activities and climate change (Roberts and Cairns, 2014).

2.2   Where Is the Knowledge
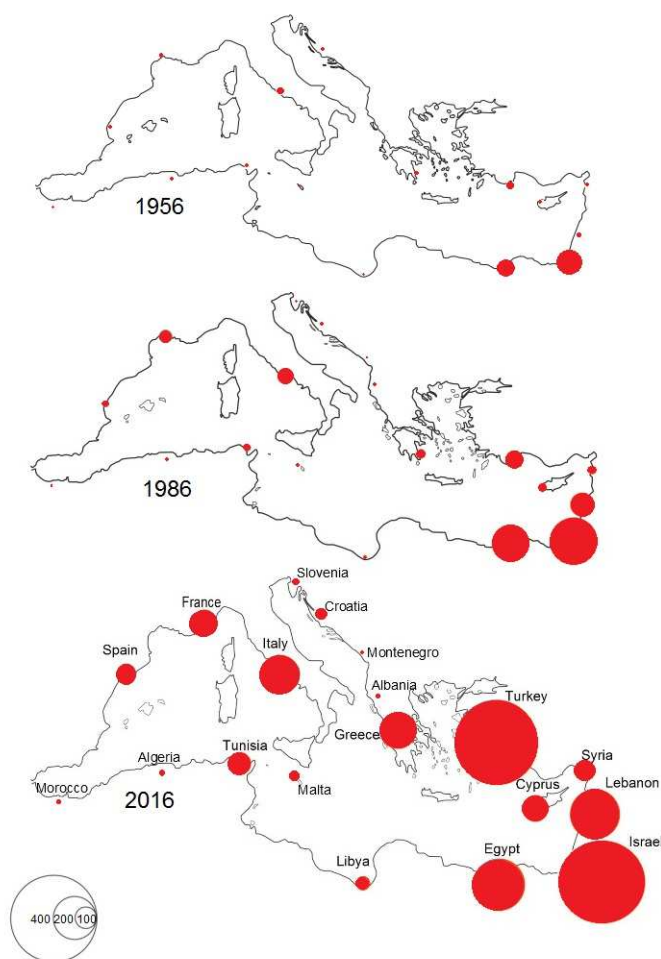
Figure 2.1. The number of recorded NIS in Mediterranean countries 1956, 1986, 2016 (Galil and Gambi, 2017).

Only in the past decade did findings of NIS populations established in deeper waters raised the spectre of a growing number of NIS on the deeper shelf, even beyond the shelf break and well into the upper slope (see above). Their number more than doubled between 1980 and 2016 and is substantially greater in the Levant than in the western Mediterranean (Fig. 2.1).

2.3 How are non indegenous species measured

2.3.1 Methodological sampling aspects

Among the objectives of the MSFD is the promotion of regionally standardized monitoring approaches capable of delivering temporal datasets reflecting long-term trends in marine ecosystem status, incorporating indicators related to the abundance, distribution, and impacts of NIS. The Commission Decision 2017/848 establishes that "Monitoring programmes shall be linked to those for Descriptors 1, 4, 5 and 6, where possible, as they typically use the same sampling methods and it is more practical to monitor non-indigenous species as part of broader biodiversity monitoring, except where sampling needs to focus on main vectors and risk areas for new introductions."

Examination of the latest assessment of the Member States' monitoring programmes under the MSFD reveals that only 5% are related to NIS, and these "will require a clear acceleration to ensure proper coverage given the MSFD Deadlines for the update of marine strategies by 2018, and achieving Good Environmental Status by 2020" (European Commission, 2017b). **Significantly, none of the NIS monitoring programmes targeted lower shelf and upper slope habitats**.

Monitoring NIS using traditional field survey and **morphological identification** methods is insufficient and inefficient. Using such methods, as recommended in Commission Decision 2017/848 (European Commission, 2017b), NIS are extremely difficult to detect at early stage of the introduction, missing incipient invasions. They are ineffective for identifying NIS lacking diagnostic features such as larval stages of many marine taxa, and yet larvae may be major contributors to propagule pressure. The same holds for microscopic taxa (meio-, micro-, nano-fauna), or taxa that are not easily sampled (e.g. in circalittoral rocky substrates, infauna etc.). In addition, they rely on expert taxonomic knowledge, which is unfortunately decreasing (see Lehtiniemi et al. (2015) for further discussion). These issues may explain in part why progress on NIS monitoring lags substantially behind other efforts aimed at achieving GES among MSFD Member States.

They also explain recent recognition of the potential utility of **molecular approaches** for monitoring, preventing, and managing biological invasions in marine systems. In response to the stated ambitions of the MSFD members of the International Convention for the Exploration of the Sea (ICES) Working Group on Introductions and Transfers of Marine Organisms (WGITMO) recently outlined 10 key requirements for NIS assessment and management (Ojaveer et al., 2013), including the development and application of molecular genetic tools. Technical guidance on MSFD monitoring initiatives have similarly recommended development of routine molecular methods (Zampoukas et al., 2014b). Already, molecular methods for targeted species detection and community profiling are being broadly applied in the context of marine biodiversity monitoring (Danovaro et al., 2016). Rapid technological advances make such tools increasingly attractive, yet efforts must be made to better integrate molecular approaches with traditional methods. Such steps would ultimately contribute to more accurate and cost-effective assessments of the distribution and impacts of deep-water NIS (Darling et al., 2017).

Targeted pilot surveys of sensitive, spatially confined slope habitats (e.g. mesophotic reefs, mud volcanoes, cold seeps) within zones noted for populations of deep-living NIS, answer the need expressed in Commission Decision 2017/848 to integrate monitoring for descriptors "except where sampling needs to focus on main vectors and risk areas for new introductions." These pilot surveys may serve as a step towards monitoring deep dwelling bioinvasions and their harm to sensitive slope habitats and help establish appropriate protocols to support baselines, targets, and their spatial variability in defining GES for descriptor 2 in slope habitats.

2.3.2 The number of non-indigenous species <u>newly introduced</u> (D2C1) - Primary

The MSFD requires that the number of non-indigenous species which are newly introduced via human activity into the wild, per assessment period (6 years), measured from the reference year as reported for the initial asessment under Article 8(1) of Directive 2008/56/EC, is minimised and where possible reduced to zero. Member States shall establish the threshold value for the number of new introductions of non-indigenous species, through regional or subregional cooperation.

'Newly-introduced' non-indigenous species shall be understood as those which were not known to be present in the area in the previous assessment period.

Where it is not clear whether the new arrival of non-indigenous species is due to human activity or natural dispersal from neighbouring areas, the introduction shall be counted under D2C1.

Unit: the number of species per assessment area which have been newly introduced in the assessment period (6 years).

Some particularly exposed communities (i.e. mesophotic corals), or regions with high NIS propagule pressure, should be monitored in order to check for the arrival of deep-dwelling NIS.

2.3.3 Abundance and spatial distribution of <u>established</u> non-indigenous species, particularly of invasive species (D2C2) - Secondary

Abundance and spatial distribution of established non-indigenous species, particularly of invasive species, contributing significantly to adverse effects on particular species groups or broad habitat types.

Established non-indigenous species, particularly invasive non-indigenous species, which include relevant species on the list of invasive alien species of Union concern adopted in accordance with Article 4(1) of Regulation (EU) No 1143/2014 and species which are relevant for use under criterion D2C3.

'Established' non-indigenous species shall be understood as those which were known to be present in the area in the previous assessment period.

When species occurrence and abundance is seasonally variable (e.g. plankton), monitoring shall be undertaken at appropriate times of year.

Unit: abundance (number of individuals, biomass in tonnes (t) or extent in square kilometres (km2)) per non-indigenous species.

Few NIS have been seen deeper than 200 m depth and can be listed:

- Erythraean portunid crab *Charybdis longicollis* (Leene, 1938) recorded in the Mediterranean since 1954, and its sacculinid parasite, *Heterosaccus dollfusi* (Boschma, 1960), were collected off Israel as deep as 250 m (Innocenti et al., 2017).

- The Erythraean portunid crab, *Gonioinfradens paucidentatus* (A. Milne Edwards, 1861) was collected off Rhodes at depth of 200 m (Corsini-Foka et al., 2010).

No initial assessment was done as yet.

2.3.4   Proportion of the species group or spatial extent of the broad habitat type which is <u>adversely altered</u> (D2C3) - Secondary

Proportion of the species group or spatial extent of the broad habitat type which is adversely altered due to non-indigenous species, particularly invasive non-indigenous species. Member States shall establish the threshold values for the adverse alteration to species groups and broad habitat types due to non-indigenous species, through regional or subregional cooperation.

Species groups and broad habitat types that are at risk from non-indigenous species, selected from those used for Descriptors 1 and 6.

Unit: the proportion of the species group (ratio of indigenous species to non-indigenous species, as number of species and/or their abundance within the group) or the spatial extent of the broad habitat type (in square kilometres (km2)) which is adversely altered.

Some of the mesophotic coral communities in the Levant (including the *Corallium rubrum* (Linnaeus, 1758) colonies in Crete and southeast Turkey), which serve as important shelter and nursery areas, are already **within the depth range of disruptive carnivorous and omnivorous NIS**. The spread of NIS to depths where these unique assemblages occur bodes ill to the already beleaguered and fragile communities.

Antipatharians, ceriantharians, zoantharians, gorgonians and pennatulaceans have been recorded from the shelf and upper slope in the Aegean and Levant seas (mainly <250 m depth) (Zibrowius, 1979b, a; Chintiroglou et al., 1989; Vafidis et al., 1994; Vafidis et al., 1997; Vafidis and Koukouras, 1998; Abdelsalam, 2014). Recently, a dense population of *Dendrophyllia ramea* (Linnaeus, 1758) has been described in the Levant from eastern Cyprus and Lebanon. The Cypriot population was recorded on soft substrate at depths between 125-155 m, whereas the deepest record has been documented in Lebanese waters, at 172 m (Jimenez et al., 2016; OCEANA, 2016; Orejas et al., 2017)

No initial assessment was done as yet.

2.4   Knowledge on Threshold

It is necessary to first identify the liable regions and habitats and carry out an initial assessment, inclusive of abundance and spatial distribution of alien species and second, analyse the data (wholly lacking at present), before setting realistic thresholds.

The Levantine Basin suffers the highest NIS propagule pressure, situated down current from the outflow of the Suez Canal, and both the LSW and LIW manifest long-term warming trends (see above). Therefore, deep-living NIS (i.e., lower shelf and upper slope) and their impacts on local native communities are likely to be encountered off the Levant coastline.

2.5   CONCLUSIONS

2.5.1   Gaps

The ongoing change of marine biodiversity that has been occurring in the context of multiple anthropogenic stressors, results in significant challenges to the sustainable management of marine resources, particularly in shelf and upper slope habitats. Acknowledgement of these has led to the initiation of policies, conventions, and various other legislative frameworks aimed at preventing future introductions and mitigating the impacts of existing marine invasions. There are several international voluntary guidelines and legislative instruments targeting evaluation and management of impacts caused by marine NIS in the Mediterranean Sea.

One of the most demanding international instruments in this regard is the European Union MSFD, setting rules to prevent and manage the introduction and spread of invasive NIS in the EU — arguably the most important policy measures taken by the EU concerning marine bioinvasions (European Parliament, 2008). The MSFD criteria for 'Good Environmental Status' include 'Impacts of non-indigenous invasive species at the level of species, habitats and ecosystem' (European Commission 2010). Regulation No 1143/2014 adopted by the European Council on the prevention and management of the introduction and spread of invasive alien species (European Commission, 2014) defines 'Invasive Alien Species' (IAS) as those with 'a significant negative impact on biodiversity as well as serious economic and social consequences', but does not clarify the levels of impact to be considered 'significant' and 'serious'. A companion document, Commission Staff Working Document, Impact Assessment (European Commission, 2013), defines IAS as 'alien species whose introduction or spread has been found, through risk assessment, to threaten biodiversity and ecosystem services, or to have a negative impact on the environment, society and the economy', again, without delineation of threat levels.

However, impacts for the vast majority of marine NIS remain unknown and have not been quantitatively or experimentally studied over sufficiently long temporal and spatial scales (Ruiz et al., 2011), and their cumulative and synergetic connections with other drivers of change affecting the marine environment are largely unknown (Stachowicz et al., 2002; Rahel and Olden, 2008). In the policy domain, species for which no impact has been detected, even if no study took place, are deemed to have no impact and, therefore, categorised incorrectly as "harmless". The application of the approach which calls for managing only NIS with demonstrated impact would result in an increased risk of harmful invasions through reliance on 'false certainties'. Limiting evaluation to demonstrable impacts is fundamentally a non-precautionary approach for marine NIS management. Rarely, if ever, is sufficient knowledge available, or the luxury of waiting for such a knowledge base to be acquired, before the window of opportunity closes for feasible management in the marine environment.

The number of marine NIS with sufficient data to satisfy the criteria for 'significant negative impact' is low, because understanding of marine ecosystems functions is constrained due to lack of appropriately designed studies. Unless impacts are conspicuous, induce direct economic cost, or impinge on human welfare, they fail to arouse public awareness or scientific analysis.

While this is true for marine bioinvasion in general, these gaps are exacerbated in the deep sea. It is unlikely bioinvasion impacts on deep-sea habitats and biodiversity will be discerned unless targeted monitoring of the presence and abundance of NIS takes place in the areas most vulnerable to bioinvasions.

2.5.2   Recommendations

In the 1980s it was commonly believed that Erythraean bioinvasions are limited to the Levant and therefore of no interest to European marine conservation. The ever increasing expansion of Erythrean NIS westwards and northwards since is already altering coastal habitats in Mediterranean riparian countries that are EU Member States. As the Suez Canal is the most significant introduction pathway into the Mediterranean Sea and following its last expansion the Suez Canal Authority is already conducting feasibility studies with the aim to increase its depth (www.suezcanal.gov.eg, viewed June 3, 2017), it is likely more deep-living Erythraean NIS may establish populations in the Mediterranean. Increasing seawater temperatures may facilitate their settlement in upper slope habitats.

Only when Erythraean NIS have established populations along the coastlines of European Union Member States bordering the Mediterranean Sea, has interest in their impacts risen among European scientific institutions and scientists. That time-lag has left us with a crippling knowledge gap that at present precludes serious management efforts. Marine scientists, policy makers and management wishing to protect the upper slope biota from NIS impacts would do well to view the Levantine region as their "miners' canary". **It is highly likely that the spread of deep-water NIS will follow the pattern of shelf dwelling NIS** (Fig. 2.1).

It is recommended to

- Use pilot surveys to **establish the current bathymetric range** of populations of deep living NIS (lower shelf and upper slope).
- Use the surveys' results and thermohaline dynamics patterns in the Eastern Mediterranean to construct **models predicting direction and extent of spread** of deep living NIS.
- Identify and map rare, notable, fragile slope habitats (e.g. mesophotic reefs, mud vulcanoes, cold seeps etc.) within the vertical and horizontal spatial reach of NIS populations, and **prioritize them for targeted monitoring**.
- Establish a **long-term Mediterranean-centered GIS platform** for monitoring, analysing and modelling data to advise monitoring actions and support management decisions (i.e. baselines, targets, frequency).
- Augment the existing monitoring toolkit with **molecular genetic tools** which offer critical assistance for monitoring, improving standard biodiversity assessments and, in the case of metabording and eDNA analysis, enabling more comprehensive analysis of ecosystem-wide impacts of deep water marine bioinvasions.

3   Descriptor 3: Populations[1] of all commercially exploited fish and shellfish[2]

Descriptor 3 stipulates that "*all populations of all commercially exploited fish and shellfish should be within safe biological limits, exhibiting a population age and size distribution that is indicative of healthy stocks*". This implies that stocks should (i) be exploited sustainably in a manner that provides high long term yields, (ii) retain their reproductive capacity so that stock biomass can be maintained, and (iii) older and larger fish / shellfish should be maintained, indicating healthy stocks. Good Environmental Status (GES) can only be achieved when all these attributes are fulfilled, implying that commercially exploited stocks should be in a healthy state and that exploitation should be sustainable, yielding the Maximum Sustainable Yield (MSY). MSY is the maximum annual catch, which can be taken year after year without reducing the productivity of the fish stock.

The Marine Strategy Framework Directive (MSFD) builds on existing EU legislation as the Common Fisheries Policy (CFP) and criteria describing stocks status are based on internationally recognised best practices. The exploitation of fisheries resources in the Mediterranean marine sub-regions is monitored internationally by the Scientific Advisory Committee (SAC) of the General Fisheries Commission for the Mediterranean (GFCM), the Scientific Technical and Economic Committee for Fisheries (STECF) of the European Commission and the International Commission for the Conservation of Atlantic Tunas (ICCAT, for those highly migratory species which count for more than 10 % of the value of the total catches in the Mediterranean). GFCM and ICCAT are two Regional Fisheries Management Organizations (RFMOs) established under the auspices of FAO. Fisheries on shared stocks are managed by two regional fisheries organisations: ICCAT for tunas and tuna-like species and the GFCM for other species. In addition, the European Commission's Scientific, Technical and Economic Committee for Fisheries (STECF) monitors the status of stocks exploited by EU fishing fleets operating in the Mediterranean, and provides scientific advice on conservation and management of living marine resources, including biological, economic, environmental, social and technical considerations to the European Commission. The fisheries and aquaculture activity in the Mediterranean Sea is divided according to the statistical divisions of FAO and GFCM in Geographical Sub-Areas (GSAs) (Fig. 3.1).

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[1] In (EU) 2017/848: The term 'populations' shall be understood as the term 'stocks' within the meaning of Regulation (EU) No 1380/2013

[2] Although Directive 2008/56/EC establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive) refers to 'fish and shellfish', all commercially exploited marine species are considered in the present report.
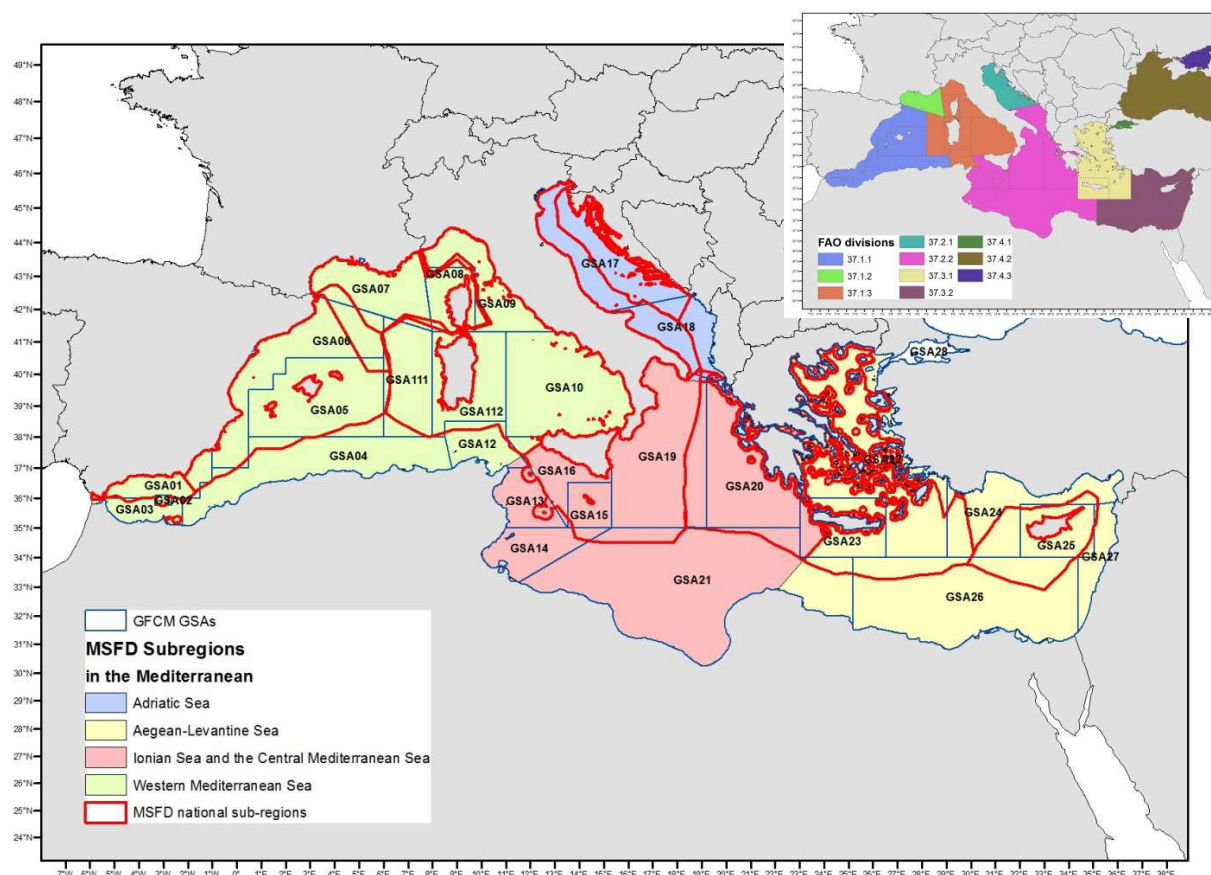
Figure 3.1 : Location of GFCM Geographical Sub-Areas and MSFD Mediterranean national sub-regions. FAO divisions are shown in thumbnail map.

In fisheries management, 'stock' refers to a harvested or managed unit of a fish population. Begg et al. (1999) stated that a stock was described by a set of characteristics of semi-discrete groups of fish with some definable attributes which are of interest to fishery managers. In practice, fish or other commercially exploited stocks of marine species are subpopulations of a particular species, for which demographic parameters (growth, recruitment, natural mortality and fishing mortality) significantly shape population dynamics while less significant factors (such as migrations) are often ignored. Typically, stocks are divided based on geographical location and, ideally, on stock identification studies. They should be studied at a spatial and temporal scale relevant to the understanding and management of the sustainability of its exploitation. As a result, one species may be composed of several stocks located in different areas and a same country may have to manage or to share several stocks of the same species.

The current divisions of the Mediterranean Sea into GFCM GSAs are generally arbitrary, often surrounding large islands (e.g. Malta, Sardinia), or coinciding with national borders (e.g. Spain/France/Italy). Knowledge of species distributions, spawning concentrations, nursery areas, distribution of fishing activity, catches and connectivity defined as the level of dependence of fish production and population dynamics on dispersal and/or migration among areas had a limited (if any) influence on the current delineation of GFCM-GSAs (STECF, 2014).

The recent EU funded StockMed project aimed to identify distinct biological units for different fish and shellfish species among different GFCM-GSAs in the Mediterranean, and to identify stock units for future stock assessments (Fiorentino et al., 2014).

There is a clear miss-match between the MSFD sub-regions and the spatial extent of GSAs (Fig. 3.1). Good environmental status is assessed on a stock scale, whilst the marine reporting unit is the marine sub-region. Good status must be achieved for each marine region or sub-region, but stock distribution patterns rarely correspond to the delimitation of such areas. The populations (stocks) of each species shall be assessed in each region or sub-region on an ecologically relevant scale as established by the relevant scientific experts with reference to Article 26 of Regulation (EC) No 1380/2013, based on the specific aggregations of GFCM geographical sub-areas. The assessment of any particular national marine sub-region (MSR) may include stocks of fishery resources whose geographical distribution is partially or wholly overlapping with it.

For example, the MSR under French responsibility covers or overlaps with several GSAs (GSA 5, 6, 7, 8, 9, 11.1, 11.2) but the stock assessments and the good environmental status evaluations are carried out at other spatial scales. Only stocks evaluated in the GSA 7 (Gulf of Lion) or much larger scale (large pelagic species) were therefore considered in the French MFSD assessment (Foucher and Delaunay, 2017). No evaluation is currently carried out in GSA 8 (Corsica). Abyssal regions in GSA 6, 9 and 11 are also in the French MSR but are either not evaluated or the available stock assessments are distributed on the Spanish or Italian continental shelves and are not relevant to waters under French scrutiny.

The lack of spatial coherence between MSFD sub-regions and the spatial extent of GSAs is even more complicated in the context of applying the MSFD to the deep-sea. Current global practices on the management of Deep-Sea Fisheries (DSF) and Vulnerable Marine Ecosystems (VMEs) in the Mediterranean, through relevant international processes and instruments such as the United Nations General Assembly (UNGA) resolutions and the FAO International Guidelines for the Management of Deep-Sea Fisheries in the High Seas (FAO, 2009a) were reviewed in 2016 and 2017 (FAO, 2016a; GFCM, 2017). DSF were considered those fisheries using bottom contacting gear or deep pelagic trawls and that target species associated with the sea floor between 300 or 400 m and 1 000 m. Shallower fisheries were considered if they also extend below 400 m and much of what is discussed in deep-sea fisheries is also relevant to other fisheries (FAO, 2009, paragraphs 8–11). The reports indicated that the main DSF in the Mediterranean targeted deep-water red shrimps (*Aristaeomorpha foliacea* and *Aristeus antennatus*), which were harvested at 400–800 m depths. In addition, there were important deep-sea trawl fisheries targeting deep-water rose shrimp (*Parapenaeus longirostris*), Norway lobster (*Nephrops norvegicus*) and European hake (*Merluccius merluccius*) at depths of 300–500 m, as well as gillnet fisheries and demersal longliners operating at around 400 m targeting European hake (*Merluccius merluccius*) and blackspot seabream (*Pagellus bogaraveo*). In Spain, Italy and other region of the eastern Mediterranean (e.g. Greece and Cyprus) deep-sea fisheries with pots and traps targeting golden shrimp (*Plesionika* spp.), and with demersal longlines targeting blackspot seabream (*Pagellus bogaraveo*), were also of local importance. Moreover in the last years swordfish longline fishery showed a significant change in fishing practices, as this fishery switched from surface (10–100 m) to mid-water (100–500 m) depth (Cambiè et al., 2013).

Although, FAO / GFCM define deep sea fisheries as operating deeper than 300/400 m, IDEM defines deep Mediterranean Sea as all waters deeper than 200 m. Many stocks are distributed over a wide depth range, and as such the distinction of depth strata and division between shallow- and deep-sea fisheries resources is irrelevant for many species. With few exceptions, such as of small pelagic stocks

which are generally exploited at depths ranging from 0-200m, many species that are primarily exploited by shallow water fisheries can also be targeted as commercially valuable species in deeper areas. Moreover, many of migratory species, such as large pelagic fish or eels, have wide depth ranges and a Mediterranean wide or even larger geographic distribution range.

IDEM is researching the implementation of the MSFD to the deep Mediterranean Sea, which was defined as all waters deeper than 200 m. Several species have wide depth ranges, and may be targeted at very different depths depending on each GSAs and specific fleets. As a result of these much contrasted situations, only very few stocks can be considered as truly limited to shallow waters and most are in line with IDEM. Here we decided to eliminate those few commercial target species which clearly have a distribution restricted to coastal waters less than 200 m (e.g. small pelagics), and which therefore are only or mostly exploited above 200m.

> All species targeted at depths below 200m, and those which are exploited both above and below 200m depth (regardless of the fishing depth) are therefore considered here.

3.1 State of the Art

The Union waters in the Mediterranean Sea are under the sovereignty and jurisdiction of the individual EU countries and do not extend to the 200-mile limit as is the case in the Atlantic Ocean. The situation only differs in the Western Mediterranean Sea, since France and Spain declared (partially overlapping) Exclusive Economic Zones in 2012 and 2013 respectively. As a result of the distribution of territorial waters, most of the surface of the Mediterranean Sea is made up of international waters and most commercial fish stocks are shared with other coastal states, many of which are not part of the EU. This shared responsibility increases from west to east and not so much from north to south. The political context can also make disciplined management difficult in cases of political instability (wars, post-war situations, migratory movements, etc.).

The high biodiversity of Mediterranean fish and shellfish communities is mirrored by the multispecies/multi-gear/seasonal nature of fisheries in the region. Most of the vessels composing the Mediterranean fleet are less than 10 m long and therefore not subjected to the most strict fisheries data collection rules applied to larger fishing vessels such as for example logbook requirements. Nevertheless, except longliners, such small boats are unlikely to target stocks up to 200 m. The vast majority of Mediterranean fishing vessels return to port every days, generally with catches mixing several species (over a hundred different species are commercialized in the Mediterranean Sea). As the total weight of catches of each species is usually below the 50 kg threshold set by the EU Control Regulation, the catches are not declared, nevertheless very few small scale fisheries are involved, considering stocks up to 200 meters. As a result, many catches are unrecorded and despite recent improvements, the number of stocks whose status is unknown for lack of data remains still large. There is also an insufficient number of scientists able to carry out stock assessments; due to this, many stock assessments are not carried out or updated although data is in fact available. According to the most recent scientific advice, only 9 % of fish stocks assessed in the Mediterranean Sea are fished at

sustainable levels (COM 396, 2016)[3]. In parallel, in 2005, GFCM prohibited the use of towed dredges and trawl nets beyond 1 000 m depth in the whole Mediterranean Sea thus banning deep bottom trawling (GFCM Recommendation 29/2005/1). In contrast, in 2016, the EU introduced a bottom trawling ban below 800m and even 400m for untouched areas (EU 2016/2336)[4], however this does not apply to the Mediterranean Sea (EU).

Eight Fishery Restricted Areas (FRA) were established by the GFCM since 2005, four of which, located both in high seas and national waters, were later established to protect deep sea sensitive habitats and fish spawning areas in Cyprus, Egypt, France, Italy and Malta, with a total area of 17678 km^2, i.e. approximately 0.7 percent of the Mediterranean Sea's surface. Three of these were established within a comprehensive multiannual management plan for deep-sea fisheries. As such, in 2016, GFCM multiannual management plan for deep-sea fisheries exploiting European hake and deep-water rose shrimp was established in the Strait of Sicily (GSA 12 to 16). On the other hand, some FRA were established independently from management plans, like in the GSA7 (FRANCE) which aims to protect spawning aggregations and deep sea sensitive habitats (GFCM, 2009b) by implementing a "freezing" of the fishing effort within the FRA. New management plans (Le Corre et al., 2013) were later established in GSA7 and now aim to protect nurseries and spawning areas during the reproduction periods of two identified stocks (M. Merluccius, M. barbatus). The existence of management plans, in particular for deep-sea fisheries, however does not necessarily imply that analytical stock evaluations are carried out.

Once geographical boundaries of different stocks or populations are defined, stock evaluations (often referred to as stock assessments) provide fisheries managers with biological and fisheries data which is required to regulate the exploitation of a stock and define reference points (such as sustainable levels of fisheries mortality rates to achieve exploitation at MSY levels). Stock related variables as well as outcomes form stock assessment analyses and data available from fishery independent surveys may include details on the age structure of the stock, age at first maturity, fecundity, sex-ratio of females to males in the stock, natural mortality (M), fishing mortality (F), growth rate of the fish, spawning behavior, critical habitats (in particular the location of nursery and spawning areas), migratory habits, food preferences, and an estimate of spawning stock biomass (SSB).

In parallel, data regarding fisheries activities are also collected: typology of the fishery, the gear used (longline, rod and reel, nets, etc.) and their respective efficiency, the age structure and sex ratio of the fish harvested by each type of fishery, the fishing effort deployed (e.g. number of fishing days or hours, engine strength, length of nets, soaking time of pots and traps, etc.), the time and geographic location of catches, the value of the fish and the way it is marketed. Mathematical and statistical assessment models combine biological and fisheries data as well as scientific fishery surveys data (for calibration of some models), to define the current status and condition of the stock and to predict how in the future (short or long-term projections), stocks will respond to varying levels of fishing pressure.

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[3] Communication from the Commission to the European Parliament and the Council - Consultation on the fishing opportunities for 2017 under the Common Fisheries Policy. See: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52016DC0396

[4] **Regulation (EU) 2016/2336 of the European Parliament and of the Council of 14 December 2016 establishing specific conditions for fishing for deep-sea stocks in the north-east Atlantic and provisions for fishing in international waters of the north-east Atlantic.**

3.1.1 Species with a stock evaluation

Mediterranean stock assessments are carried out by working groups convened under the auspices of GFCM, ICCAT, and STECF, and cover a large number of species and GSAs relevant to MFSD (with the exception of Black Sea and certain southern Mediterranean stocks). Some species such as Clupeidae, *Spicara* spp., *S. solea* or *Trachurus* spp. are primarily localized in shallow waters and are not relevant to the deep Mediterranean focus. While some species such as Norway lobster (*N. norvegicus*) and European hake (*M. merluccius)* may be exploited over large bathymetric ranges and are evaluated at that scale. On the other hand, deep water shrimps such as *A. antennatus, A. foliacea* are often (but not exclusively) found in area deeper than 200m.

STECF EWG 17-08 (STECF, 2017b) compiled stock assessment parameters for all STECF, GFCM and ICCAT assessments carried out for the evaluation years 2013-2016. Those that are relevant to the implementation of the MSFD in the deep Mediterranean Sea are listed below (Table 3.1). Overall, during this period, 85 stocks were assessed, covering 20 different taxa. Six are exclusively exploited above 200m and four only are exclusively exploited below 200m. All other assessed taxa may be exploited over a wide depth range.

Table 3.1: List of STECF, GFCM and ICCAT assessments in2013-2016 relevant to the implementation of the MSFD in the deep Mediterranean Sea. Source: stock assessment database compiled during STECF EWG 17-08 (STECF, 2017b); information provided by working group chair G. Scarcella. Violin: Only or mostly exploited below 200 m; Blue: Only or mostly exploited above 200 m; White: Exploited above and below 200 m.

Order	Family	Scientific name	English name	French name	Spanish name	GSAs	Source	Reporting year
GADIFORMES (Demersal)	Gadidae	*Micromesistius poutassou*	Blue whiting(=Poutassou)	Merlan bleu	Bacaladilla	6	STECF	2014
	Gadidae	*Micromesistius poutassou*	Blue whiting(=Poutassou)	Merlan bleu	Bacaladilla	9	STECF	2014
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	1	STECF	2013
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	1-3	GFCM	2016
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	18	STECF	2013
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	10	STECF	2013
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	9	GFCM	2016
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	7	GFCM	2016
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	1-5-6-7	STECF	2015
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	6	GFCM	2016
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	5	GFCM	2016
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	19	STECF	2015
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	9-10-11	STECF	2015
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	12-13-14-15-16	GFCM	2016

Order	Family	Scientific name	English name	French name	Spanish name	GSAs	Source	Reporting year
	Merlucciidae	*Merluccius merluccius*	European hake	Merlu européen	Merluza europea	17-18	GFCM	2016
LOPHIIFORMES (Demersal)	Lophiidae	*Lophiidae*	Anglerfishes nei	Baudroies, etc. nca	Rapes, etc. nep	6	STECF	2014
	Lophiidae	*Lophiidae*	Anglerfishes nei	Baudroies, etc. nca	Rapes, etc. nep	5	STECF	2014
	Lophiidae	*Lophius budegassa*	Blackbellied angler	Baudroie rousse	Rape negro	5	STECF	2014
	Lophiidae	*Lophius budegassa*	Blackbellied angler	Baudroie rousse	Rape negro	6	STECF	2014
	Lophiidae	*Lophius piscatorius*	Angler(=Monk)	Baudroie commune	Rape	1-5-6-7	STECF	2016
	Lophiidae	*Lophius spp*	Monkfishes nei	Baudroies nca	Rapes nep	5	STECF	2014
	Lophiidae	*Lophius spp*	Monkfishes nei	Baudroies nca	Rapes nep	1-5-6-7	STECF	2016
	Lophiidae	*Lophius spp*	Monkfishes nei	Baudroies nca	Rapes nep	6	STECF	2014
NATANTIA (Demersal)	Aristaeidae	*Aristaeomorpha foliacea*	Giant red shrimp	Gambon rouge	Gamba española	11	STECF	2015
	Aristaeidae	*Aristaeomorpha foliacea*	Giant red shrimp	Gambon rouge	Gamba española	10	STECF	2015
	Aristaeidae	*Aristaeomorpha foliacea*	Giant red shrimp	Gambon rouge	Gamba española	18-19	STECF	2015
	Aristaeidae	*Aristaeomorpha foliacea*	Giant red shrimp	Gambon rouge	Gamba española	9	GFCM	2016
	Aristaeidae	*Aristeus antennatus*	Blue and red shrimp	Crevette rouge	Gamba rosada	5	GFCM	2016
	Aristaeidae	*Aristeus antennatus*	Blue and red shrimp	Crevette rouge	Gamba rosada	9	GFCM	2016
	Aristaeidae	*Aristeus antennatus*	Blue and red shrimp	Crevette rouge	Gamba rosada	6	GFCM	2016
	Aristaeidae	*Aristeus antennatus*	Blue and red shrimp	Crevette rouge	Gamba rosada	1	GFCM	2016
	Penaeidae	*Parapenaeus longirostris*	Deep-water rose shrimp	Crevette rose du large	Gamba de altura	5	STECF	2013
	Penaeidae	*Parapenaeus longirostris*	Deep-water rose shrimp	Crevette rose du large	Gamba de altura	1	STECF	2016
	Penaeidae	*Parapenaeus longirostris*	Deep-water rose shrimp	Crevette rose du large	Gamba de altura	10	STECF	2016
	Penaeidae	*Parapenaeus longirostris*	Deep-water rose shrimp	Crevette rose du large	Gamba de altura	12-13-14-15-16	GFCM	2016
	Penaeidae	*Parapenaeus longirostris*	Deep-water rose shrimp	Crevette rose du large	Gamba de altura	19	GFCM	2016
	Penaeidae	*Parapenaeus longirostris*	Deep-water rose shrimp	Crevette rose du large	Gamba de altura	6	STECF	2013
	Penaeidae	*Parapenaeus longirostris*	Deep-water rose shrimp	Crevette rose du large	Gamba de altura	17-18-19	STECF	2015
	Penaeidae	*Parapenaeus longirostris*	Deep-water rose shrimp	Crevette rose du large	Gamba de altura	17-18	GFCM	2016
	Penaeidae	*Parapenaeus longirostris*	Deep-water rose shrimp	Crevette rose du large	Gamba de altura	9-10-11	STECF	2016
	Penaeidae	*Parapenaeus longirostris*	Deep-water rose shrimp	Crevette rose du large	Gamba de altura	9	STECF	2016
PERCOIDEI (Demersal)	Centracanthidae	*Spicara smaris*	Picarel	Picarel	Caramel	25	GFCM	2016
	Moronidae	*Dicentrarchus labrax*	European seabass	Bar européen	Lubina	7	STECF	2016
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	17-18	STECF	2015
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	15-16	GFCM	2016
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	25	GFCM	2016
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	6	GFCM	2016
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	1	STECF	2014
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	13	GFCM	2016

Order	Family	Scientific name	English name	French name	Spanish name	GSAs	Source	Reporting year
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	19	STECF	2015
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	18	GFCM	2016
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	17	GFCM	2016
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	5	STECF	2013
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	10	GFCM	2014
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	9	STECF	2014
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	7	GFCM	2016
	Mullidae	*Mullus barbatus*	Red mullet	Rouget de vase	Salmonete de fango	11	STECF	2013
	Mullidae	*Mullus surmuletus*	Surmullet	Rouget de roche	Salmonete de roca	5	GFCM	2016
	Mullidae	*Mullus surmuletus*	Surmullet	Rouget de roche	Salmonete de roca	15-16	STECF	2013
	Mullidae	*Mullus surmuletus*	Surmullet	Rouget de roche	Salmonete de roca	9	STECF	2016
	Sparidae	*Sparus aurata*	Gilthead seabream	Dorade royale	Dorada	7	STECF	2016
PLEURONECTI-FORMES (Demersal)	Soleidae	*Solea solea*	Common sole	Sole commune	Lenguado común	7	STECF	2016
	Soleidae	*Solea solea*	Common sole	Sole commune	Lenguado común	17	GFCM	2016
REPTANTIA (Demersal)	Nephropidae	*Nephrops norvegicus*	Norway lobster	Langoustine	Cigala	15-16	STECF	2013
	Nephropidae	*Nephrops norvegicus*	Norway lobster	Langoustine	Cigala	11	STECF	2016
	Nephropidae	*Nephrops norvegicus*	Norway lobster	Langoustine	Cigala	9	STECF	2016
	Nephropidae	*Nephrops norvegicus*	Norway lobster	Langoustine	Cigala	6	STECF	2016
	Nephropidae	*Nephrops norvegicus*	Norway lobster	Langoustine	Cigala	5	STECF	2014
	Nephropidae	*Nephrops norvegicus*	Norway lobster	Langoustine	Cigala	17-18	STECF	2016
STOMATOPODA (Demersal)	Squillidae	*Squilla mantis*	Spottail mantis squillid	Squille ocellée	Galera ocelada	18	STECF	2015
	Squillidae	*Squilla mantis*	Spottail mantis squillid	Squille ocellée	Galera ocelada	17	STECF	2015
	Squillidae	*Squilla mantis*	Spottail mantis squillid	Squille ocellée	Galera ocelada	17-18	STECF	2015
SCOMBROIDEI (Large Pelagic)	Scombridae	*Thunnus thynnus*	Atlantic bluefin tuna	Thon rouge de l'Atlantique	Atún rojo del Atlántico	ALL	ICCAT	2014
	Xiphiidae	*Xiphias gladius*	Swordfish	Espadon	Pez espada	ALL	ICCAT	2016
CLUPEIFORMES (Small Pelagic)	Clupeidae	*Sardina pilchardus*	European pilchard(=Sardine)	Sardine commune	Sardina europea	17-18	GFCM	2016
	Clupeidae	*Sardina pilchardus*	European pilchard(=Sardine)	Sardine commune	Sardina europea	1-3	GFCM	2016
	Clupeidae	*Sardina pilchardus*	European pilchard(=Sardine)	Sardine commune	Sardina europea	1	GFCM	2016
	Clupeidae	*Sardina pilchardus*	European pilchard(=Sardine)	Sardine commune	Sardina europea	17-18	STECF	2016
	Clupeidae	*Sardina pilchardus*	European pilchard(=Sardine)	Sardine commune	Sardina europea	6	STECF	2016
	Clupeidae	*Sardina pilchardus*	European pilchard(=Sardine)	Sardine commune	Sardina europea	6	GFCM	2016
	Clupeidae	*Sardina pilchardus*	European pilchard(=Sardine)	Sardine commune	Sardina europea	16	GFCM	2015
	Engraulidae	*Engraulis encrasicolus*	European anchovy	Anchois	Boquerón	17-18	STECF	2016
	Engraulidae	*Engraulis encrasicolus*	European anchovy	Anchois	Boquerón	9	STECF	2016

Order	Family	Scientific name	English name	French name	Spanish name	GSAs	Source	Reporting year
	Engraulidae	*Engraulis encrasicolus*	European anchovy	Anchois	Boquerón	6	STECF	2016
	Engraulidae	*Engraulis encrasicolus*	European anchovy	Anchois	Boquerón	17-18	GFCM	2016

Only or mostly exploited above 200m
Only or mostly exploited below 200m
Exploited above and below 200m

In 2016, the GFCM working groups assessed 26 stocks (populations exploited in both shallow and deep waters) of demersal fishery resources (WGSAD) in the Mediterranean Sea (Jadaud and Saraux, 2016) out of which, 20 stocks are in a state of overexploitation and 6 are exploited sustainably. Cardinale and Scarcella (2017) collated more than 500 stock assessments results over the 2007-2015 periods (from both STECF and GFCM SAC) and reported their continued over-exploitation and the failure of the fishery management system in the Mediterranean Sea (through ineffective national management plans and effort reductions to control fishing mortalities and continuous non-adherence to the scientific advice). Colloca et al. (2017) compiled data for more than 80 stocks of fish and crustaceans assessed in the period 2002–2014 (from both STECF and GFCM SAC) and showed that for 90% of them the current fishing mortality (F) is higher than the fishing mortality at MSY (F_{MSY}). Demersal fish, particularly hake (*Merluccius merluccius),* black bellied anglerfish (*Lophius boudegassa*), and red mullet (*Mullus barbatus*) were the most over-exploited species. Finally, reviewing stock assessments over the 2003-2014 periods (from both STECF and GFCM SAC), Ulrich (2017) reported status of 90 stocks and found that 69 were over-exploited, 13 had no reference points and only 8 were sustainably exploited. In the western and central Mediterranean, respectively 78% and 77% of the stocks were over exploited and no assessment were carried out in the eastern Mediterranean since 2012, mostly because of the suspension of the sampling from the Data Collection Framework funds due to economic reasons in Greece.

In addition, large pelagic stocks are assessed by the International Commission for the Conservation of Atlantic Tunas (ICCAT) at large geographical scale: eastern Atlantic and Mediterranean for bluefin tuna (*Thunnus thynnus*) and albacore tuna (*Thunnus alalunga*), and Mediterranean for swordfish (*Xiphias gladius*), which necessitates the combination of data collected by both EU Member-States and third countries fishing these stocks (ICES, 2014). The Atlantic bluefin tuna stock is in good environmental status considering the requirements of descriptor 3 of the MFSD following the successful implementation of a recovery plan. The swordfish stock is in a state of overexploitation with insufficient fertile biomass. The quantity of data available does not allow a quantitative assessment of Mediterranean albacore tuna.

Finally, the international Commission for the exploration of the Sea (ICES) is also investigating the European eel (*Anguilla anguilla*) stock at the scale of the North Atlantic, North Sea, Baltic, Mediterranean and North African areas. Also little is known about its status, the eel stock remains in a critical situation (ICES, 2016d).

3.1.2 Species without Stock Evaluation

The number of stocks benefiting from an assessment in the Mediterranean is very low. Only 48 of the 235 stocks exploited in the western Mediterranean benefit from a scientific evaluation (Foucher and Delaunay, 2017). The proportion is even lower in the eastern Mediterranean. Lleonart (2015) reported that 285 different species or group of species were landed in Mediterranean fisheries, out of which only 17 taxa, account for more than 1% of total catch, and only two represent more than 10%: sardines and anchovies. In total only 27 species are currently assessed, with only nine belonging to the group with more than 1% of Mediterranean landings. The other 18 have particular economic importance or local interest or both (Lleonart, 2015).

Nevertheless, many unevaluated species may contribute significantly to the landings in both weight or value (Fig. 3.2). For example, in the GSA 7 (Gulf of Lion), cephalopods such as *Octopus* sp. and *Eledone* sp. are the first groups of species landed in 2015 since they represent up to 13% of landings in weight and are not subjected to stock evaluation. Other species caught with hake and red mullet, like anglerfish (*Lophius* sp.), gurnards or Norway lobsters (*Nephrops norvegicus*) also occur on the deeper areas of the continental shelves or slopes, and are not evaluated by the GFCM at the GSA 7 scale.
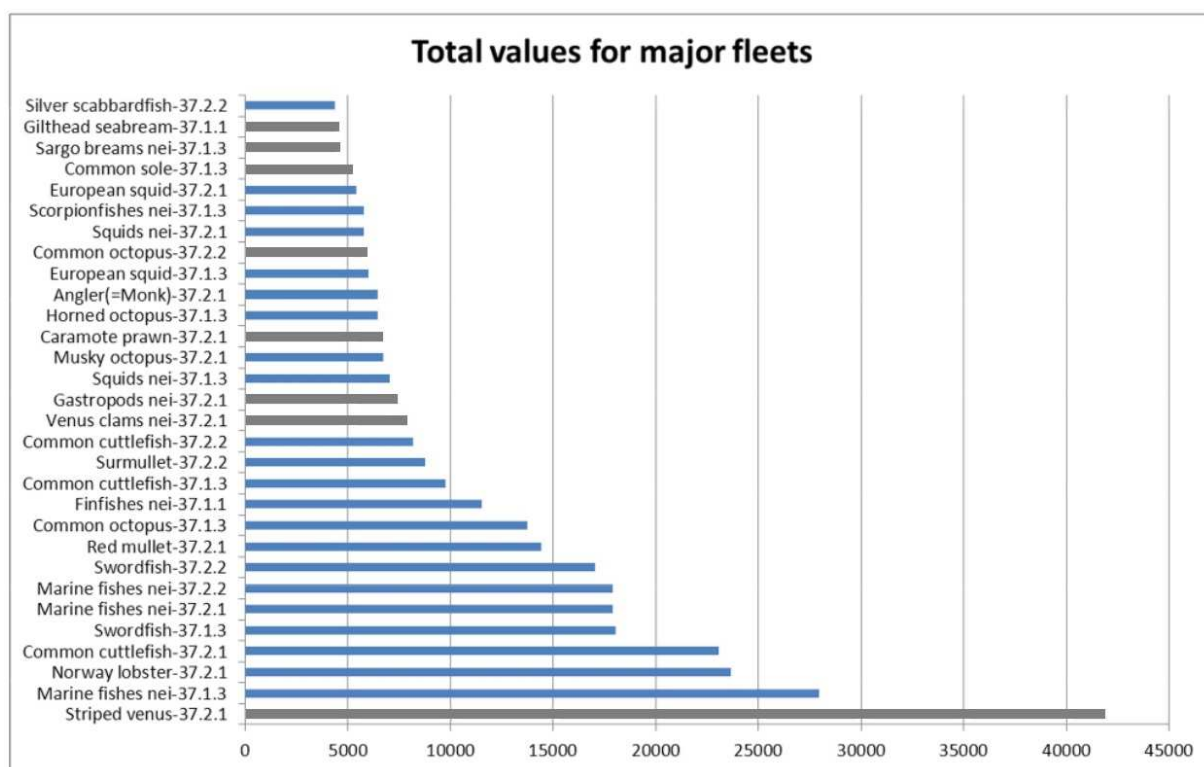
Figure 3.2 The thirty most important stocks in FAO major fishing Area 37 – Mediterranean and Black Sea (based on catch values) targeted by EU fleet segments which together generated 80% of total landings values and for which no stock assessment data was available in 2014. X-axis shows value in Euros- source (STECF, 2015). Species that may be caught by deep-sea fisheries are highlighted in blue.

Furthermore, following the decline of shallow coastal water resources a shift of fishing effort to deeper waters has been document in many fisheries, facilitated by increasing fishing vessel engine powers and technological advances (Koslow et al., 2000; Garibaldi and Limongelli, 2003; Morato et al., 2006). This trend of increasingly targeting previously unexploited deep-water species has also been noted in the Mediterranean Sea, where it is for instance known that trawlers in Greece, Italy and Spain have expanded the median depth of their fishing operations from waters shallower than 100 m to depths exceeding 300 m (Damalas et al., 2015). Similarly, swordfish longline fisheries in Italy, Greece and the Balearic Islands have switched from surface to mid-water fishing grounds extending to depths of up to 500 m (Cambiè et al., 2013). For some fleet however, the local regulation limiting the fishing trip to 14h duration may slow down and hopefully even prevent this evolution.

3.2 Where is the knowledge

The European Commission Decision (EU) 2017/848 specifies that the assessment of good environmental status shall apply to all stocks covered by Commission Implementing Decisions (EU) 2016/1251, adopting a multiannual Union programme for the collection, management and use of data in the fisheries and aquaculture sectors for the period 2017-2019. Regulation (EU) 2016/1701 lays down rules on the format for the submission of work plans for data collection, management and use of data in the fisheries and aquaculture sectors (EU-DCMAP) and for obtaining the scientific advice necessary for the implementation CFP (Regulation (EU) No 1380/2013 as amended by Regulation (EU) 2015/812).

This Data Collection Multiannual Programme (DC-MAP) includes, among others, catches and landings of the most important *"métiers"* in the EU Mediterranean Member States, the biological data of the most important species, the collection of socio-economic data, and the estimate of ecosystem indicators. It goes beyond the objective of collecting data for commercial species but also include '*Species to be monitored under protection programmes in the Union or under international obligations*' to cater for the revised Common Fishery Policy's increased focus on environmental effects of fishing. Full species lists may be found in Commission Implementing Decision (EU) 2016/1251, adopting a multiannual Union programme for the collection, management and use of data in the fisheries and aquaculture sectors for the period 2017-2019[5]. These lists were filtered to only retain species occurring in the Mediterranean Sea and have been sorted by depth range using FishBase (2017) and expert judgment (Table 3.2). The DC-MAP requirements in Mediterranean EU waters concern 53 exploited stocks species for which biological data on exploited stocks species is required for the international management process including species under EU management, recovery or multi-annual plans or EU action plans for conservation and management based on Regulation (EU) No 1380/2013 as amended by Regulation (EU) 2015/812. Seventeen stocks are exclusively found and exploited above 200 m (e.g: sole, octopus, picarels, sea bass, small pelagics) and four are exclusively deep species (three deep water shrimps species and blue whiting). The actual species for which Member States collect data ultimately depend on catches and species distribution patterns vary across the Mediterranean MSFD sub-regions (EU 2016/1251).

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[5] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016D1251&from=EN

Table 3.2: Mediterranean Species listed as stocks in EU waters (EU 2016/1251)

	Common name	Scientific name	Region/RFMO
Bony fishes	Wahoo	*Acanthocybium solandri*	Atlantic Ocean and adjacent seas
Bony fishes	*Thunnus alalunga*	Albacore tuna	Atlantic Ocean and adjacent seas
Bony fishes	European eel	*Anguilla anguilla*	all areas in the Med
Bony fishes	Transparent gobid	*Aphia minuta*	GSA 9,10,16 and 19
Bony fishes	Sand smelt	*Atherina* spp.	GSA 9,10,16 and 19
Bony fishes	*Euthynnus alleteratus*	Atlantic back skipjack	Atlantic Ocean and adjacent seas
Bony fishes	Bullet tuna	*Auxis rochei*	Atlantic Ocean and adjacent seas
Bony fishes	Frigate tuna	*Auxis thazard*	Atlantic Ocean and adjacent seas
Bony fishes	Bogue	*Boops boops*	1.3, 2.1, 2.2, 3.1, 3.2
Bony fishes	Dolphinfish	*Coryphaena equiselis*	all areas in the Med
Bony fishes	Dolphinfish	*Coryphaena hippurus*	all areas in the Med
Bony fishes	Sea bass	*Dicentrarchus labrax*	all areas in the Med
Bony fishes	Anchovy	*Engraulis encrasicolus*	all areas in the Med
Bony fishes	Grey gurnard	*Eutrigla gurnardus*	2.2, 3.1
Bony fishes	Billfish	*Istiophoridae*	all areas in the Med
Bony fishes	Black-bellied angler	*Lophius budegassa*	1.1, 1.2, 1.3, 2.2, 3.1
Bony fishes	Anglerfish	*Lophius piscatorius*	1.1, 1.2, 1.3, 2.2, 3.1
Bony fishes	Blue marlin	*Makaira nigricans (or mazara)*	Atlantic Ocean and adjacent seas
Bony fishes	Hake	*Merluccius merluccius*	all areas in the Med
Bony fishes	Blue whiting	*Micromesistius poutassou*	1.1, 3.1
Bony fishes	Grey mullets	*Mugilidae*	1.3, 2.1, 2.2, 3.1
Bony fishes	Red mullet	*Mullus barbatus*	all areas in the Med
Bony fishes	Striped red mullet	*Mullus surmuletus*	all areas in the Med
Bony fishes	Plain bonito	*Orcynopsis unicolor*	Atlantic Ocean and adjacent seas
Bony fishes	Pandora	*Pagellus erythrinus*	all areas in the Med
Bony fishes	Atlantic bonito	*Sarda sarda*	Atlantic Ocean and adjacent seas
Bony fishes	Sardine	*Sardina pilchardus*	all areas in the Med
Bony fishes	Mackerel	*Scomber* spp.	all areas in the Med
Bony fishes	*Katsuwonus pelamis*	Skipjack tuna	Atlantic Ocean and adjacent seas
Bony fishes	Sole	*Solea vulgaris*	1.2, 2.1, 3.1
Bony fishes	Gilthead sea bream	*Sparus aurata*	1.2, 3.1
Bony fishes	Picarels	*Spicara smaris*	2.1, 3.1, 3.2
Bony fishes	White marlin	*Tetrapturus albidus*	Atlantic Ocean and adjacent seas
Bony fishes	Bluefin tuna	*Thunnus thymus*	Atlantic Ocean and adjacent seas
Bony fishes	Mediterranean horse mackerel	*Trachurus mediterraneus*	All areas in the Med
Bony fishes	Horse mackerel	*Trachurus trachurus*	all areas in the Med
Bony fishes	Tub gurnard	*Trigla lucerna*	1.3, 2.2, 3.1
Bony fishes	Poor cod	*Trisopterus minutus*	All regions
Bony fishes	Swordfish	*Xiphias gladius*	Atlantic Ocean and adjacent seas
Cartilaginous fishes	Blue shark	*Prionace glauca*	Atlantic Ocean and adjacent seas
Cartilaginous fishes	All commercial sharks, rays & skates ([4])	*Selachii, Rajidae*	All regions
Crustaceans	Giant red shrimp	*Aristeomorpha foliacea*	all areas in the Med

	Common name	Scientific name	Region/RFMO
Crustaceans	Red shrimp	*Aristeus antennatus*	all areas in the Med
Crustaceans	Norway lobster	*Nephrops norvegicus*	all areas in the Med
Crustaceans	Deepwater rose shrimp	*Parapenaeus longirostris*	all areas in the Med
Crustaceans	Caramote prawn	*Penaeus kerathurus*	3.1
Crustaceans	Mantis shrimp	*Squilla mantis*	1.3, 2.1, 2.2
Molluscs	Horned/curled octopus	*Eledone cirrhosa*	1.1, 1.3, 2.1, 2.2, 3.1
Molluscs	Musky octopus	*Eledone moschata*	1.3, 2.1, 2.2, 3.1
Molluscs	Squid	*Illex* spp., *Todarodes* spp.	all areas in the Med
Molluscs	Common squid	*Loligo vulgaris*	all areas in the Med
Molluscs	Common octopus	*Octopus vulgaris*	all areas in the Med
Molluscs	Clam	*Veneridae*	2.1, 2.2

(blue)	Only or mostly exploited above 200m
(purple)	Only or mostly exploited below 200m
(white)	Exploited above and below 200m

In 2016 GFCM adopted a Recommendation on the progressive implementation of data submission in line with the GFCM Data Collection Reference Framework (DCRF) (REC.DIR-GFCM/40/2016/2). The DCRF represents a comprehensive framework for the collection and submission of fisheries-related data in the Mediterranean and Black Sea. Full species lists are available in the GFCM-DCRF manual[6]. These lists were filtered to only retain species occurring in the Mediterranean Sea and have been sorted by depth range based on FishBase (2017) and expert knowledge (see Table 3.3). In order to fulfill the GFCM objectives, the data collected within the DCRF encompass area-based information on national fishing fleets and their activities, catch and effort data, biological information on the main target species, and data on incidental catches of vulnerable species as well as discards. In addition, contracting parties are required to collect socio-economic data in order to assess the economic situation of fishing enterprises and employment trends.

The GFCM-DCRF distinguishes between several types of priority species:

• Group 1 species (n= 11, Table 3.3 A). Species that drive the fishery and for which assessment is regularly carried out. Only height species are relevant to the Mediterranean Sea, out of which two are exclusively exploited above 200m (sardine and anchovy) and one is exclusively deep (deepwater rose shrimp).

• Group 2 species (n= 33, Table 3.3 B). Species which are important in terms of landing and/or economic values at regional and sub-regional level, and for which assessment is not regularly carried out. Thirty species are relevant to the Mediterranean Sea, out of which ten are exclusively exploited above 200m (horse mackerel, sole, picarel, sea bream, octopus,…) and four are exclusively deep (2 deepwater shrimps species, blue whiting, black-bellied angler)

• Group 3 species (n=27, Table 3.3 C). Species within international/ national management plans and recovery and/or conservation action plans; non-indigenous species with the greatest potential impact. All these are relevant to the Mediterranean Sea and only two are exclusively exploited above 200m (starry smooth-hound and red coral).

TABLE 3.3: Mediterranean Species (excluding sea birds, marine mammals and turtles) to be monitored under GFCM Data Collection Reference Framework (DCRF) (REC.DIR-GFCM/40/2016/2)

Table 3.3 A - Group 1 species. Species that drive the fishery and for which assessment is regularly carried out

	GFCM subregions	Western Mediterranean Sea	Central Mediterranean Sea	Adriatic Sea	Eastern Mediterranean Sea
	GSAs	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	12, 13, 14, 15, 16, 19, 20, 21	17, 18	22, 23, 24, 25, 26, 27
	Countries	Algeria, France, Italy, Monaco, Morocco, Spain	Italy, Greece, Libya, Malta, Tunisia	Albania, Bosnia and Herzegovina, Croatia, Italy, Montenegro, Slovenia	Cyprus, Egypt, Greece, Israel, Lebanon, Syria, Turkey
Scientific name	FAO 3-alpha code				
Engraulis encrasicolus	ANE	X	X	X	X
Merluccius merluccius	HKE	X	X	X	X
Mullus barbatus	MUT	X	X	X	X
Mullus surmuletus	MUR	X	X		X
Nephrops norvegicus	NEP	X	X	X	
Parapenaeus longirostris	DPS	X	X	X	X
Sardina pilchardus	PIL	X	X	X	X
Squalus acanthias*	DGS				

Only or mostly exploited above 200m
Only or mostly exploited below 200m
Exploited above and below 200m

* Species included in Appendix III (species whose exploitation is regulated) of the Barcelona Convention (protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean).

Table 3.3 B-Group 2 species. Species which Species which are important in terms of landing and/or economic values at regional and subregional level, and for which assessment is not regularly carried out.

GFCM subregions	Western Mediterranean Sea	Central Mediterranean Sea	Adriatic Sea	Eastern Mediterranean Sea
GSAs	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	12, 13, 14, 15, 16, 19, 20, 21	17, 18	22, 23, 24, 25, 26, 27
Countries	Algeria, France, Italy, Monaco, Morocco, Spain	Italy, Greece, Libya, Malta, Tunisia	Albania, Bosnia and Herzegovina, Croatia, Italy, Montenegro, Slovenia	Cyprus, Egypt, Greece, Israel, Lebanon, Syria, Turkey
Scientific name — FAO 3-alpha code				
Aristaeomorpha foliacea — ARS		X		
Aristeus antennatus — ARA	X			
Boops boops — BOG	X	X	X	X
Chamelea gallina — SVE			X	
Coryphaena hippurus — DOL		X		
Diplodus annularis — ANN		X		
Eledone cirrhosa — EOI	X		X	
Eledone moschata — EDT			X	
Galeus melastomus — SHO	X			
Lophius budegassa — ANK	X	X		
Micromesistius poutassou — WHB	X			
Octopus vulgaris — OCC	X	X	X	X
Pagellus bogaraveo — SBR	X			
Pagellus erythrinus — PAC	X	X	X	X
Raja asterias — JRS	X			
Raja clavata — RJC	X	X		
Sardinella aurita — SAA	X	X		X
Saurida undosquamis — LIB				X
Scomber japonicus — MAS	X			X
Scomber scombrus — MAC	X	X		
Sepia officinalis — CTC	X	X	X	
Siganus luridus — IGU				X
Siganus rivulatus — SRI				X
Solea vulgaris — SOL			X	X
Sphyraena sphyraena — YRS		X		
Spicara smaris — SPC			X	X
Squilla mantis — MTS			X	
Trachurus mediterraneus — HMM	X			
Trachurus picturatus — JAA	X			
Trachurus trachurus — HOM	X	X		X

Only or mostly exploited above 200m
Only or mostly exploited below 200m
Exploited above and below 200m

Table 3.3 C - Group 3 species. Species within international/ national management plans and recovery and/or conservation action plans; non-indigenous species with the greatest potential impact.

	GFCM subregions	Western Mediterranean Sea	Central Mediterranean Sea	Adriatic Sea	Eastern Mediterranean Sea
	GSAs	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	12, 13, 14, 15, 16, 19, 20, 21	17, 18	22, 23, 24, 25, 26, 27
	Countries	Algeria, France, Italy, Monaco, Morocco, Spain	Italy, Greece, Libya, Malta, Tunisia	Albania, Bosnia and Herzegovina, Croatia, Italy, Montenegro, Slovenia	Cyprus, Egypt, Greece, Israel, Lebanon, Syria, Turkey
Scientific name	FAO 3-alpha code				
Dalatias licha	SCK	X	X	X	X
Dipturus oxyrinchus	RJO	X	X	X	X
Etmopterus spinax	ETX	X	X	X	X
Galeus melastomus	SHO		X	X	X
Hexanchus griseus	SBL	X	X	X	X
Mustelus asterias*	SDS	X	X	X	X
Mustelus mustelus*	SMD	X	X	X	X
Mustelus punctulatus*	MPT	X	X	X	X
Myliobatis aquila	MYL	X	X	X	X
Prionace glauca *	BSH	X	X	X	X
Pteroplatytrygon violacea	PLS	X	X	X	X
Raja asterias	JRS		X	X	X
Raja clavata	RJC			X	X
Raja miraletus	JAI	X	X	X	X
Scyliorhinus canicula	SYC	X	X	X	X
Scyliorhinus stellaris	SYT	X	X	X	X
Squalus acanthias*	DGS	X	X	X	X
Squalus blainvillei	QUB	X	X	X	X
Torpedo marmorata	TTR	X	X	X	X
Torpedo torpedo	TTV	X	X	X	X
Fistularia commersonii	FIO				X
Lagocephalus sceleratus	LFZ				X
Marsupenaeus japonicus	KUP				X
Metapenaeus stebbingi	MNG				X
Scomberomorus commerson	COM				X
Corallium rubrum	COL	X	X	X	X
Anguilla anguilla	ELE	X	X	X	X

Only or mostly exploited above 200m
Only or mostly exploited below 200m
Exploited above and below 200m

* Species included in Appendix III (species whose exploitation is regulated) of the Barcelona Convention (protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean).

It is worth noting that both in the European DC-MAP and GFCM DCRF, no direct information on the depth needs to be provided and deep-sea fisheries are only identified on the basis of the landing composition (resource-based identification).

The DCMAP also includes the collection of trawl-survey (MEDITS) and acoustic data (MEDIAS) for the assessment of demersal fish species and stock biomass of small pelagics, respectively. The MEDITS program aims at conducting coordinated surveys from bottom trawling in the Mediterranean Sea and to produce basic information on benthic and demersal species in term of population distribution as well as demographic structure, on the continental shelves and along the upper slopes at a global scale in the Mediterranean Sea, through systematic bottom trawl surveys. The surveys intend to include as much as possible all the trawlable areas over the shelves and the upper slopes from 10 to 800 m depth off the coasts of the partner countries (Fig. 3.3). The survey data may be used in the future to produce indicator proxies in the absence of stock evaluation for many deep species.
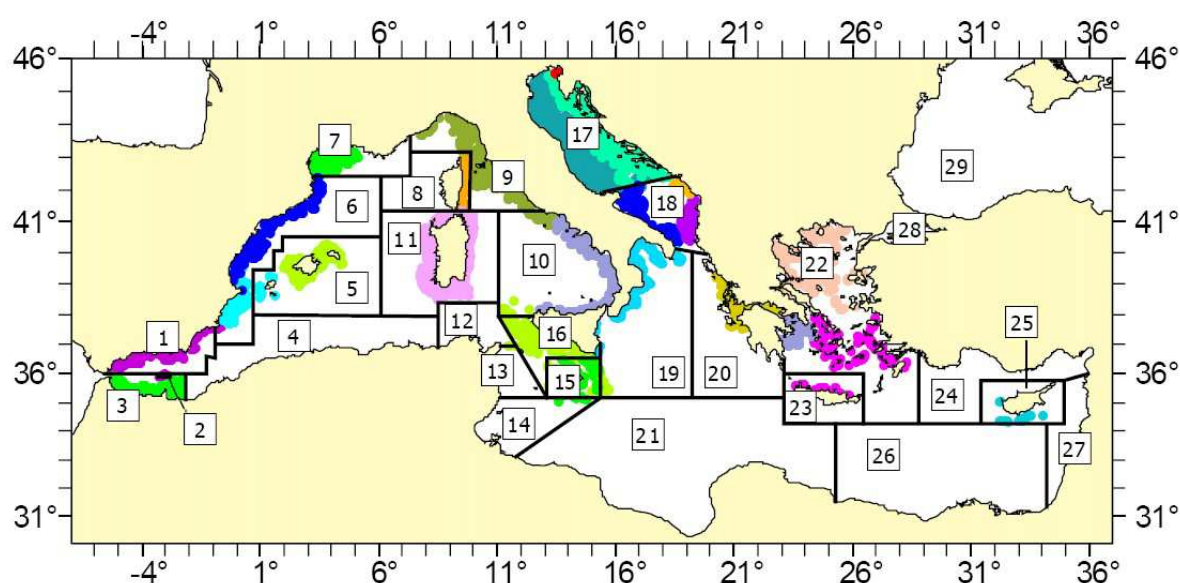
Figure 3.3: GFCM geographical sub areas (GSA). Coloured: areas covered by the MEDITS surveys (source http://www.sibm.it/SITO%20MEDITS/principalegeo.htm)

With the Annex II "monitoring Programs" in the declaration of February 11 2015, the Italian Ministry of Environment invited the seven Italian GSA involved in the MEDITS program to also collect biological data (length, sex and maturity) for some deep-sea fishes such as *Phycis blennoides*, *Micromesistius poutassou*, *Nezumia* sp., *Hymenocephalus italicus*, *Trachyrhynchus* sp., *Helicolenus dactylopterus* and *Hoplostetus mediterraneus*.

3.3 How is D3 MFSD Evaluation done

3.3.1 Methodological sampling aspects

> The indicators of the D3 were developed to be applied to entire stocks regardless of their bathymetric distribution. Therefore, **the distinction of depth strata is irrelevant** to the way the evaluation of this descriptor is done. Moreover, many stocks are distributed and exploited over large depth range (not exclusively above or below the 200 meters limit), and are evaluated at that scale.

The developed indicators that allow the assessment of good environmental status are calculated on the scale of each stock. This condition requires co-operation at European or international level within the International Council for the Exploration of the Sea (ICES), the General Fisheries Commission for the Mediterranean (GFCM) or the International Commission for the Conservation of Atlantic Tunas (ICCAT). Some so-called "national" stocks are however routinely assessed on a finer scale.

The first step in the assessment of descriptor 3 is to define a list of stocks for each Mediterranean Sub Region (MSR) in accordance with the first specification in the Annex to the Commission Decision (EU Decision 2017/848 Commission of 17 May 2017) describing the descriptor 3. This selection should take into account stocks that are assessed at international level and also the specific importance of the landings (by weight) of each MSR.

The second step consists of gathering the information available for each stock that will enable an assessment of the good environmental status to be carried out under the MSFD. Depending on the data available and the nature of the assessment, stocks may be classified into 6 categories (ICES, 2012)

- Category 1: stocks with comprehensive analytical estimates and forecasts;
- Category 2: stocks with negligible landings compared to discards;
- Category 3: stocks with qualitative analytical assessments and forecasts including quantitative assessments and forecasts which, for various reasons, are merely indicative of trends in fishing mortality, recruitment and biomass;
- Categories 4 to 6: stocks with trends from scientific surveys (robust indices on total mortality, recruitment, biomass); stocks with solid catch data on short time series; data-limited stocks (only landings available).

The next step is to evaluate each stock using the various criteria defined by the Directive. To the extent that the available data permit, conducting diagnoses leads to estimates of a few indicators to monitor the evolution of resources and their exploitation over time. The two main indicators are:

• fishing mortality (F), which gives an estimate of the pressure that fishing has on a stock;
• spawning stock biomass (SSB), which is the biomass of fish beyond the age or size class in which 50% of the individuals are mature.

Finally, the final step is to integrate stock assessments to define a global status of the ecological status of commercial species in each MSR.

The Annex to the Commission Decision (Commission Decision (EU) 2017/848, 17 May 2017) laying down criteria and methodological standards for the good environmental status of marine waters and the specifications and standardized monitoring methods, and repealing Directive 2010/477 / EU stipulates that, for descriptor 3, good environmental status shall be achieved when the following conditions are fulfilled:

3.3.2 Criterion D3C1 - Fishing Mortality (Primary[7])

Fishing mortality (F) gives an estimate of the pressure that fishing has on a stock. The fishing mortality rate (F) of commercially exploited species is at or below the level of maximum sustainable yield (MSY). This indicator is calculable for category 1 and 2 stocks. The unit of measurement is the annual fishing mortality rate. If yield values based on quantitative assessments are not available for fishing mortality, due to inadequate data, other variables such as catch-to-biomass ratio may be used as a substitute method (e.g. for category 3 stocks). In this case, an appropriate method of trend analysis is adopted (for example, the value at the time of valuation can be compared to the long-term historical average).

3.3.3 Criterion D3C2 - Spawning stock biomass (Primary[3])

The amount of spawners (Spawning Stock Biomass, SSB) measures the ability of a stock to reproduce. Although there is generally no directly proportional relationship between the number of spawners and the number of recruits, it is established that below a certain spawning biomass threshold, the risk of recruitment collapse and therefore non-renewal of the stock, are high. SSB is computed from quantitative models (based on the evolution of the total biomass in respect to the catches) or analytical models (structured by age or length). Such models enable the estimation of abundance indices that reflect the biomass. Several models are currently in use, but the cohort analysis (Virtual Population Analysis and other derived methods) are the most commonly used method (Hilborn and Walters, 1992; Trenkel, 2006).

Spawning Stock Biomass should be used as criterion for category 1 stocks, spawning stock biomass index for category 2 and 3 stocks. The biomass unit of measure is in tons or number of individuals per species. If yield values based on quantitative assessments are not available for spawning stock biomass due to inadequate data, other biomass indices such as catch per unit effort or abundance from studies can be used as a substitute method. In this case, an appropriate method of trend analysis is adopted (for example, the value at the time of valuation can be compared to the long-term historical average).

3.3.4 Criterion D3C3 - Demographic Characteristics (Primary[3])

The distribution by age and size of individuals in populations of commercially exploited species demonstrates the good health of the stock (Shin et al., 2005). This is characterized by a high proportion of old / large individuals and limited adverse effects of exploitation on genetic diversity (ICES, 2015a).

Member States shall cooperate at regional or sub-regional level with a view to establishing threshold values for each species population on the basis of the scientific opinion obtained in accordance with Article 26 of Regulation (EU) No 1380 / 2013.

D3C3 should reflect that healthy stocks are characterized by a high proportion of elderly and large individuals. The relevant characteristics are:

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[7] Commission Decision (EU) 2017/848 of 17 May 2017 on criteria and methodological standards on good environmental status of marine waters makes a distinction between "primary" and "secondary" criteria. While primary criteria should be used to ensure consistency across the Union, flexibility should be granted with respect to secondary criteria. The use of a secondary criterion should be decided upon by the Member States, where necessary, to complement a primary criterion or when, for a particular criterion, the marine environment is at risk of not achieving or not maintaining a good environmental status.

• the size distribution of individuals within the population that can be expressed by (i) the proportion of fish larger than the average size of the first sexual maturation or (ii) the 95th percentile of the fish size distribution for each population, as found by research vessels or in other studies;

• the genetic effects of the exploitation of the species, for example on the size of the first sexual maturation, if necessary and to the extent possible.

Other expressions of relevant characteristics may be used when scientific and technical knowledge concerning this criterion has been consolidated.

The annex to the decision on the criteria for good environmental status states that the degree of achievement of good environmental status for descriptor 3 must be expressed as follows for each area assessed:

• assessed populations, values obtained for each criterion and whether or not the levels of criteria D3C1 and D3C2 and the threshold values of criterion D3C3 are met and the overall state of the stock defined on the basis of the rules for the integration of the criteria agreed at the level of the European Union; and

• populations of commercially exploited species not subject to assessment in the assessed area.

If the species are relevant for the assessment of particular benthic habitat groups and habitat types, the results of these population assessments also contribute to the assessments under descriptors 1 and 6.

A list of commercially exploited species to which the criteria apply in each assessment area shall be established by the Member States through regional or sub-regional cooperation and updated for each six-year evaluation period, taking into account Council Regulation (EU) 2016/1251 and the following:

(A) all stocks managed in accordance with Regulation (EU) No 1380/2013 and (EU) No 1343/2011 and within the framework of ICCAT and GFCM (http://www.fao.org/gfcm/data/dcrf/en/);

(B) species for which fishing opportunities (total allowable catches and quotas) are fixed by the Council in accordance with Article 43 (3) of the Treaty on the Functioning of the European Union;

(C) species for which minimum conservation reference sizes are fixed in accordance with Regulations (EC) No 1967/2006 and COM/2013/0889;

(D) species covered by multiannual plans drawn up in accordance with Article 9 of Regulation (EU) No 1380/2013;

(E) species covered by national management plans adopted in accordance with Article 19 of Regulation (EC) No 1967/2006;

(F) any species of regional or national importance for artisanal fisheries or local inshore fishing.

For the purposes of this decision, non-native species commercially exploited in each assessment area are excluded from the list and are therefore not considered for the determination of good environmental status under the descriptor 3.

3.4 Knowledge on Threshold

Descriptor 3 of the MSFD focuses on the status of populations of commercial species, subject to fishing activity as the source of human pressure on the marine environment. Good environmental status (GES) is achieved when populations of all commercially exploited fish and crustaceans are within safe biological limits, with a distribution of population by age and size that reflects good health of the stock (European Commission (EU) 2017/848). The threshold value used must comply with Article 2 (2) of Regulation (EU) No 1380/2013. However, the quality of available data, as highlighted by the Scientific, Technical and Economic Committee for Fisheries (STECF, 2017a), in some cases is not sufficient to allow some analytical approaches to be applied and that biological parameters for a same species across different areas may vary greatly.

MSFD is a cyclical process with each *cycle* taking 6 years. The evolution of these indicators during the period under review gives initial information on the state of resources and their exploitation. The situation of these indicators in relation to reference thresholds, when these have been defined, completes the diagnosis. In the Mediterranean, for each stock, maximum sustainable yield threshold (MSY) for fishery mortality (F_{MSY}) and Biomass (B_{MSY}) at maximum sustainable yield threshold should be estimated. B_{MSY} is not available for all the stocks for reasons that are detailed in the following D3C2 section. In fisheries science, a stock is considered to be sustainably exploited when the spawning biomass is greater than BPA and the fishing mortality rate is less than FPA. Since the Johannesburg summit in 2002, the Common Fishery Policy (CFP) has defined the objective of achieving maximum sustainable yield by 2020 as the management objective for European fisheries. The MSFD set the same objectives for the assessment of descriptor 3 for commercial species.

D3C1 threshold: The GFCM has extensively used $F_{0.1}$ as target reference point for demersal stocks which is the fishing mortality rate corresponding to 10% of the slope of the yield-per-recruit curve at the origin (Gulland and Boerema, 1973). $F_{0.1}$ as proxy of F_{MSY} has been adopted as limit and basis for management advice on demersal stocks in EU Mediterranean waters also by STECF. The difference or ratio between the actual F and the F_{MSY} proxy is used to evaluate the fishing mortality status and the exploitation level of a stock. GES aims at a fishing mortality level (F) that would be below or equal to the F_{MSY} proxy.

D3C2 threshold: B_{MSY} is the Spawning stock biomass (SSB) that results from fishing at F_{MSY} for a long time. The actual spawning stock biomass should be equal or higher than B_{MSY} to indicate a sufficient number of mature individuals (spawners) to enable safe population renewal and maintain sustainable yield. In the Mediterranean, stocks assessments are performed using standardized approaches and F_{MSY} reference points, however B_{MSY} estimates are generally lacking, due to the lack of established Stock/Recruitment relationships as well as the lack of long time series on landings/catches data. The determination of the threshold value is generally difficult as it should be determined after the analysis of a period during which the stock was exploited at F_{MSY}. Precaution approach biomass (B_{PA}) may be used as a proxy but also requires long-time series including period where the SSB was very high to be determined (ICES, 2015b).

D3C3 threshold: This indicator is not yet operational as there is no threshold defined to this day (ICES, 2016e). The interpretation of this descriptor in the event of large recruitments may also prove difficult. The definition of thresholds would require developing a generic concept to identify the reference demographic values of each stock in respect to the GES. In order to do so, one needs to observe or simulate the population structure in age and size when exploited at MSY (information which is generally not available as fishery monitoring started after the beginning of exploitation, and since simulations could lead to analyses that may be statistically redundant with those necessary with D3C1 and D3C2 (ICES, 2017a, b). The recommendation is to monitor trends in this descriptor to prevent stock demographic degradation (ICES, 2015b).

As a result of these uncertainties, ICES recommends that the MFSD GES evaluation should be based only on criteria D3C1 (Fishery Caused Mortality) and D3C2 (Spawning Stock Biomass) when available. This evaluation should only consider stocks for which these primary indicators exist, i.e. stocks for which reference points (at maximum sustainable yield) could be computed (ICES, 2016c).

The criteria are grouped for each stock. The integration method used to evaluate the MFSD GES is that of the « One Out All Out » (OOAO). Therefore, all evaluated criteria should be in the limit values describing the GES (i.e. the maximum sustainable yield). The global status is computed at the scale of a stock: « Good environmental status (GES) achieved », « GES not achieved » and « no evaluation ». There should be no integration at the descriptor level of the different stocks status for any given MSR. At that scale, only the number of stocks in the categories « GES achieved », « GES not achieved », « not evaluated » should be presented (ICES, 2016a; ABPmer, 2017).

3.5   First applications

In its first evaluation exercise, ICES (2014) tried to assess the D3 GES and highlighted a number of difficulties in the Mediterranean that have not yet been overcome. Even though the aim of achieving GES for all commercial species is increasingly recognized as important, the proportion of stocks assessed as achieving GES is generally low, when adopting indicators D3C1 and D3C2. Data were available for 56 demersal fish, 14 small pelagics and 34 shellfish stocks, which showed that the vast majority of assessed stocks were overexploited. Overall, only 11 stocks out of 104 were estimated to be sustainably exploited. In particular, only about 11% of demersal fish stocks and 6% of shellfish were in good status (ICES, 2014). They noted the spatial imbalance in the availability of stock assessments, with 50 stocks assessed within the Western Mediterranean sub-region and 36 in the Ionian and Central Mediterranean compared to 12 and 6 stocks for the Adriatic Sea and Aegean-Levantine sub-regions, respectively. Available knowledge on the status of the stocks is still poor in some GSAs. Raicevich et al. (2017) compared the number and typology of stocks considered for GES assessment by each member state in relation to available data and the proportion of landings they represented. They found that the number of stocks considered by the member states per assessment area ranged between 7 and 43, while the share of landings corresponding to these selected stocks ranged from 23 to 95%. They also highlighted inconsistencies between GES definitions among the member states with environmental targets that were less ambitious than MSFD and CFP requirements thus reducing the likelihood of achieving fishery sustainability in the Mediterranean by 2020. Although this study was carried out regardless of stocks' depth ranges, their conclusions most definitely apply to deep Mediterranean stocks.

The first application of the D3 indicators at national levels and therefore the first evaluation of GES is due in 2018. French preliminary results of the evaluation are presented as examples below; information relevant to the evaluation of D3 in the deep Mediterranean Sea were available for six stocks only. These results need to be finalized, evaluated and aggregated with those of other European countries to produce a first assessment. It is however impossible to focus only on the deep Mediterranean areas. In coherence with the definition of the D3 objectives, the populations of each species are assessed at the ecologically relevant scales in the region or sub-regions. The stocks presented below are known to occur both above and below 200m depth. However, indicators produced for small pelagic stocks that are assessed only above 200m are not presented here.

In the Gulf of Lion (GSA7), the stock of hake (*Merluccius merluccius*) is exploited from 0 to 1000m by the means of bottom trawls, gillnets and longlines. The current exploitation level is above the level estimated to be sustainable, with the highest levels in the series since 2010. Reference point $F_{0.1}$ (0.15), selected as proxy of F_{MSY} is much lower than the current fishing mortality (F = 1.92). The spawning stock biomass (SSB) shows a downward trend over the period analyzed (1998-2015), particularly in recent years. Despite a significant decrease in the number of French trawlers since 1998, which has accelerated since 2011 (reduction of almost 50%), but an activity that targets juveniles in particular, the stock is in a state of high overexploitation with a low relative biomass (Jadaud and Saraux, 2016).

The red mullet of GSA7 (*Mullus barbatus*) in the Gulf of Lion: This stock is exploited mainly from 10 to around 400 m by the means of bottom trawls, gillnets and longlines. It is also in a state of overexploitation but with relatively high biomass. Recruitment of this species has been particularly high in recent years (2010, 2013 and 2015). The level of exploitation is currently higher than the level estimated to be sustainable ($F_{0.1}$, F_{MSY} proxy) but the current fishing mortality is the lowest in the series (2004 - 2015). The spawning stock biomass is on an upward trend with signs of stabilization in 2014 and 2015. As with hake, exploitation is mainly concentrated on young individuals (0-2 years), but 75% of recruitment is mature (Jadaud and Saraux, 2016).

Bluefin tuna mainly lives in the pelagic ecosystem of the entire North Atlantic and its adjacent seas, mostly in the Mediterranean Sea. Breeding stock biomass (SSB) recorded a record number of more than 300000 tons in the late 1950s and early 1970s before falling to approximately 150000 tons until the middle of 2000. In the most recent period, SSB is showing clear signs of a sharp increase to almost 585000 t in 2013. However, the uncertainty around this estimate is significant because historical catch data are of poor quality. Trends in fishing mortality have shown a steady decline in recent years for the different age classes of bluefin tuna. Estimates of the current stock status relative to the maximum sustainable yield benchmarks are very sensitive to the selectivity pattern and assumptions about recruitment levels. The Atlantic bluefin tuna stock is in good ecological status when considering MSFD descriptor 3 criteria (Foucher and Delaunay, 2017).

Considering Mediterranean swordfish stock, the latest ICCAT advice is based on the results of the 2014 assessment. Fishing mortality tends to decline with a recent decline in younger individuals (ages 1 and 2). During the last decade, the biomass of the stock remains stable but remains well below the reference value (the SSB represents less than 30% of B_{MSY}). The swordfish stock is in a state of overexploitation with insufficient fertile biomass (Foucher and Delaunay, 2017).

The first assessment of Mediterranean albacore tuna was carried out in 2011 using data until 2010. The quantity of data available does not allow a quantitative assessment. However, this limited information has resulted in a relatively stable pattern of albacore biomass and a decrease in mortality rates compared to the early 2000s (Foucher and Delaunay, 2017).

Finally, and also little is known about its status, the eel stock remains in a critical situation with very low recruitment indices compared to the reference period (1960 - 1979) (Foucher and Delaunay, 2017).

3.6 CONCLUSIONS

According to the Zampoukas et al. (2014a), in general, Mediterranean stock assessments are affected by a lack of data (in particular age readings that could allow an age-based assessment as well as the shortness of available time-series) and economic resources, together with difficulties in calculation of stock-recruit relationships. Accordingly, data collected in the EU Mediterranean waters within DC-MAP do not allow to fully meet MSFD requirements in regard to the calculation of indicators proposed within descriptor 3.

In coherence with the definition of the D3 objectives, the populations of each species are assessed at the ecologically relevant scales in the region or sub-regions based on aggregations of GFCM geographical sub-areas where relevant. Although a few species are almost exclusively found in deep-water habitats such as for example the crustaceans *Aristeus antennatus* and *Aristaeomorpha foliacea*, many species targeted by commercial fisheries in the Mediterranean are found over a wide depth-range. As such, stock assessments do not distinguish between populations found in shallow waters (0-200 m) and the deep-sea (>200 m). Moreover several stocks are not restricted to single or regionally aggregated GSAs, but are present throughout the Mediterranean Sea basin. Examples are large pelagics (bluefin tuna, swordfish) and European eels, for which stock assessments are consequently carried out at large spatial scales.

Moreover, during their draft assessment exercise, ICES (2014) found that the list of the species evaluated by the different countries were often different even in the same sub-region. This resulted from the different approaches adopted in the selection of the species and indicators to be used for GES assessment. There was no agreed strategy and approach to a coherent assessment of GES in the Mediterranean sub-regions at the time, and to our knowledge, it is still the case today. Different interpretations across member states, resulting from lack of sub-regional and regional coordination, induced a lack of consistency in the selection of stocks, application of reference points, and definition of GES and their associated environmental targets (Raicevich et al., 2017).

Enhanced international coordination is needed at the Mediterranean level to achieve standardized and coherent approach to GES of the populations of commercial fish species as required by the MSFD. According to the ICES (2014) "There is an urgent need to establish an overarching strategic framework to ensure the coordination of approaches toward GES assessment and monitoring programmes at the Mediterranean Sea regional scale, by collaboration between GFCM, EC and the Barcelona Convention." Similarly the STECF reiterates 'the strong need for a better coordination and full harmonization among the scientific bodies of FAO-GFCM and EU, in order to develop common approaches and make the best use of the human resources' in its conclusions on the work done by EWG 16-05 on Methodology for the stock assessments in the Mediterranean Sea (STECF, 2016).

4   Descriptor 4 : Ecosystems, including Food webs

Descriptor 4 of the Marine Strategy Framework Directive (MSFD) requires that "*all elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity*".

Food webs describe biological communities in terms of their consumer–resource relationships, and are often referred to in terms of their structure (e.g. diversity, trophic levels) and dynamics (e.g. robustness, resilience) (Rombouts et al., 2013b). In contrast to single-species approaches, a system-level approach is attractive because both the direct and indirect effects of disturbance can be considered in a single interaction network (Rombouts et al., 2013b). However, considering the high functional diversity in marine ecosystems and, consequently, of the high food-web complexity, practical application remains a challenge (Rombouts et al., 2013b).

Different approaches are currently being used to investigate food web properties: (1) data-based approaches are applied to reveal natural processes, using stomach contents, stable isotope analyses and/or fatty acid trophic markers; (2) modelling techniques increase our knowledge of food web theory to provide a more integrative image of the structure, functioning and dynamics of systems (Rombouts et al., 2013b). Whilst an ecosystem perspective is increasingly used in fisheries management to study ecosystem responses to different stressors and to assure sustainable use of resources (Coll et al., 2008), similar holistic approaches to evaluate the combined influences of other anthropogenic stressors on food webs has received less attention to date (Rombouts et al., 2013b).

4.1   *State Of The Art*

The Mediterranean Sea is a semi-enclosed basin characterized by peculiar morphological-topographical features. Water circulation is driven mainly by the presence of physical thresholds, such as the Strait of Gibraltar and the Sicilian Channel. This structuring results in strong environmental gradients in both space (latitude and longitude) and time (seasons) (Pranovi et al., 2014). The Mediterranean deep-water fauna is based on a region-wide species pool, from which assemblages are drawn in each basin (western, central, and eastern) according to basin-specific environmental drivers, which include surface primary production and food availability at the seafloor (Sarda et al., 2004; Tecchio et al., 2011b). With the exception of chemosynthetic communities, deep-sea benthic food-webs are essentially sustained by particulate organic matter (POM) derived from the water column (Nomaki et al., 2010; Tecchio et al., 2015). This food input occurs in the form of direct sedimentation of phytoplankton, or through fecal pellets, zooplankton carcasses, molts, and marine snow. Species may adapt to the scarceness of food resources by applying a wide variety of responses, such as feeding specialization, niche width variation, and reduction in metabolic rates (Fanelli et al., 2013a; Papiol et al., 2013; Tecchio et al., 2013b).

Some other species have complex life history. Among macro/megabenthic species, many have a larval/juvenile stage which inhabits surface or near surface waters (e.g. *Peristeion cataphractum* has pelagic larvae, juveniles live in coastal waters, migrating later to deeper waters, ditto leptocephali of *Nemichthys scolopaceus*). Some species are going to the surface at night to feed in upper layers (e.g. *Stomias boa*, *Chauliodus sloani*). Some other species spawn in the deep sea, have a larval phase and a transition from the pelagic to the demersal phase in relatively shallow water, and settle on the deep

seabed hundreds of kms from the spawning grounds (i.e. *Lophius piscatorius*) (Hislop et al., 2001). The management of these populations are not limited to the deep seafloor.

4.1.1 Stomach content and stable isotopes approach

Stable isotope analyses are based on the link between the isotopic ratio of a consumer and the ratio of its diet, with a known fractionation factor between them. For carbon, this factor is rather low (theoretically +1‰ at each trophic level), and allows the use of carbon as a tracer of the origin of OM supporting the consumer. Fractionation factor is more important for nitrogen (theoretically from 2.5 to +3.4‰ at each trophic level). Nitrogen is thus commonly used as a proxy of trophic level. Nevertheless, recently published studies demonstrated that these fractionation factors can vary with feeding behavior, trophic position, metabolism, body size or temperature (Wyatt et al., 2010; Fanelli et al., 2011a; Fanelli et al., 2011b; Cresson et al., 2014; Hussey et al., 2014).

Using stable isotopes is appropriate for understanding trophic levels in deep-sea species in addition to the gut content analyses, because the latter technique by itself suffers drawbacks (Polunin et al., 2001; Fanelli et al., 2011a). Indeed, due to rapid pressure change, deep-sea species commonly regurgitate their stomach content when caught. Feeding is also sporadic for carnivorous species. Extensive sampling is thus needed to avoid empty or regurgitated stomachs and to get reliable data (Cresson et al., 2014) or alternatively intestine contents can be examined (Papiol et al., 2014). In a fisheries management context, the ^{15}N assay can be useful to demonstrate, albeit indirectly, the impacts of fishing pressure on marine food webs (Fry, 2006; Fanelli et al., 2010; Sinopoli et al., 2012). Fishing removes large fish from the oceans selectively, thereby reducing the mean trophic level creating a phenomenon known as "fishing down the food web" (Pauly et al., 1998).

Due to the influence of benthic processes on energy flows and nutrient cycling within food webs (Covich et al., 1999), indicators based on the structure (abundance and diversity) and processes (production and metabolism) of benthic groups can help to describe trophic functioning (Borja and Dauer, 2008; Frid et al., 2008; Rombouts et al., 2013a; Rombouts et al., 2013b).

The role of meiofauna in deep-sea benthic food webs has not been investigated as intensively as for megafauna and macrofauna because of the difficulty of sample collection and analysis, and a generally low standing stock of biomass at deep-sea floor compared to macrofauna (Nomaki et al., 2010). Benthic macrofauna (i.e. bivalves, polychaetes, amphipods, gastropods) mix the sediments, aerate deeper layers of sediments and increase rates of recycling nutrients by bioturbation and fecal production (Covich et al., 1999). Even if small invertebrates are functionally important in deep-sea ecosystems because they burrow deeply into layered sediments and accelerate nutrient cycling, there is very few published data on their trophic ecology in the deep Mediterranean Sea (Pape et al., 2013). With regards to megafauna, an update of the feeding habits of fish in the Mediterranean Sea has just been published, though this does not focus exclusively on deep waters (Stergiou and Karpouzi, 2002; Karachle and Stergiou, 2017).

Trophic ecology of macro and megafauna has been investigated mainly in fauna collected together with fish (accompanying fauna - crustaceans, cephalopods - in trawls, see Table 4.1) but also in epibentic and infaunal invertebrates, and in zooplankton, mainly in western Mediterranean Sea (Table 4.1). However, in central and eastern part a few studies are also reported either at some specific deep locations like in the central Mediterranean Sea in cold-water coral communities, at Santa Maria di Leuca (Carlier et al., 2009) and South Malta (Naumann et al., 2015), or at cold-seeps in the eastern Mediterranean Sea (Carlier et al., 2010).

Trophic ecology of fish under 200m depth has been investigated with stable isotopes especially in the western part of the Mediterranean Sea (Table 4.1), while only two studies are reported in the eastern part (Madurell and Cartes, 2005, 2006). A recent review reports on the feeding ecology of deep-sea fishes and lists the general trophic guilds (Drazen et al., 2017), not specifically in the Mediterranean Sea.

In the Mediterranean Sea, broad regional studies on trophic habitats are almost absent; only three studies comparing the three basins of the deep Mediterranean Sea have been published. Those studies emphasize on the megabenthos (Tecchio et al., 2013b), the foraminiferans (Theodor et al., 2016a) or the deep-sea shrimp *Aristeomorpha foliacea* (Cartes et al., 2014). The Mediterranean Sea presents a distinct environment in each of its three basins, and continental slope areas are considered oceanographically dynamic and influenced by local-scale events like river input coastal atmospheric events (Company et al., 2008) and in contrast, the deepest areas are subject to a considerably lower nutrient input and are thus more oligotrophic (Sarda et al., 2004). This factor, coupled with the longitudinal gradient, leads the two axes of food availability, which was fairly represented in the density of megafaunal samples (Tecchio et al., 2013b). Two distinct pathways were observed, in a more pronounced way in the western basin. A split of the deep benthic food-web at the suprabenthic level was observed, with a benthic detritus-based chain and a more pelagic-linked one (Tecchio et al., 2013b). The δ^{13}C signal of deep-sea benthic foraminifera from different areas of the western, central, and eastern Mediterranean Sea reflects an integration of various environmental and biological signals (Theodor et al., 2016a). Comparison with sediment trap data reveals underestimation of satellite-derived organic Carbon fluxes for the marginal areas of the central and northern Aegean Sea and the canyon systems of the Gulf of Lion. In these ecosystems, additional lateral transport of resuspended and terrestrial OM contributes substantially to organic Carbon fluxes (Theodor et al., 2016a). The δ^{15}N trends observed in deep-sea shrimps *A. foliacea* indicates that its optimal ecological habitat appears to be located in the Tyrrhenian Sea and the Sicily Channel, where the highest gut fullness, the greatest trophic diversity and the highest densities were found, and where *A. foliacea* was in the best biological condition (Cartes et al., 2014).

4.1.2   Modelling techniques

Ecosystem models are particularly useful because they allow the study of marine ecosystems as a whole. For example, the Ecopath modelling approach, a mass-balance model integrated in the Ecopath with Ecosim software that provides a representation of the food web, can be used to depict the structure and functioning of marine ecosystem and calculate different ecological indicators (Christensen and Walters, 2004; Heymans et al., 2016). The use of mass-balance models and stable isotope analysis has confirmed that trophic level estimated by the model and δ^{15}N were highly correlated (Navarro et al., 2011).

A total of 40 Ecopath models (www.ecopath.org) describing Mediterranean ecosystems have been fully developed and documented (Coll and Libralato, 2012), among which only four of them considered bathymetry deeper than 200m. In the Catalan Sea, three models ranged from 0 to 400 m depth (Coll et al., 2006a; Coll et al., 2006b; Coll et al., 2008; Coll et al., 2009), and in the Aegean Sea one model ranged from 20 to 300 m (Tsagarakis et al., 2010). In 2013 new trophic models were developed in the deep-sea (Table 4.2): in the lower continental slope of the Balearic Sea from 1000 to 1400 m depth (Tecchio et al., 2013a), in the Gulf of Lion from 0 to 2500 m (Bănaru et al., 2013) and in the Greek Ionian Sea from 50 to 1100 m depth (Moutopoulos et al., 2013).

Recently another model was built from 0 to 1000 m depth that included previously modeled areas (Gulf of Lion and Catalan Sea) in the NW Mediterranean Sea and extended their ranges (Corrales et al., 2015). The model is composed of 54 functional groups, from primary producers to top predators, and Spanish and French fishing fleets were considered. A comparative approach with previous models developed (in western, central and eastern Mediterranean Sea) highlighted that despite productivity differences, the ecosystems shared common features in structure and functioning traits such as the important role of detritus, the dominance of the pelagic fraction in terms of flows and the importance of benthic-pelagic coupling (Corrales et al., 2015).

4.2 Where Is the Knowledge

Most of the studies using stomach contents and isotope approach have focused on the western Mediterranean Sea while only four of them have focused on the eastern basin (Table 4.1). In addition three of them have compared the three basins, one occurred on cold-water coral in Santa Maria di Leuca Province and one on chemosynthetic ecosystems of mud volcanoes (Table 4.1).

*Table 4.1. Studies based on **stable isotopes** dedicated to food webs in deep Mediterranean Sea. In grey: studies out of western Mediterranean Sea. In bold: studies dealing with the three basins. POM: Particulate Organic Matter.*

Where	What	Date	Depth	Who
Baleares Sea, off Ibiza	Fish, Crustaceans, suprabenthos, plancton	1996-1998	200 to 1800 m	(Polunin et al., 2001)
Catalan slope	13 species of fish and their prey (macro and megafauna)	1987-1991	1000-2500 m	(Carrasson and Cartes, 2002)
Catalan and Balearic slopes (Balearic basin)	18 species of fish and 14 species of decapod crustaceans - Trophic guilds -	1987-1989	862-2261 m	(Cartes and Carrassón, 2004)
Eastern Ionian Sea	8 demersal fish (gut content) 2 sharks and 6 osteichyes	1999-2000	473-603 m	(Madurell and Cartes, 2005)
Eastern Ionian Sea	3 macrourids (gut content)		473-603 m	(Madurell and Cartes, 2006)
Off Mallorca	2 shrimp species *Plesionika heterocarpus* *Plesionika martia*	2003-2004	247 and 752 m	(Fanelli and Cartes, 2008)
NW Balearic islands	24 Suprabenthic fauna species 5 mysids, 12 amphipods, 2 cumaceans, 2 isopods, 1 euphausiid, 1 decapod and 1 fish. and 1 broad taxa (Copepoda)	2003-2004	350-780 m	(Madurell et al., 2008)
South-East of Mallorca	34 species of zooplankton and suprabenthic macrofauna 2 broad taxa (Copepoda and Cumacea)	2003-2004	650 and 780 m	(Fanelli et al., 2009a)
Off Iberian Peninsula (from Gibraltar to Cape Creus)	2 shark species	1999	50-500 m	(Fanelli et al., 2009b)
Levantin basin	Mesozooplankton (copepods, ostracods, chaetognaths) and POM	2001	0-4200 m	(Koppelmann et al., 2009)
Central Med, Santa Maria di Leuca	Sponges, Cnidarians, Polychaetes, Crustaceans, Echinoderms, zooplankton	2006	550-700 m	(Carlier et al., 2009)
Napoli and Amsterdam Mud Volcanoes	Benthic invertebrates (sponges, bivalves, gastropods, polychaetes and crustaceans)	2007	1950 and 2025 m	(Carlier et al., 2010)
Balearic basin	Three species of deep.-sea fish *Hoplostethus mediterraneus*, Nezumia aequalis, *Hymenocephalus italicus*	2003-2004	550-750 m	(Fanelli and Cartes, 2010)

Where	What	Date	Depth	Who
Catalan margin, off Barcelona	Macroplankton/micronekton (34 taxa)	2007-2008	650-800 m	(Fanelli et al., 2011a)
Catalan slope, off Barcelona	Epibenthic and infaunal invertebrates 9 broad taxa (Nemertina, Polychaetes, Sipuncula, Mollusca, Cirripedia, Amphipoda, Isopoda, Decapoda, Echinodermata)	2007-2008	650-800 m	(Fanelli et al., 2011b)
Balearic Sea	Fish , *Polycheles* spp., crab *Chaceon mediterraneaus*	2009	2800 m	(Jeffreys et al., 2011)
Catalan-Balearic basin	Pelagic and bottom-dwelling Cephalopods	1985-1992 2007-2010	450-2200m	(Fanelli et al., 2012)
Catalan slope and Besos canyon	Benthopelagic megafauna, macrofauna and zooplankton (29 species)	2007-2008	423-1175 m	(Papiol et al., 2013)
Catalan and Balearic slopes (Balearic basin)	62 Fish species 52 decapods species POM and TOC	2010	445-2198 m	(Fanelli et al., 2013a)
Catalan and Balearic slopes (Balearic basin)	71 species 26 Fish species, 19 decapod species, 4 cephalopods, bivalves, sponges, zooplankton, 6 echinoderm species	2010	445-2198 m	(Fanelli et al., 2013b)
South Balearic Islands	Meiofauna	2009	1582 m	(Pape et al., 2013)
3 basins	**Benthic megabenthos (fish and crustaceans), plankton and POM**	**2009**	**1200, 2000 and 3000 m**	**(Tecchio et al., 2013b)**
3 basins	**1 Crustacean species** ***Aristaeomorpha foliacea***	**2009-2012**	**455 and 600 m**	**(Cartes et al., 2014)**
Gulf of Lion	7 Fish, 1 crustacean	2012	284-816 m	(Cresson et al., 2014)
Catalan slope and Besos canyon	7 fish (3 trophic guilds)	2007-2008	500-1000m	(Papiol et al., 2014)
Balearic basin	Lantern fish *Lampanyctus crocodilus* and 17 mesopelagic fish species (mostly Myctophidae and Stomiiformes)	2007-2011	450-2200	(Fanelli et al., 2014)
South Malta Coral Province	Scleractinians *Desmophyllum dianthus*, *Madrepora oculata*, POM	2009	490-690 m	(Naumann et al., 2015)
Levantine Sea (SW Turkey)	1 species *Aristaeomorpha foliacea*	2012-2013	442-600 m	(Bayhan et al., 2015)
Catalan margin	Sediment (organic matter) and meiofauna	2007	60, 600 and 800 m	(Rumolo et al., 2015)
Gulf of Lion	3 fish and 2 shark species	2013	284-816	(Cresson et al., 2016)
Balearic basin	2 notacanthids fish (*Notacanthus bonapartei* and *Polyacanthonotus rissoanus*)	2008-2014	579-2233 m	(Romeu et al., 2016)

Where	What	Date	Depth	Who
Catalan and Balearic slopes (Balearic basin)	Fish, decapods and other invertebrates (21 species)	1985-1989 and 2007-2011	1000 and 2250 m	(Fanelli et al., 2016)
Alboran Sea Mallorca channel	Benthic foraminifera	2006	500-1000m	(Theodor et al., 2016b)
3 basins	**Benthic foraminifera**	**compilation**	**400-1500m**	**(Theodor et al., 2016a)**
Gulf of Lion	*Merluccius merluccius* and its preys (stomach contents)	2004-2006	30-600 m	(Mellon-Duval et al., 2017)
Western Med/ from Gibraltar to Cape Creus	3 deep sea fish	2004	50-800 m	(Louzao et al., 2017)
Balearic Sea	18 cephalopod species 5 elasmobranch species	2007-2016	200-900 m	(Valls et al., 2017)

Most of the studies based on models (ecopath model) are located in the Western Mediterranean Sea, while only two of them are located in the eastern basin and one of them focus on Santa Maria di Leuca (Table 4.2).

Table 4.2. Studies based on **models** used to understand food webs in deep Mediterranean Sea.

Where	Name and What	Date	Depth (m)	Who
Southern Catalan Sea	CAT_94	1990	50-4000	(Coll et al., 2006a) (Coll et al., 2006b)
Southern Catalan Sea	CAT_03	2003	50-400	(Coll et al., 2008) (Coll et al., 2009)
Southern Catalan Sea	CAT_NF	No Fishing	50-400	(Coll et al., 2009)
Northern Aegean Sea	AEG_03 40 functional groups: 2 detritus groups (detritus, discards) Commercial invertebrates and fish 3 groups of threatened species (dolphins, turtles, seabrids) 5 groups of non-commercial fish	2003-2006	20-300	(Tsagarakis et al., 2010)
Lower continental slope of the Catalan Sea	DCS 18 consumers groups 1 Marine snow group 1 Sediment detritus group	2009	1000-1400	(Tecchio et al., 2013a)
Gulf of Lion	GoL 40 compartments 1 group of seabird 2 group of cetaceans 18 groups of fish 12 groups of invertebrates 5 groups of primary producers Detritus and discards	2000-2009	0-2500	(Bănaru et al., 2013)
Greek Ionian Sea	IS 39 functional groups	1998-2006	50-1100	(Moutopoulos et al., 2013)
NW Mediterranean Sea	NWM 54 functional groups: 3 primary producers, 17 groups of invertebrates, 27 groups of fish, 1 group of sea-turtles, 1 group of seabirds, 2 groups of Marine Mammals and 3 groups of detritus natural, discards, by-catch)	1990-2003 for Southern Catalan Sea And		(Corrales et al., 2015)
Santa Maria di Leuca Central Med Sea	15 cephalopods, 25 crustaceans, 7 chondrichthyes and 54 osteichthyes.	2005-2010	100-541	(Vassallo et al., 2017)

4.3 How is food web measured

4.3.1 Methodological sampling aspects

Member States shall establish the list of trophic guilds through subregional cooperation in the Mediterranean Sea.
The trophic guilds selected under criteria elements shall take into account the ICES list of trophic guilds and shall meet the following conditions:
(a) include at least three trophic guilds;
(b) two shall be non-fish trophic guilds;
(c) at least one shall be a primary producer trophic guild;
(d) preferably represent at least the top, middle and bottom of the food chain.

Descriptor 4 indicators are required to consider the differing influences of environmental variability and anthropogenic activity on considerations of GES. Indicator development should specifically investigate the role of lower trophic guilds on the likely assessment of GES for descriptor 4, the role of size in foodweb stability, and management strategy evaluations of the sensitivity of descriptor 4 indicators to anthropogenic pressures.

A guild is any group of species that exploit the same resources or different resources in related ways (benthic-feeder fish, filter-feeding benthos, omnivorous zooplankton, etc.). Guilds are defined according to the locations, attributes, or activities of their component species. For example, the mode of acquiring nutrients, the mobility, and the habitat zones that the species occupy or exploit can be used to define a guild. The number of guilds occupying an ecosystem is termed its *disparity*. Members of a guild within a given ecosystem could be competing for resources.

Not all trophic guilds in each ecosystem need to be assessed but, by region, a minimum of at least three representative trophic guilds should be monitored. The choice of trophic guilds is expected to reflect regional differences in priorities and ecosystem dynamics.

According to the ICES (advice 2015) the concepts of trophic guild, taxonomic grouping, habitat type, and fish stock need to be combined in a way that accounts for the functional requirements of the state descriptors to ensure efficient implementation of the MSFD.

4.3.2   Diversity of the trophic guild (D4C1) - Primary

Member States shall establish threshold values of the diversity (species composition and their relative abundance) of the trophic guild to determine if the diversity is adversely affected due to anthropogenic pressures. Species composition shall be understood to refer to the lowest taxonomic level appropriate for the assessment.

The selection criteria stipulate that at least one trophic guild should be at the primary producer trophic guild, but this does not make much scientific sense for the vast majority of the deep sea areas, since the only deep-sea primary producers are chemosynthetic species which have very localised distributions. In addition, connectivity among deep-sea ecosystems is poorly known. Therefore we suggest **no monitoring of primary producers in the deep sea (only for chemosynthetic ecosystems)**.

Trophic guilds in the deep Sea have only been investigated in few areas as the Balearic basin (Cartes and Carrassón, 2004; Papiol et al., 2014) and mainly in species groups of fish (Drazen et al., 2017). Their diversity and threshold values of the diversity are far from being established in the deep-sea.

Trophic guilds have not been much investigated on **benthic habitats**, although few studies have measured isotopic values in some specimen (Table 4.1). For benthic habitats we propose a list of broad functional compartment/trophic guilds that could be considered on each habitats described in descriptor 1 (i.e. Large cnidarians on outcroping hard rocks, bivalves on outcroping hard rocks, large cnidarians on open slopes, mud volcanoes, hydrothermal vents, seamounts and seamount-like structures). On those habitats the following groups can potentially be found:

Decomposers (i.e. heterotrophic bacteria)
Passive suspension feeders (i.e. cnidarians, crinoids, barnacles)
Active filter feeders (i.e. sponges, bivalves)
Zooplankton (i.e. juveniles crustaceans or larvae)
Deposit/Detritus-feeders (i.e. holothurians)
Planktivores (i.e. fish)
Low-level carnivores (including scavengers) (i.e. fish, crustaceans, sea stars, sea urchins, gastropods, and some polychaetes)
Sub-apex mesopelagic and bathy-pelagic predators
Sub-apex demersal predators
Apex carnivores

A minimum of three trophic guilds have to be considered for the MSFD. Food chains are often far shorter on land, with their apices usually limited to the third trophic level. In the ocean, there are many more trophic levels and therefore a choice will have to be made, especially since it will not be feasible to attempt to monitor all trophic guilds. The trophic guilds to be considered will depend on the benthic habitat, but in general, it would be appropriate to include guilds from different positions within the food web, while keeping in mind practical considerations for implementation of monitoring programmes. It is recommended to include passive suspension feeders as one of the trophic guilds towards the base of the food chain, when they are present, because many species belonging to this guild (e.g. large benthic cnidarians) also have a role as autogenic ecosystem engineers and would therefore be an important ecosystem component to monitor under descriptor 1. Choice of other two guilds may vary according to the particular benthic ecosystem, but should include at least one higher level predatory guild.

4.3.3   Balance of total <u>abundance</u> between the trophic guilds (D4C2) - Primary

Member States shall establish threshold values of the balance of total abundance between the trophic guilds to determine if the balance is adversely affected due to anthropogenic pressures.

Because of the lack of data on the response of deep-sea communities to anthropogenic stress, no thresholds are presently available.

4.3.4   <u>Size distribution</u> of individuals across the trophic guild (D4C3) - Secondary

Member States shall establish threshold values of the size distribution of individuals across the trophic guild to determine if the size distribution is adversely affected due to anthropogenic pressures.
Total abundance should be understood as the number of individuals or biomass in tonnes (t)) across all species within the trophic guild.

Because of the lack of data on the response of deep-sea communities to anthropogenic stress, no thresholds are presently available.

4.3.5   <u>Productivity</u> of the trophic guild (D4C4) - Secondary

Member States shall establish threshold values of the Productivity of the trophic guild to determine if the productivity is adversely affected due to anthropogenic pressures.

Because of the lack of data on the response of deep-sea communities to anthropogenic stress, no thresholds are presently available.

4.4   Knowledge on Threshold

Because of the lack of data on the response of deep-sea communities to anthropogenic stress, no threshold are presently available.

4.5 CONCLUSIONS

Human activities cause direct, indirect, diffuse and emergent changes in food webs. In fact, events such as overexploitation (Pauly et al., 1998; Pauly and Palomares, 2005), pollution, eutrophication, habitat fragmentation and destruction (Melian and Bascompte, 2002; Layman et al., 2007), invasions of species and anthropogenic climate change (Kirby et al., 2009; Fanelli et al., 2016) all pose potential threats to the structure and dynamics of food webs, acting at variable spatial scales and affecting food webs in different ways (Moloney et al., 2011). Nutrient enrichment, for example, can drive bottom-up effects, propagated up food webs from lower trophic levels (Davis et al., 2010), whereas the removal of top-predators can initiate top-down cascade effects through to basal trophic levels (Rombouts et al., 2013b).

4.5.1 Gaps

Whilst the current criteria and indicators of descriptor 4 (D4) under the MSFD can be informative on trophic functioning, they are, at their current state of development, possibly insufficient to assess whether marine food webs in European waters really are at, what the MSFD defines as, "Good Environmental Status" (GES) (Rombouts et al., 2013b).

Existing information is mainly focused on western Mediterranean Sea, and information is lacking in the eastern basin. Food webs in benthic habitats, as defined in descriptor 1, is also lacking.

4.5.2 Recommendations

Increasing the spatial resolution of many of the current models would further improve our under-standing of the direct effect of fishing and other activities (such as decommissioning of oil rigs) on seafloor integrity (D6). However, most of the models do not explicitly include descriptions of these types of pressures on the marine environment, they do not link to benthic habitat layers (Piroddi et al., 2015).

There is still the need to collect and analyze high resolution data. In effect, direct measurements can provide complementary information when combined with ecological modelling techniques. For example, empirical analyses provide ground truth data for validating dynamic models; abundance indices collected during monitoring programs can be compared to abundances calculated from models to help model validation (Rombouts et al., 2013a). In this respect, it is also important to ensure that the scales of data collection and model resolution are compatible. Since empirical approaches mostly pertain to local assessments, large-scale surveys and regional approaches should be adopted in order to facilitate their integration with models (Pelletier et al., 2008).

Member States need to cooperate to ensure a coordinated effort in the study and development of management strategies for the different marine regions and sub-regions. This is the case for ecological models developed for understanding and forecasting the marine ecosystem response to pressures (Piroddi et al., 2015). The assessment of the environmental status can benefit considerably from greater use of ecological modelling (Piroddi et al., 2015).

5 Descriptor 5 : Eutrophication

In its original use and etymology, 'eutrophic' meant 'good nourishment', and eutrophication meant the process by which water bodies become more productive (Thienneman, 1918; Naumann, 1919). However, about 50 years ago, it has been recognized that this 'good nourishment' had considerable environmental impacts in freshwater and coastal environments (see Ferreira et al. (2011) for a review). By the end of the 20th Century, eutrophication had acquired a scientific and legal meaning, which in Europe was enshrined in several European Directives and Regional Sea Conventions, as well as a decision by the European Court of Justice in 2004 (Ferreira et al., 2011).

In the context of the MSFD, eutrophication has been defined as "a process driven by enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, leading to increased primary production and biomass of algae, changes in the balance of organisms, and water quality degradation" (Ferreira et al., 2010).

According to the MSFD, GES with respect to eutrophication is reached when "*Human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters*" (MSFD, 2008/56/EC, Annex I).

5.1 State Of The Art

5.1.1 Pressures

In coastal environments, there is no doubt that the main cause that induces eutrophication is nutrient input. Generally, a natural nutrient input is not continuous in time but it is a single pulse phenomenon and the biogeochemical processes (i.e., denitrification and phosphorus precipitation) of the ecosystem, acting as negative feedback, can buffer and neutralize it in a relatively short time (UNEP-WG.321, 2007). However, anthropogenic activities can cause nutrient loading in a such short time that there is no possibility for the ecosystem to recover rapidly (Ramirez-Llodra et al., 2011). Such effects do not always follow from nutrient enrichment, and can result from other causes, including climate change, removal of top predators by fishing, enrichment by allochthonous organic matter loads and contamination by harmful substances. Although these shifts may not be harmful in themselves, the consequent increased nutrient loading and thus production can have an impact on ecosystem structure, biodiversity and function and, thus, on the provision of ecosystem goods and services (UNEP WG.417, 2015).

Most of the pressures resulting in eutrophication come from coastal areas, producing a strong gradient from coastal to offshore waters. Most of the offshore areas target of the MSFD generally show limited eutrophication symptoms (Ærtebjerg et al., 2001; Frid et al., 2003). However, multiple effects of human activities and climate change may lead to a modification of nutrient inputs and organic loads with consequences such as hypoxic or anoxic conditions, similar to those observed in coastal eutrophicated environments.

5.1.2 Possible Impacts

Coastal eutrophication may result in sinking and decomposition of the excess organic matter produced, leading to oxygen deficiency. In Baltic Sea, for example, coastal eutrophication has caused, beside increased biomass of phytoplankton and frequencies of toxic algal blooms, also the reduction of oxygen levels in the deep waters (Ahtiainen et al., 2014). Oxygen deficiency can also come about from

other causes, including discharges of allochthonous organics (Danovaro et al., 2014) and from decrease in deep water ventilation of caused for example by climate change (Ferreira et al., 2010).

Recent studies highlighted that present climate change may affect also deep-sea ecosystems in the near future. Accordingly to Macias et al. (2014), which explored potential changes in primary productivity in the Mediterranean Sea under scenarios of future climate change, the basin will become warmer and more saline with consequences on the productivity of the region. The Western basin is expected to become more oligotrophic, associated to a surface density decrease influenced by the Atlantic waters, while the Eastern basin, on the contrary, is predicted to become more eutrophic due to a surface water density rise caused by increasing evaporation rate (Ramirez-Llodra et al., 2011). Because most deep-sea organisms are heterotrophic, this change would have significant effects on the trophic structure of deep-sea communities (Ramirez-Llodra et al., 2011; Fanelli et al., 2016). Since these results are expected to alter the structural and functional processes shaping the Mediterranean food webs, modeling tools are necessary in predicting the effects of climate change on the future food web configurations in the deep sea (Piroddi et al., 2017).

Moreover, predicted increasing surface temperatures may affect the formation of cold oxygenated deep water, modifying global ocean circulation and the dissolved oxygen availability in deep-water masses, increasing the existing natural Oxygen Minimum Zones (OMZs). Although not all scientists agree with these predictions, the ultimate effect of a significant temperature rise might be the break (or minimization) of the deep thermohaline circulation that ensures the oxygenation of the deep sea and nutrient availability to shallower ecosystems (Ramirez-Llodra et al., 2011). Current models predict an oxygen decline of 1% to 7% in the next 100 years (Keeling et al., 2010) with an expansion of pelagic and benthic OMZs (Stramma et al., 2008). Expansion of OMZs will undoubtedly alter the diversity, taxonomic composition, and functional properties of bathyal ecosystems. For the majority of pelagic species that are not tolerant to hypoxia, a shoaling of OMZs causes vertical habitat compression and possibly reduced vertical migratory range.

5.2 Where is the Knowledge

Scientific and grey literature dealing with eutrophication in the Mediterranean Sea is related exclusively to coastal environments. Overall, satellite images of the Mediterranean Sea reveal that the highest levels of autotrophic biomass correspond to the areas close to river deltas or those off large urban agglomerations. The northern coastline presents most eutrophication hot spots, whereas open seawaters in the Eastern Mediterranean are extremely oligotrophic (UNEP-WG.321, 2007). In the coastal areas affected by eutrophication, the anthropogenic and natural origins of the various nutrient sources were identified and an estimation of the inputs into the Mediterranean waters was attempted. This estimation considers all sources: rivers, wastewaters from urban and industrial plants, agriculture, atmospheric precipitation and uncultivated land/forests. The phenomenon was recorded all around the Mediterranean Sea and this was apparent from the review of the publications from various countries. In the EEA report on "Priority issues in the Mediterranean environment" (European Environment Agency, 2006) it is stated again that eutrophication is still a major environmental problem in the coastal zone of Mediterranean. In fact 15 coastal countries had reported on facing eutrophication problems, among which 11 countries characterized these problems as medium (Albania, Algeria, Greece, France, Israel, Morocco, Gaza Strip, Slovenia, Spain, Syria and Tunisia) and 5 countries as important (Croatia, Egypt, Italy, Turkey). In many cases, the increased trophic level observed in coastal areas was creating problems of various kinds for the local inhabitants (UNEP-WG.321, 2007).

However, the conclusion derived on the basis of the existing information at that time regarding eutrophication in Mediterranean Sea was that its main body would not be seriously threatened by eutrophication over the next few decades, although even large basins as the Adriatic, the Gulf of Lion and the northern Aegean Sea could suffer from eutrophication problems in their coastal waters (UNEP-WG.321, 2007).

Regarding the deep sea, this ecosystem has been historically considered as a food-poor environment. However, massive phytodetritus exports from surface waters to the sedimentary deep-sea floor have been reported (Billet et al., 1983), with important consequences on the abundance, biomass, biodiversity, metabolism, and distribution of deep-sea species (Danovaro et al., 2014). Researchers have long debated whether these events are highly episodic and limited to short time scales, or occur more frequently. Additionally, clear evidence shows that lateral advection delivers much of the organic flux on continental margins with massive and frequent downward transport (Canals et al., 2006). These processes explain the absence of consistent trends in sediment organic matter with increasing depth in most systems, comprising the Mediterranean Sea (Pusceddu et al., 2010; Danovaro et al., 2014). In short, some deep-sea areas are more eutrophic than previously thought.

In the past, food availability to the benthos has been quantified simply by measuring bulk organic matter, or characterizing its composition in detail but without considering the importance of food bioavailability to consumers. In systems rich in organic matter, rapid transformation of organic molecules, and particularly biopolymers, leads to complexation processes that produce high molecular weight compounds (e.g., humic and fulvic acids) that consumers cannot digest easily. Therefore, the higher palatable deep-sea fraction might significantly offset the low overall quantity of organic carbon observed in many deep-sea sediments, reducing differences from their shallow counterparts (Danovaro et al., 2014).

5.3 How is eutrophication measured

5.3.1 Methodological sampling aspects

In the coastal environments, most eutrophication assessment studies recognize that the immediate biological response is increased primary production reflected as increased chlorophyll-a (Chl-a) and/or macroalgal biomass (Ferreira et al., 2007; Xiao et al., 2007; Borja et al., 2008; Bricker et al., 2008; OSPAR, 2008; Claussen et al., 2009; HELCOM, 2009). Methods for detecting eutrophication effects in the water column are typically based on nutrients quantification. However, ideally, a sound assessment should provide indications not only on the source(s) of nutrients but also on the effects and causes of observed impairments (Ferreira et al., 2011).

Studies addressing the analysis of trophic state of coastal environments have been mostly focused on inorganic nutrient concentrations (mainly, N and P), phytoplankton community analysis (structure, primary production, growth rates) or alteration of chemical and physical parameters (e.g., turbidity, oxygen concentration; Justic, (1991); Zurlini, (1996)). Based on this approach, a synthetic trophic index (TRIX, which utilizes chlorophyll-a, total N and P concentrations, oxygen saturation) has been proposed to assess the environmental quality of marine coastal waters (Vollenweider et al., 1998). A similar approach including the analysis of chlorophyll-a, total N and P concentrations, oxygen saturation can be

applied also for deep-sea waters. However, the assessment of trophic status using only variables measured in the water column can lead to misleading classifications (Dell'Anno et al., 2002). In this regard, marine sediments underlying shallow waters can be considered a sort of "recorder" of the biological processes that occur in the overlying water column (Dell'Anno et al., 2002).

Eutrophication indeed is a problem for the GES of marine ecosystems as a result of the impact that the excess of biomass has when undergoes decomposition processes determining a rapid free oxygen consumption (due to chemical and biological oxidations processes) which determines, in turn, a reduction in dissolved oxygen availability, with important and in some cases lethal effect on marine organisms (Vaquer-Sunyer and Duarte, 2008). Similarly an excess of organic matter deriving from any source, comprising primary production, and exported to the sediments, including the deep-sea floor, might determine detrimental effects when the excess of organic causes a reduction in oxygen availability (Dell'Anno et al., 2002; Pusceddu et al., 2010).

In the context of the Water Framework Directive (2000/60/EC) it was recommended that the status of coastal waters has been assessed by using all variables (both physico-chemical and biological) potentially affected by eutrophication. This has been complemented, within the MSFD, using phytoplankton and physico-chemical (e.g. nutrients, Secchi disc, etc.) indicators also in offshore and open marine waters (Borja et al., 2010). However, in the deep-sea ecosystems, being under 200 m water depth, this approach can be only partially applied. Indeed, all indicators related to photosynthetically primary producers (e.g., macroalgae, harmful algal bloom) cannot be used.

More specifically, the MSFD calls for an assessment of the environmental status based on a list of physical and chemical characteristics listed in Table 1 of Annex III to the Directive, including: i) annual and seasonal temperature regime; ii) spatial and temporal distribution of salinity; iii) spatial and temporal distribution of nutrients (DIN, TN, DIP, TP, TOC), iv) dissolved oxygen and v) pH. The monitoring factsheet also enables assessment of 'Nutrient and Organic Matter enrichment' as a pressure on the marine environment as listed in Table 2 of Annex III to the Directive, including i) inputs of fertilisers and other nitrogen- and phosphorous-rich substances (e.g. from point and diffuse sources, including agriculture, aquaculture and atmospheric deposition) and ii) inputs of organic matter (e.g. sewers, mariculture, riverine inputs).

Although UNEP(DEC)/MED WG.282/Inf.5 in 2007 stated that "In the shallow and medium depth ecosystems the benthic community could be directly affected. In deep waters, since a complete mineralization occurs in the water column, the bottom community is unlikely to be affected." this is not the case. The conceptual framework of eutrophication mentioned above, suggests the need of the introduction of new parameters and indicators also related to the deep-sea ecosystem. Indeed, monitoring of benthic habitats will generate data to assess indirect effects of organic loads (Ferreira et al., 2011). For example, there is the need to identify the occurrence and extension of the hypoxic/anoxic events as well as the impacts of such events on benthic communities. Some clues as to the structure of ecosystems impacted by eutrophication may be found in the OMZs, where low pH and low oxygen occur naturally. In these areas, biomass, body size and diversity, particularly of crustaceans and fishes were found reduced, whereas squid, jellyfish and annelids do well. Because jellyfish are relatively tolerant of hypoxia and can store oxygen in their mesoglea, the jelly plankton may also benefit in a lower-oxygen ocean (Ramirez-Llodra et al., 2011). Benthic communities within OMZs are typically composed by nematodes, annelids and molluscs, with few crustaceans and echinoderms (Levin, 2003), and bacterial mats may cover the seabed in patches (Jørgensen and Gallardo, 1999).

For all the above mentioned reasons, it is important to collect sediment samples to analyze physical-chemical characteristics in terms of quantity and quality of sedimentary organic matter, as well as their impacts on the biodiversity and taxonomic composition of benthic assemblages (Mercado et al., 2015).

Regarding the monitoring of eutrophication in open waters, a core group of indicators has been already in use at European level, as: i) Nutrient (Nitrate, Ammonium, Phosphate), ii) Dissolved oxygen and iii) Phytoplankton (Chlorophyll a, Dominance). Zooplankton biomass is considered a potentially useful indicator but not yet mature (UNEP(DEPI)/MED, (2007) and references therein). For benthic ecosystems, the monitoring approach should include i) quantity and quality of organic matter in the sediments and the ii) biodiversity and taxonomic composition of benthic invertebrates (Dell'Anno et al., 2002; Pusceddu et al., 2009; Pusceddu et al., 2011; Pusceddu et al., 2014; Bianchelli et al., 2016).

Among the indicators recently proposed to assess the benthic trophic status of marine ecosystems, the one based on the quantity and biochemical composition of the sedimentary organic matter has been increasingly applied, both in coastal and deep-sea ecosystems. The sedimentary contents of the main biochemical organic matter compounds as protein, carbohydrate, lipid, biopolymeric C (the sum of C deriving from protein, carbohydrate and lipid) and its algal fraction have been repeatedly utilized to assess the benthic trophic status of several marine coastal ecosystems in Mediterranean sub-basins, also impacted by human activities (Dell'Anno et al., 2002; Pusceddu et al., 2009; Pusceddu et al., 2011; Pusceddu et al., 2014; Bianchelli et al., 2016). Considering that changes in the amount and biochemical characteristics of nutrients and organic loads could have an impact on deep-sea benthic communities, it is also important to analyze biodiversity and taxonomic composition of benthic invertebrates such as macrozoobenthos (e.g., AMBI/BENTIX indices: Borja et al. (2000); Simboura and Zenetos (2002)). Besides macrofauna, also meiofauna, including foraminifera, are good indicators of organic enrichment, caused by eutrophication (Vollenweider et al., 1998; Naeher et al., 2012; Bianchelli et al., 2016).

5.3.2 Nutrients in the water column (D5C1) - Primary

The MSFD states that the Nutrient concentrations are not at levels that indicate adverse eutrophication effects. Nutrients in the water column are Dissolved Inorganic Nitrogen (DIN), Total Nitrogen (TN), Dissolved Inorganic Phosphorus (DIP), Total Phosphorus (TP). Beyond coastal waters, Member States may decide at regional or subregional level to not use one or several of these nutrient elements.

The threshold values beyond coastal waters are values consistent with those for coastal waters under Directive 2000/60/EC. Member States shall establish those values through regional or subregional cooperation.

The extent to which good environmental status has been achieved shall be expressed beyond coastal waters, as an estimate of the extent of the area (as a proportion (percentage)) that is not subject to eutrophication (as indicated by the results of all criteria used, integrated in a manner agreed where possible at Union level, but at least at regional or subregional level).

Nutrient concentrations in micromoles per litre (µmol/l).

The D5C2 can be applied to the deep sea but provide very limited indications on the potential effect of eutrophication and these measures should be considered only as support measures.

5.3.3 Chlorophyll a in the water column and sediments (D5C2) - Primary

The MSFD states that the Chlorophyll a concentrations are not at levels that indicate adverse effects of nutrient enrichment. *The threshold values beyond coastal waters are values consistent with those for coastal waters under Directive 2000/60/EC. Member States shall establish those values through regional or subregional cooperation.*

The extent to which good environmental status has been achieved shall be expressed beyond coastal waters, as an estimate of the extent of the area (as a proportion (percentage)) that is not subject to eutrophication (as indicated by the results of all criteria used, integrated in a manner agreed where possible at Union level, but at least at regional or subregional level).

The outcomes of the assessments shall also contribute to assessments for pelagic habitats under descriptor 1 as the distribution and an estimate of the area that is subject to eutrophication in the water column.

Chlorophyll a concentrations (biomass) in micrograms per litre (μg/l).

The D5C2 is not applicable as it is to the deep sea. However, the determination of chl-a concentration in sediments can provide important insights into the trophic state of the area. Different methods for assessing chlorophyll-a concentrations in marine sediments can provide different under-or over-estimates, also because of the relative importance of the chlorophylls' degradation products (Pinckney et al.,1994). For consistency with previous studies, chloroplastic pigments (chlorophyll-a and phaeopigments) can be analysed fluorometrically (Danovaro et al., 2010). Total phytopigment concentrations can be utilized as a proxy for the organic material of algal origin and are defined as the sum of chlorophyll-a and phaeopigment concentrations, after conversion into C equivalents (Pusceddu et al., 2009; Pusceddu et al., 2010). The percentage contribution of total phytopigments to biopolymeric C is an estimate of the freshness of the organic material deposited in the sediment: since photosynthetic pigments and their degradation products are assumed to be labile compounds in a trophodynamic perspective, the lower their contribution to sediment organic C the more aged the organic material (Pusceddu et al., 2010).

5.3.4 Harmfull algal blooms (e.g. cyanobacteria) in the water column (D5C3) - Secondary

The MSFD states that the number, spatial extent and duration of harmful algal bloom events are not at levels that indicate adverse effects of nutrient enrichment.

The extent to which good environmental status has been achieved shall be expressed beyond coastal waters, as an estimate of the extent of the area (as a proportion (percentage)) that is not subject to eutrophication (as indicated by the results of all criteria used, integrated in a manner agreed where possible at Union level, but at least at regional or subregional level).

The outcomes of the assessments shall also contribute to assessments for pelagic habitats under descriptor 1 as the distribution and an estimate of the area that is subject to eutrophication in the water column.

Bloom events as number of events, duration in days and spatial extent in square kilometres (km²) per year.

Deep-sea sediments can be repositories of cysts of harmful algae and their presence can be monitored through standard international approaches and methodologies. These cysts (either in quiescence of diapauses) can suddenly determine bloom reaching the coastal areas through upwellings.

5.3.5 Photic limit (transparency) of the water column (D5C4) - Secondary

The MSFD states that the photic limit (transparency) of the water column is not reduced, due to increases in suspended algae, to a level that indicates adverse effects of nutrient enrichment.

*The outcomes of the assessments shall also contribute to assessments for **pelagic** habitats under descriptor 1 as the distribution and an estimate of the area that is subject to eutrophication in the water column.*

*The outcomes of the assessments shall also contribute to assessments for **benthic** habitats under Descriptors 1 and 6 as the distribution and an estimate of the extent of the areas that is subject to eutrophication on the seabed.*

Photic limit a4 depth in metres (m).

The criteria D5C4 is not applicable to the deep sea.

5.3.6 Dissolved oxygen in the bottom of the water column (D5C5) - Primary

- This Criterion may be substituted by D5D8 -

The MSFD states that the concentration of dissolved oxygen is not reduced, due to nutrient enrichment, to levels that indicate adverse effects on benthic habitats (including on associated biota and mobile species) or other eutrophication effects.

The threshold values beyond coastal waters are values consistent with those for coastal waters under Directive 2000/60/EC. Member States shall establish those values through regional or subregional cooperation.

The extent to which good environmental status has been achieved shall be expressed beyond coastal waters, as an estimate of the extent of the area (as a proportion (percentage)) that is not subject to eutrophication (as indicated by the results of all criteria used, integrated in a manner agreed where possible at Union level, but at least at regional or subregional level).

*The outcomes of the assessments shall also contribute to assessments for **benthic** habitats under Descriptors 1 and 6 as the distribution and an estimate of the extent of the areas that is subject to eutrophication on the seabed.*

Oxygen concentration in the bottom of the water column in milligrams per litre (mg/l).

In shallow ecosystems oxygen deficiency can result from the sinking and decomposition of the excess organic matter produced as a result of eutrophication. It can also come about from other causes, including discharges of allochthonous organics and from decreases in the ventilation of waters caused for example by climate change.

Considering that oxygen depletion is one of the main cause of benthic faunal mortality, it is important to measure oxygen concentrations also in the sediments. This is also true for deep-sea ecosystems, because benthic communities could be negatively affected by oxygen depletion. The upper oxidised part of the sediment is separated from the reduced sediment occurring below by a recognisable division zone known as the redox potential discontinuity (RPD) (Fenchel and Riedl, 1970). The measure of RPD gives useful information especially in relation to sedimentary pattern and organic loading processes which can affect benthic assemblages. Thus it is highly recommended to measure temporal and spatial evolutions of redox potential in deep-sea ecosystems. The position of the RPD can be analysed by electrodes (Fenchel, 1969) or by digitally analysing the apparent RPD (aRPD) in sediment profile images (SPIs) (Rhoads and Germano, 1986).

5.3.7 Opportunistic macroalgae of benthic habitats (D5C6) - Secondary

The MSFD states that the abundance of opportunistic macroalgae is not at levels that indicate adverse effects of nutrient enrichment.

The threshold values beyond coastal waters are values consistent with those for coastal waters under Directive 2000/60/EC. Member States shall establish those values through regional or subregional cooperation.

The extent to which good environmental status has been achieved shall be expressed beyond coastal waters, as an estimate of the extent of the area (as a proportion (percentage)) that is not subject to eutrophication (as indicated by the results of all criteria used, integrated in a manner agreed where possible at Union level, but at least at regional or subregional level).

*The outcomes of the assessments shall also contribute to assessments for **benthic** habitats under Descriptors 1 and 6 as the distribution and an estimate of the extent of the areas that is subject to eutrophication on the seabed.*

Ecological Quality Ratio for macroalgal abundance or spatial cover. Extent of adverse effects in square kilometres (km2) or as a proportion (percentage) of the assessment area.

The criteria D5C6 is not applicable for deep sea.

5.3.8 Macrophyte communities of benthic habitats (D5C7) - Secondary

The MSFD states that the species composition and relative abundance or depth distribution of macrophyte communities achieve values that indicate there is no adverse effect due to nutrient enrichment including via a decrease in water transparency.

*The outcomes of the assessments shall also contribute to assessments for **benthic** habitats under Descriptors 1 and 6 as the distribution and an estimate of the extent of the areas that is subject to eutrophication on the seabed.*

Ecological Quality Ratio for species composition and relative abundance assessments or for maximum depth of macrophyte growth. Extent of adverse effects in square kilometres (km2) or as a proportion (percentage) of the assessment area.

The criteria D5C7 is not applicable for deep sea.

5.3.9 Macrofaunal communities of benthic habitats (D5C8) - Secondary

The MSFD states that species composition and relative abundance of macrofaunal communities, achieve values that indicate that there is no adverse effect due to nutrient and organic enrichment.

Beyond coastal waters, values shall be consistent with those for coastal waters under Directive 2000/60/EC. Member States shall establish those values through regional or subregional cooperation.

*The outcomes of the assessments shall also contribute to assessments for **benthic** habitats under Descriptors 1 and 6 as the distribution and an estimate of the extent of the areas that is subject to eutrophication on the seabed.*

Ecological Quality Ratio for species composition and relative abundance assessments. Extent of adverse effects in square kilometres (km2) or as a proportion (percentage) of the assessment area.

The organic matter enrichment, which leads to hypoxic/anoxic conditions, can drastically affect the benthic populations, with a reduction of the most sensitive components being the result (Kemp and Boynton, 1992; Heip, 1995; Ritter and Montagna, 1999). All marine benthic groups are affected by the consequences of eutrophication if anoxia occurs. The benthic community represents a source of information at different food-web levels, and can be utilized to investigate and characterize the habitat where the community exists, in both coastal an deep-sea ecosystems. Indeed, benthic fauna plays a pivotal role in sedimentary organic matter diagenesis, nutrient cycling and ecosystem functioning and, at the same time, is a very sensitive component to environmental changes (Brown et al., 2004).

Macrofaunal biodiversity, whose ecological traits have been widely associated to ecological alteration, is commonly utilized for the classification of the ecological status of marine benthic ecosystems (Borja et al., 2008). Macro-invertebrates are sensitive to disturbances of habitat such that the communities respond fairly quickly with changes in species composition and abundance. Each macro-invertebrate species has sensitive life stages that respond to stress and integrate effects of short-term environmental variations, whereas community composition depends on long-term environmental conditions.

In addition to macrofauna, meiofauna, due to their high diversity and standing stocks, high turnover rates and lack of larval pelagic dispersal, have attracted increasing attention as a tool for detecting anthropogenic impact and for ranking the environmental quality status both in coastal and deep-sea ecosystems (Danovaro et al., 1995; Mazzola et al., 2000; Fraschetti et al., 2006; Pusceddu et al., 2007; Gambi et al., 2009; Mirto et al., 2010; Pusceddu et al., 2011; Mirto et al., 2014; Pusceddu et al., 2014; Bianchelli et al., 2016; Pusceddu et al., 2016). Meiofauna are ubiquitous and the numerically dominant metazoan components of the deep-sea benthos (Vincx et al., 1994) and play important roles in the processing and redistribution of food reaching the abyssal seafloor (Rex and Etter, 2010). Meiofauna are very sensitive to environmental disturbances, particularly to organic enrichment and eutrophication (Pusceddu et al., 2011; Bianchelli et al., 2016), at temporal scales much narrower than those exhibited by macrofauna.

Since meiofauna and macrofauna have different ecological roles in marine ecosystems (Coull and Palmer, 1984), they may respond to environmental changes at different spatial and temporal scales. Indeed, with its planktonic larval dispersal, macrofauna could be indicative of effects over larger spatial and longer temporal scales. On the other hand, with direct benthic development, and short generation times, meiofauna (i.e., metazoan component) may indicate effects over smaller spatial and shorter temporal scales (Coull and Palmer, 1984).

Nevertheless, there are still some limitations of benthic fauna monitoring. Highly specialized taxonomic expertise is needed to support extensive monitoring activities. Current methods can distinguish severely impaired sites from those that are minimally impaired, however, it can be difficult to discriminate between slightly or moderately impaired areas. The cost and effort to sort, count, and identify benthic invertebrates can be significant (especially meiofauna), requiring tradeoffs between expenses and the desired level of confidence. In addition to taxonomic identification, benthic invertebrate metrics may require knowledge of the feeding group to which a species belongs, for example, suspension and deposit feeders. For the analysis of biodiversity and taxonomic composition of deep-sea benthic invertebrates, sediment samples could be collected by using box corer or even better multicorer.

5.4   Knowledge On Threshold

Because of the lack of data on the response of deep-sea communities to anthropogenic stress, and particularly to changes in trophic status, no general threshold are available until now. It is indeed evident that threshold might change considerably according to the geographic area considered and the regional productivity and biodiversity values.

Regarding oxygen depletion, the conventional 2 mg O2/liter limit serves to separate ''dead zones,'' from waters supporting significant benthic animal communities. However, it fails to reflect the oxygen threshold at which these communities experience hypoxia-derived mortality. Vaquer-Sunyer and Duarte (2008) found a broad, order of-magnitude variability in the thresholds of oxygen concentrations for hypoxia among coastal benthic marine organisms, which cannot be adequately captured by a single, universal threshold. This variability partially derived from significant differences in oxygen thresholds across taxa. The most sensitive organisms are crustaceans, which showed the highest LC50 (2.45 ± 0.14 mg O2/liter) and the shortest LT50 (55.5 ± 12.4 h). On the other hand, molluscs (gastropods), with the lowest LC50 (0.89 ± 0.11 mg O2/liter) are the most tolerant organisms to hypoxia, together with cnidarians, and priapulids, which showed the longest LT50 (1512.0 ± 684.0 h). Differential tolerance to hypoxia among invertebrate taxa (annelids>molluscs>crustaceans>echinoderms) has been previously reported also in deep waters (Levin, 2003). In addition, a recent study (Levin et al., 2009) performed on the Pakistan margin OMZ, provided evidences of clear oxygen thresholds for macrofaunal abundance and burrowing activity at ~0.11–0.13 mL−1, suggesting that this level of oxygen is required to support macrofaunal populations. Primarily spionid and amphinomid polychaetes appear at the lowest oxygen concentrations. The threshold for molluscs and crustaceans appears to be between 0.13 and 0.15 mL−1 while echinoderms appear to require 0.15–0.17 mL−1. Taxa most tolerant of severe oxygen depletion (<0.2 ml L-1) in seafloor OMZs include calcareous foraminiferans, nematodes and annelids (Levin, 2003; Levin et al., 2009).

5.5   CONCLUSIONS

Despite it is generally assumed that deep-sea ecosystems are oligotrophic, some deep-sea areas either along the water column and at the water sediment interface or within the sediment areas may experience severe effects of oxygen depletion linked to the excess of organic matter deposition deriving from eutrophication (Danovaro et al., 2014). So far, eutrophication effects have been so far almost completely ignored in the Mediterranean deep sea. However, recent studies highlighted that actual climate change may affect also deep-sea trophic status in the near future, with the Western basin expected to become more oligotrophic and the Eastern basin more eutrophic (Piroddi et al., 2017). A parallel decrease of both surface Chl-a concentration and oxygen in deep waters (at the Benthic Boundary Layer and in the LIW) were found among the environmental drivers causing a drop in the trophic level of several megafaunal species in the western Mediterranean (i.e. Balearic basin, Fanelli et al. (2016)). Moreover, climate change and coastal eutrophication may increase oxygen deficiency in some regions, also in deep waters, as observed for instance in the Pacific region facing California (Netburn and Koslow, 2015). This could be particularly relevant in the Ionian sea receiving inputs from the Adriatic Sea, or in deep-sea sediments facing highly productive areas such as Gulf of Lion or in the northern Aegean Sea.

5.5.1 Gaps

The main gaps related to descriptor 5 in the deep Mediterranean Sea are: i) lack of major data concerning either the availability of information pertaining to descriptor 5 and to specific sub-basins in the deep sea, ii) the lack of thresholds of indicators to identify the GES in the deep Mediterranean Sea.

Regarding the lack of data, the available information are almost completely related to coastal areas for which most countries have extensive datasets on eutrophication acquired through national monitoring programs in the framework of WFD and MSFD implementation or the Regional Sea Conventions, national or international research programs, technical reports, scientific publications and satellite imagery, and particularly for the initial assessment (Crise et al., 2015). Conversely, open waters and deep-sea environments lack spatial coverage of data, quantitative data on pressures (monthly/seasonal variation, natural/anthropogenic sources) and appropriate monitoring programs to allow the application of appropriate indicators (Crise et al., 2015). In the open sea pressures related to nutrient dynamics and deep ocean circulation (related but not equivalent to eutrophication and hydrographical conditions) are supported by a sufficient data base (Crise et al., 2015), whereas only data on benthic trophic status are available in the scientific literature for the deep sea (Pusceddu et al., 2010; 2014). This leads to the tentative conclusion that the deep-sea initial assessments, aimed at responding to the requirements of the MSFD criteria, tended to extrapolate the existing information (mainly along the coasts) to the open seas. This is a major pitfall in initial assessments, since the analysis of the pressure/impact show how, even considering similar pressures, the states, gaps and impacts may be different, particularly in deep-sea ecosystems (Crise et al., 2015). In this regard, mechanisms of impact of change in the trophic status and oxygen availability on the structure of the deep-sea communities, food web and carbon fluxes are still poorly studied (Crise et al., 2015).

It has to be mentioned that in the available scientific literature regarding eutrophication, also the use of the precise terminology may represent a gap, since 'shallow waters' refers to water depths between ca. 5 and 100 m, and the term 'deep water' to water deeper than 100 m (Druon et al., 2004).

5.5.2 Recommendations

Member States should follow agreed monitoring approaches, particularly within the same sub-region and/or region. Ideally, they would monitor a common regional set of elements, following agreed frequencies, comparable spatial resolution and agreed sampling methods.

The use of CTD profilers is proposed to obtain data of oxygen concentration along the water column till the deep-sea floor. Oxygen concentration across the top 20 cm of the sediments is also recommended.

Sediment samples could be collected by using box corer or even better multicorer, in order to analyse physical-chemical characteristics of sediments (including oxygen concentration and profiles, chlorophyll-a concentrations, organic loads) as well as meio- and macrofaunal abundance diversity and composition.

The monitoring of indicators related to descriptor 5, should be carried out, at least, in critical deep ecosystems such as the southern Adriatic sea (Pomo pit), slopes of the Gulf of Lion and the deeper portion of the northern Aegean Sea.

Sampling stations should be established after an analysis of the available data or, alternatively, after a prospective study. It is also encouraged to establish reference areas (i.e. stations that are not affected by direct human activities).

Regarding the number and position of the sampling stations in each area selected, it is proposed to obtain samples at least in three stations of each selected area.

Regarding to the minimal requirements of data, at least 2 samplings per year (in opposite productivity periods) should be carried out (although higher frequency is recommended), the data should allow to do calculations of temporal trends and to be enough to detect changes over a period of ten years.

It has been demonstrated that one of the world's largest discharge of municipal sewage sludge on the deep-sea floor (2500 m depth) off New York, has a strong impact on benthic ecology. More in details, the sludge-derived organic matter caused a variation in the abundance and taxonomic composition of benthic assemblages. Opportunistic species, such as polychaetes, extended their normal depth range in response to favorable organic enrichment of the sediments (Van Dover et al., 1992; Bothner et al., 1994).

Recently disposal of sediments from industrial ports has been planned in deep-sea regions and these highly enriched sediments might very likely cause effects of eutrophication in the disposal areas, which should be carefully investigated through before – after investigations.

6   Descriptor 6 : Seafloor Integrity

Descriptor 6 of the Marine Strategy Framework Directive (MSFD) requires that seafloor integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems, in particular, are not adversely affected (European Parliament, 2008). Based on Decision (EU) 2017/848 (European Commission, 2017a), this chapter deals with the pressures "physical loss" and "physical disturbance" to the seabed (criteria D6C1, D6C2, D6C3) whilst the overall assessment of ecological status of "Benthic habitat" (D6C4 and D6C5) is described under descriptor 1 - Biodiversity.

The Mediterranean Sea, with extensive parts of its shores being densely populated, provides valuable ecosystem services while supporting many human activities (Lloret, 2010). The Mediterranean seafloor is consequently exposed to a number of physical disturbances such as abrasion and silting. Many investigations show that both fisheries and exploitation of non-living marine resources are the most important pressures both for rocky and sedimentary bottom habitats (Bo et al., 2014a; D'Onghia et al., 2016a; Holler et al., 2017; Lauria et al., 2017). Physical disturbances of the seafloor may directly and indirectly affect the benthic habitats and biodiversity of the deep Mediterranean Sea (see descriptor 1-Biodiversity as mentioned above for the ecological status of benthic habitats).

6.1   State Of The Art

Habitat destruction is one of the main threats to environmental integrity (Claudet and Fraschetti, 2010). Assessing the consequences of human impacts is crucial both to predict and to prevent structural and functional changes of habitats. However, to date, all studies on marine threats have mostly focused on coastal habitats (Claudet and Fraschetti, 2010). This document will attempt to gather information on marine threats in the deep sea, with a special focus on the deep Mediterranean Sea.

6.1.1   Physical restructuring of seabed morphology (Abrasion, bottom trawling)

While several studies have looked at the impacts of bottom trawling in various parts of the Mediterranean Sea, these have mostly focused on biological impacts (as described in the descriptor 1-Biodiversity report); studies on the physical changes are much more limited, although this aspect has recently received attention in the northwestern Mediterranean (Revenga Martinez de Pazos, 2012).

In the Mediterranean, several studies have shown the impact of trawling on the benthos, which is much more alarming in the deep sea than on the continental shelf because the deep seafloor is in general characterized by a low resilience (de Juan and Demestre, 2012; Dimech et al., 2012; Pusceddu et al., 2014). Seafloor disturbance by bottom fishing is mainly attributable to trawling (or dredging) on soft substrate bottoms and to fishing using long lines (or other set gears) mostly where rocky substrates occur. In both cases abrasion is the direct impact, and it can be so intense that in some areas it has modified the large, medium and small-scale morphology of the seafloor, as illustrated in the northwestern Mediterranean Sea (Puig et al., 2012; Martin et al., 2014b). Bottom trawling has become an important driver of the deep seascape evolution, and given the global dimension of this type of fishery, it is anticipated that the morphology of the upper continental slope in many parts of the World's oceans has been already altered and continues to be so due to intensive bottom trawling, producing comparable effects on the deep seafloor to those generated by agricultural ploughing on land (Puig et

al., 2012). Compared with untrawled areas, chronically trawled sedimentary bottoms along the continental slope of the northwestern Mediterranean Sea are characterized by significant decreases in organic carbon content and benthic fauna abundance and diversity (50% to 80% decline) (Pusceddu et al., 2014). The organic carbon removed daily by trawling in the La Fonera Canyon area, in the north Catalan margin, is estimated to represent as much as 60-100% of the input flux. Such an impact is anticipated to cause degradation of deep-sea sedimentary habitats and infaunal impoverishment (Pusceddu et al., 2014).

In canyons of the Gulf of Lion a comprehensive study based on videos shows that trawling ploughmarks are mostly located at a mean depth of 300 m (Fabri et al., 2014). In the north Catalan margin, southwest of the Gulf of Lion, the impact of persistent bottom trawling is clearly visible down to 800 m depth (Puig et al., 2012). In Maltese waters trawling is regulated and there are specific designated areas where trawling can take place (see figure 6 of (MEPA, 2013)).

In the Eastern part of the Mediterranean Sea, most fishing activities do not operate at depth greater than 250 m (Ibrahim et al., 2011; Farrag, 2016). Yet, scientific studies based on fishing operations from Italian bottom trawlers at depth ranging from 350 to 800 m have shown that deep-sea shrimps (*Aristaeomorpha foliacea* and *Aristeus antennatus*) represents 78-84% of the total catch and could be economically exploited by the Egyptian government (Ibrahim et al., 2011; Farrag, 2016).

The extent and intensity of bottom trawling on the European continental shelf (0-1000 m) was analysed from logbook statistics and vessel monitoring system data for 2010-2012 at a grid cell resolution of 1 x 1 min longitude and latitude (Fig. 6.1) (Eigaard et al., 2017). The footprint of the management areas ranged between 6-94% for the depth zone from 201 to 1000 m (Deep). Largest footprints per unit landings were observed off Portugal and in the Mediterranean Sea. Highest intensities were recorded in the Skagerrak-Kattegat, Iberian Portuguese area, Tyrrhenian Sea and Adriatic Sea. Bottom trawling was highly aggregated. The methods developed in this study integrate official fishing effort statistics and industry-based gear information to provide high-resolution pressure maps and indicators, which greatly improve the basis for assessing and managing benthic pressure from bottom trawling.

As a Regional Fisheries Management Organisation (RFMO), the General Fishery Commission for the Mediterranean (GFCM) is entitled to adopt spatial management measures that regulate and/or restrict fishing activities in its area of application, e.g. by establishing total closures or prohibiting the use of some fishing gear.

Since 2006, seven Fishery Restricted Areas (FRAs) have been established to ensure the protection of deep-sea sensitive habitats and of essential fish habitats (EFH) in well-defined sites (http://www.fao.org/gfcm/data/map-fisheries-restricted-areas/en/). In addition, in 2005, the GFCM prohibited the use of towed dredges and trawl nets in all waters deeper than 1000 metres to protect little-known deep-sea benthic habitats in the Mediterranean. In 2016, this large protected area below 1000 metres was officially declared a FRA by the Commission.

It is to be noted that in December 2016 the European Union agreed to ban bottom trawling below 800 m in all its waters. The regulation also lays down separate rules to protect vulnerable marine ecosystems (VMEs) at depths below 400 metres. If a catch exceeds set amounts of VME indicator species, then the vessel will have to stop fishing immediately and resume only when it has moved at least five nautical miles away from where it encountered a VME.
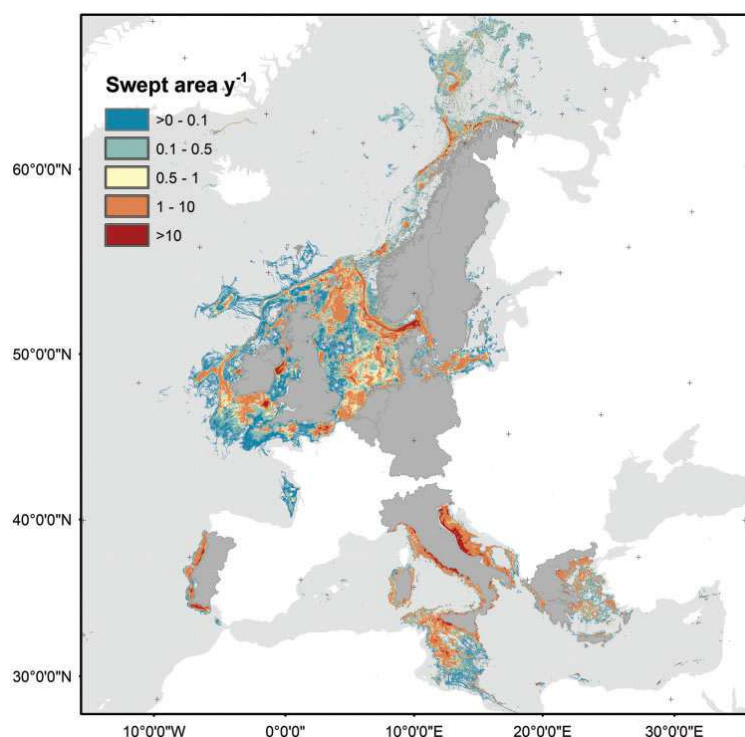
Figure 6.1. Mean annual trawling intensity in the period 2010–2012 at the surface level (sediment abrasion < 2 cm). The intensity is estimated from VMS and logbook data of bottom trawl fleets as the total area swept yearly in grid cells of 1 x 1 min divided by grid cell size. Countries marked dark grey provided data, from Eigaard, et al. *(2017).*

Bottom trawling is generally not undertaken in deep-water rocky areas due to the risk of losing the fishing gear. Other types of gear such as bottom longlines and gillnets contribute less to the total fisheries activities, but have also a considerable effect on Vulnerable Marine Ecosystems (VME) (*Isidella elongata* meadows, sponge fields, black corals, gorgonians and scleractinians) (GFCM, 2017). Those fishing gears are used on some specific areas where they can damage ecosystems that may be patchily distributed and thus the impact on the habitat may be significant. They may result in entanglement and breakage of coral colonies/frameworks, which can be considered to be both a change in the physical structure and a biological effect; this impact is discussed in the descriptor 1-Biodiversity report.

In addition to abrasion, bottom trawling had been shown to remobilize surface sediments in canyon rims and plains, generate sediment turbidity far from the specific fishing ground and increase sediment accumulation rates and suspension beyond the trawled areas, altering many vulnerable communities and habitat forming species (GFCM, 2017) (see the following paragraph).

6.1.2   Physical restructuring of seabed morphology (Resuspension, Silting-up)

Natural sediment accumulation in the deep sea is mostly dominated by fine-grained size-classes and naturally results from a variety of hydrosedimentary and sedimentary processes such as turbidity currents, cascading of dense shelf water and other gravity flows.

Mainly in the last decades (i.e. from the industrialization of bottom trawling fleets since the mid 1960's), bottom trawling substantially contributed to increase fine resuspension and silting-up in the

deep sea (GFCM, 2017). More powerful engines and more resistant trawl nets allowed, in addition, extending fishing activities to more distant, deeper and rougher seabed types.

Bottom trawling can modify the physical properties of seafloor sediments and also the sediment fluxes (Puig et al., 2012). It has been shown that near-bottom turbidity peaks were linked to working-days and working-hours of the trawling fleet, in La Fonera Canyon (off Spain) (Martin et al., 2014c; Payo-Payo et al., 2017). Sediment resuspension can propagate from fishing grounds to wider and deeper areas, including places where vulnerable marine ecosystems may occur, which can therefore result in their suffocation and burial (Martin et al., 2008). Resuspension by bottom trawling also involves a massive supply of generally old or fossil organic matter, which is not as fresh and nutritional as more recent material coming from the sea surface (Pusceddu et al., 2014); therefore this could impact the food web and the fauna distribution, diversity and composition on the flanks of canyons subjected to intense trawling. Furthermore, reiterative trawling in given areas could lead to the quasi-permanent cloudiness of near-bottom waters, which may potentially affect pelagic, benthopelagic and benthic organisms and communities, to the point that the original ones could be replaced by others that are better adapted to such conditions. This is in particular the case of the bathyal muddy bottoms of the north Catalan margin, where the highly priced deep-water shrimp *Aristeus antennatus* is thriving and sustains an important fishery whereas the original diversity has been seriously damaged (Canals et al., 2006; Company et al., 2008; Puig et al., 2012; Gorelli et al., 2016)
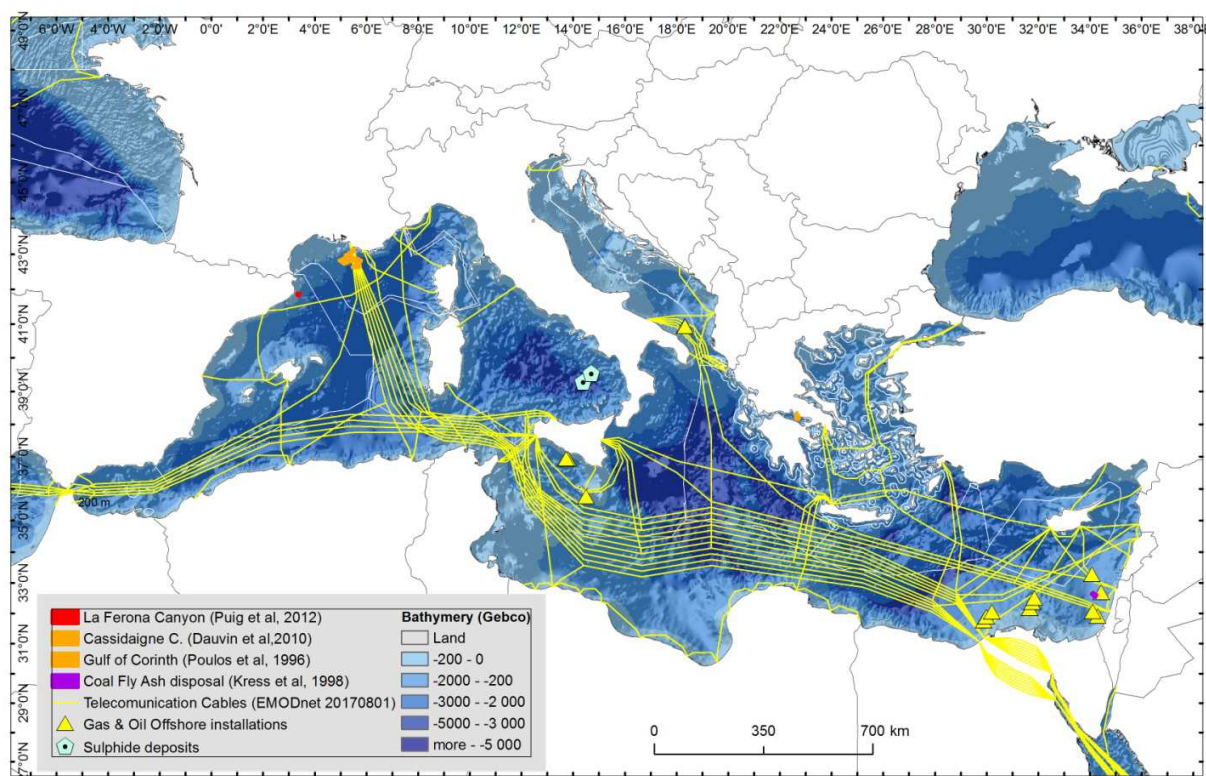
Figure 6.2. Location of known impacts affecting GES descriptor 6 in the deep Mediterranean Sea (i.e. deeper than 200 m). In red, bottom trawling. In orange, industrial waste disposal. Note that the total area impacted by bottom trawling is much larger, as shown by VMS data where available (Fig. 6.1). As yellow lines, telecommunication cables routes. In blue pentagons, sulphide deposits. As yelow triangles, offshore installations for Gas and Oil extraction(from EMODnet and internet). Figure by Marie-Claire Fabri based on the sources quoted.

6.1.3   Industrial uses (Waste disposal)

The direct disposal of industrial waste and tailings from land also takes place directly in the deep sea, either in relation to coastal mining (Koski, 2012; Vogt, 2013) or other types of processing plants onshore. Even dumping of mine tailings on the shoreline and on the inner shelf may lead to the escape of metals and other pollutants both in dissolved and particulate phases (e.g. on-going studies in Portmán Bay and nearby area in SE Spain (Oyarzun et al., 2013; Mestre et al., 2017)). In this context, concern on the need to mitigate environmental impacts of mine wastes is growing globally, including the marine component (Hudson-Edwards, 2016). One key point that, generally speaking, has been poorly addressed in this field is the actual bioavailability of released pollutants.

Deep fjords and submarine canyons with their heads close to the shoreline are preferred sites for direct deep sea disposal due to their natural capacity to act as conduits to deep basins –i.e. "out of sight, out of mind"- (Ramirez-Llodra et al., 2015). Currently, two aluminium industries discharged their red waste (called red mud but also "killing muds") in the deep Mediterranean Sea: one in France (Gulf of Lion, Cassidaigne Canyon) and one in Greece (Gulf of Corinth, Antikyra Bay, N38°20, E22°40') (Fig. 6.2).

In France a pipeline transported residues into the Cassidaigne Canyon down to 320 meters deep from 1965 to 2015. During 50 years the red mud spread on the bottom of the canyon and over the surrounding open slope and bathyal plain, covering the previous seabed (Dauvin, 2010). The ecosystem impact of such disposal is essentially a physical disturbance by oversilting and suffocation, while possible chemical pollution has not been proved (Stora et al., 2011; Fontanier et al., 2012; Fabri et al., 2014; Fontanier et al., 2014). Since January 2016 the factory stopped direct discharge in the canyon but obtained a dispensation to expel a fluid instead. No scientific study of the potential impact of that fluid is available yet.

In Greece red mud resulting from bauxite processing is discharged in the Gulf of Corinth through a pipeline opening at a depth of 100 m. There, waste forms a massive deposit that extends 17 km beyond the mouth of the pipeline and down to the basin floor at a water depth of 860 m. The current 1-7 m thick red mud deposit is a direct result of *in situ* discharge between 1974 and 1988 (15 years) (Varnavas et al., 1986; Varnavas and Achilleopoulos, 1995; Poulos et al., 1996).

Israel's *Ministry* of *Environmental Protection* has long condoned waste disposal in the deep sea. Coal fly ash (CFA) from the Hadera power plant, Israel, was dumped in the Mediterranean Sea some 70 km offshore, at a water depth of 1400 m, since 1988 (Kress et al., 1993; Kress et al., 1996; Kress et al., 1998). The disposal site (16 km²) contained areas which were covered by a 0.5-1.0 cm thick layer of ash. CFA on the seabed ranged from fine powder, small aggregates and even as large blocks. Comparison of the benthic fauna at the centre of the disposal site with that of a control area indicated a severe impoverishment of the benthos in the disposal area. Despite the negative impact on the biota and the fact that CFA was dumped widely outside the 200 km² allocated site, the environmental ministry allowed the disposal to continue over a decade.

The Israel's ministry permitted also long term disposal of polluted dredged sediments and industrial waste (1,900,000 m³) at a site 1300 m deep (Herut et al., 2010). The sediments there were polluted with Hg, Cd, Pb, TBT and organotins, PCBs. Cadmium levels were significantly higher in decapod crustaceans than in the control site. Dredged sediments were retrieved also at a distance of 3 km from the allotted site (Herut et al., 2010).

6.1.4 Extraction of non-living resources (oil, gas, sulphide deposits)

Other existing or emerging activities in the deep Mediterranean Sea include exploration for, and mining of, seabed mineral resources such as sulphide deposits, oil, and gas (FAO, 2016b).

Metalliferous sulphidic muds and massive consolidated sulphides are deposits associated with hydrothermal activity on the seafloor. The metal composition of massive sulphide deposits is highly variable, the principal metals of value being copper, lead and zinc. However, hydrothermal vents are not all of potential economic significance. Sulphide deposits are found in the Tyrrhenian Sea (see distribution in Fig. 6.1) (Boschen et al., 2013).

Deep-sea mining and drilling can impact the seafloor as it generates sediment plumes and, in some cases, mobilizes heavy metals, which may affect larger areas of the seabed. In particular areas, the offshore industry (i.e. for hydrocarbon drilling) further contributed to the enhancement of resuspension and silting up of deep environments, with a relevant role played by drilling muds (fluid mixture) and drill cuttings (broken bits of solid material), the latter especially when no mud recovery is implemented.

In central Mediterranean Sea, oil and gas offshore installations are located south of Sicily and in the Ionian Sea (http://unmig.mise.gov.it/unmig/strutturemarine/elenco.asp) (Fig. 6.2). Malta's central location in the Mediterranean and its proximity to proven hydrocarbon systems with producing oil and gas fields in offshore Sicily, Libya and Tunisia makes Malta's offshore acreage attractive for petroleum exploration but there are currently no active extraction activities since so far, no commercially-exploitable discovery has been made (https://continentalshelf.gov.mt/en/Pages/Oil-and-Gas-Exploration.aspx). Nonetheless, it is expected that exploration activity will continue; for instance in September 2017 the Government of Malta issued a new notice regarding availability of areas for authorization on a permanent basis under either an exploration license or an exploration and production license (Official Journal of the European Union, 2017/C 313/02).

The eastern Mediterranean Sea has several large natural gas deposits. The Levant's deepsea continues its rapid growth as the Mediterranean's most prolific gas province (Fig. 6.3). Gas exploration was pioneered on the Egyptian Mediterranean Shelf in the early 1980s. During that period, two significant gas discoveries were made, Port Fouad Marine and Wakar in the northeastern offshore area of the Nile Delta. The discovered gas potential of these fields encouraged exploration activities offshore throughout the region. The deeper areas off Egypt were initially explored in 1975, with the intensive prospecting starting in 1995. These exploration activities resulted in the drilling of several hundred wells. Israel's first commercially recoverable discovery of natural gas was in 1999. Beginning in 2009, a series of natural gas discoveries off Israel's northern coast, culminating with the *Leviathan* gas field, have greatly increased the region's natural gas reserves. There will likely be additional offshore oil and natural gas discoveries elsewhere in the Levant. The U.S. Geological Survey estimated a mean of 1.7 billion barrels of recoverable oil and a mean of 122 trillion cubic feet of recoverable gas in the Levant Basin Province using a geology based assessment methodology (https://pubs.usgs.gov/fs/2010/3014/pdf/FS10-3014.pdf). Egypt starts producing this year at the giant Zohr field, the largest gas field in the Mediterranean Sea (100 km², *depth* 1,450 m), expecting to reach production of 6.2 billion cubic feet per day in 2018. The already intense and still increasing activities, of great economic and social importance, have grave environmental implications. No independently validated environmental risk assessment was performed .
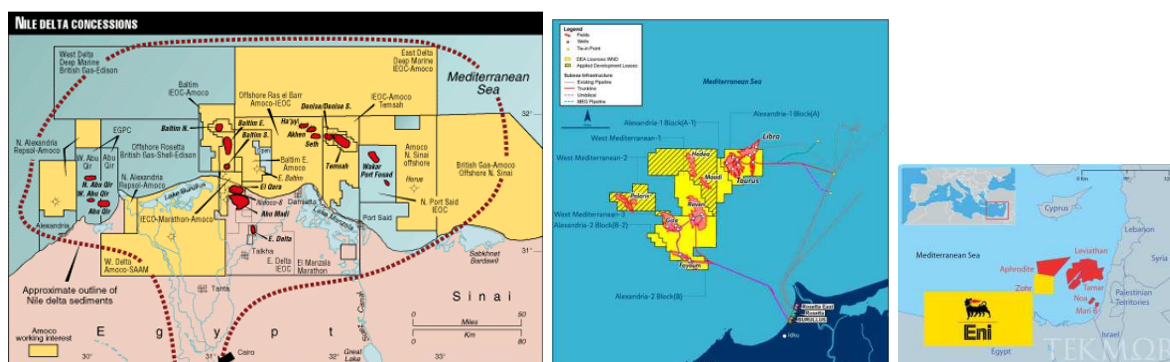
Figure 6.3: Maps of gas fields in the Eastern Mediterranean Sea (Source: Internet)

6.1.5   Shipping, laying of cables, marine research and injection of carbon dioxide

Other sources of impacts may come from shipping, whose risks include pollution from accidental spills and intentional discharges; the laying of cables and pipelines (Benn et al., 2010), which may generate pollution and sedimentation, while the cable-laying operation and the heavy anchors used during such operations can cause physical damage to i.e. corals; dumping of wastes and other material, where the physical impact (sedimentation, etc.) can damage or destroy habitats; and bioprospecting, whose bottom-sampling equipment can also negatively affect habitats. Submarine telecommunication cables are shown on Fig. 6.2.

Marine scientific research may also impact habitats depending on the sampling gear and techniques used. Additionally, emerging activities may eventually impact the seafloor, including, for example, the injection of carbon dioxide into deep ocean waters in order to mitigate the greenhouse effect, ocean fertilization, and energy generation, among others, as well as environmental changes such as global climate change (FAO, 2016b). The toxic effects of carbon dioxide disposal in the deep sea are different from other pollutants. The introduction of gaseous or liquid carbon dioxide will cause a strong acidification of extended pelagic or near bottom water masses. Additionally, sterilization of the deep sea floor and the near bottom water layers is to be expected inside and around deposits of liquid carbon dioxide (Ahnert and Borowski, 2000).

6.2 Where Is the knowledge

The western part of the Gulf of Lion (off Spain) is the location where most of the studies have occurred with regards to physical restructuring of the seabed morphology (bottom trawling). Very few studies have occurred elsewhere, or with regards to other pressures (Table 6.1).

Table 6.1. Census of studies dealing with seafloor integrity, ordered by pressures and locations. In grey, studies in central or eastern Mediterranean Sea.

Pressure	Where	Western	Central	Eastern
Physical restructuring of seabed morphology (abrasion, bottom trawling)	La Fonera canyon (Spain)	(Martin et al., 2008) (Martin et al., 2014b) (Martin et al., 2014c) (Puig et al., 2012) (Lastras et al., 2016) (Revenga Martinez de Pazos, 2012)		
	Italy		(Eigaard et al., 2017)	
	Malta		(Dimech et al., 2012)	
	Greece		(Eigaard et al., 2017)	
Physical restructuring of seabed morphology (waste disposal)	Cassidaigne canyon (France)	(Dauvin, 2010) (Fontanier et al., 2014)		
	Gulf of Corinth (Greece)			(Varnavas et al., 1986) (Varnavas and Achilleopoulos, 1995) (Poulos et al., 1996)
	Off Israel (polluted dredged sediments)			(Herut et al., 2010)
	Off Israel (Coal fly ash)			(Kress et al., 1990) (Kress et al., 1991) (Kress et al., 1993) (Kress et al., 1996) (Kress et al., 1998)
Extraction of non living resources	South of Sicily and in the Ionian Sea		EMODnet http://www.emodnet.eu/	
	Off Israel			Internet wikipedia https://en.wikipedia.org/wiki/Natural_gas_in_Israel
	Off Egypt			http://www.ogj.com/articles/2017/05/bp-begins-gas-production-from-west-nile-delta.html
Laying of cables	Med. Sea	EMODnet http://www.emodnet.eu/	EMODnet http://www.emodnet.eu/	EMODnet http://www.emodnet.eu/

6.3   How is Seafloor integrity measured

6.3.1   Methodological sampling aspects

The MSFD requires the application of the ecosystem approach to the management of human activities, covering all sectors having an impact on the marine environment (Martin et al., 2014d). Ecosystem-based management of activities affecting the seafloor will require knowledge of a wide variety of ecological variables supported by abiotic measurements and detailed habitat mapping (Danovaro et al., 2017). An evaluation of the spatial extent of human activities on the deep seafloor has been assessed in the North East Atlantic, and could be used for an evaluation in the Mediterranean deep Sea (Benn et al., 2010).

See descriptor 1 for more details on the methodologies with regards to ecosystems.

6.3.2   Spatial extent and distribution of the physical <u>loss</u> of the natural seabed (D6C1)

A physical loss of the natural seabed due to permanent change of seabed substrate or morphology, shall be understood as a permanent change to the seabed which has lasted or is expected to last for a period of two reporting cycles (12 years) or more. The area lost shall be assessed in relation to the total natural extent of all benthic habitats in the assessment area (i.e. extent of anthropogenic modification). The unit of measurement of the area physically lost should be in km².

It is noteworthy that a 12 years timing in the deep sea is very short. Impact on the deep-seafloor integrity is of much greater concern than on the continental shelf because of the low resilience of deep-sea benthic ecosystems.

<u>Physical restructuring of seabed morphology (abrasion, bottom trawling)</u>

Heavily impacted fishing grounds, such as the one on La Fonera Canyon (Martin et al., 2008; Martin et al., 2014b; Martin et al., 2014c), will probably take millennia to recover if the pressure stops. The seafloor is so ploughed that the morphology of the continental slope has been dramatically changed (Puig et al., 2012; Lastras et al., 2016) beyond a tipping point (i.e. no recovery within a reasonable timeframe).

Areas trawled by the commercial fisheries can be identified using the Vessel Monitoring System (VMS) with which every vessel exceeding 15 m of overall length has been equipped since 2005 (Martin et al., 2014d). Year by year data can be retrieved (when governments are not reluctant to release them) to identify fishing grounds and non-trawled sites and to measure the fishing effort. This effort is calculated from the VMS data after filtering by considering vessel speeds of between 2 and 4 knots to be indicative of trawling on the deep slope, because trawlers travel at an average speed of 2 to 3 knots when trawling at a depth around 600 m (4.5 knots on the continental shelf) (Dimech et al., 2012). Trawling grounds can be mapped using GIS techniques.

The spatial extent, and hence impact, of bottom trawling has been found to be orders of magnitude greater than that for other human activities in the north-East Atlantic (Benn et al., 2010), and it is probably the same in the deep Mediterranean Sea but it still has to be calculated.

Industrial uses (Waste disposal)

Industrial deep-sea tailing is a pressure that is disturbing the seafloor by sedimentation. The measure of the extent of the disturbance can be evaluated by the mean of sediment cores collected from the site covered by mine tailing deposits. For example the extent of the Cassidaigne Canyon disturbance was evaluated using box-cores (Dauvin, 2010; Fontanier et al., 2014) and the thickness of the artificial layer was evaluated using gravity-corers (Dauvin, 2010). Sediment traps can also be used to measure the sedimentation rate. Chemical analyses of the cores allows the evaluation of the composition of the sediments and the delineation of the contaminated area. The known extent of the three waste disposal sites are reported in Table 6.2.

Table 6.2: Extent of waste disposal on the deep-sea floor of the Mediterranean Sea.

Location	thickness	Extent off shore	surface
Cassidaigne Canyon	Several meters	50 km	A least 18.3 km² extrapolated from (Dauvin, 2010)
Gulf of Corinth	1 to 7 m	17 km	1.4 km² (Poulos et al., 1996)
70 km off Israel	0.5 - 1 cm		16 km² (Kress et al., 1990; Kress et al., 1991; Kress et al., 1993; Kress et al., 1996; Kress et al., 1998)

Extraction of non-living resources (oil, gas, sulphide deposits)

The effects of anthropogenic disturbance, such as oil drilling activity, on the deep-sea benthic environment are conventionally assessed by sampling sediments (typically by grab) and then measuring chemical parameters (e.g. heavy metal content) and occasionally macrofauna (for diversity analyses) from the origin of the impact along four radiating transects at geometrically increasing distances (Hughes et al., 2010).

Deep-water drilling activities cause physical changes to the seafloor as drill cuttings composed of drilling mud, chemicals, and fragments of rock are discharged onto the seabed. Assessment of the extent of drilling disturbance around deep oil and gas structures under exploitation in the Mediterranean Sea is not reported in the literature. In the North-East Atlantic a radius of 83m around each well was considered to estimate the area covered by the physical presence of cuttings (different from the extent of biological impact) (Benn et al., 2010).

In the Méditerranean Sea massive sulphides can be found in the Tyrrhenian Sea but are not under exploitation (Boschen et al., 2013).

6.3.3 Spatial extent and distribution of the physical <u>disturbance</u> pressures on the seabed (D6C2)

A physical disturbance shall be understood as a change to the seabed from which it can recover if the activity causing the disturbance pressure ceases. The unit of measurement of the area physically disturbed should be in km².

Physical restructuring of seabed morphology (resuspension, silting-up)

Sediment resuspension can propagate from fishing grounds or mining waste deposits to wider areas. The spatial extent of these areas impacted can be measured by the disturbance caused to the structure of the communities living in and around the direct pressure.

Shipping, laying of cables, marine research and injection of carbon dioxide

The spatial extent of telecommunication cables is low to moderate depending on whether cable burial is included in the calculation (Benn et al., 2010).

Non fisheries marine scientific research has a relatively small footprint, but the spatial extent of fisheries marine scientific research is moderate and should be evaluated (Benn et al., 2010).

6.3.4 Spatial extent of each <u>habitat type adversely impacted</u> by physical disturbance (D6C3)

The extent of habitat adversely impacted shall be assessed in relation to total natural extent of each benthic habitat type assessed. Species composition shall be understood to refer to the lowest taxonomic level appropriate for the assessment. The extent of each habitat type adversely affected should be in km² or as a proportion (percentage) of the total natural extent of the habitat in the assessment area.

Quantifying the extent of human activities, and more accurately the extent of each habitat type impacted by a physical disturbance, imply the routine use of a large variety of tools that so far have only been used for research. While some activities have an immediate impact after which seafloor communities may be re-established albeit after a long time period, other activities, such as waste disposal, may have an effect for many decades and the impact is likely to extend far beyond the physical disturbance (Benn et al., 2010).

A set of technical tools for assessing seafloor integrity is available and has already been used in research. High resolution maps of benthic substrata and habitats are in increasing demand both to underpin environmental and socioeconomic impact assessments and to help in the development of effective management measures (Kenny et al., 2003; Brown et al., 2011; Stephens and Diesing, 2014; Holler et al., 2017). Over the last decades, developments in acoustic remote sensing techniques, coupled with *in situ* sampling, have enabled extensive seafloor mapping, and we are now able to map the seabed at high spatial resolution and accuracy (Micallef et al., 2012; Puig et al., 2012; Lastras et al., 2016). Acoustic mapping equipments, such as multibeam echosounders (MES) and sidescan sonars (SSS), can ensonify areas of seabed with 100% spatial coverage at a resolution finer than 1 m² depending on the altitude at which the data is collected (Kenny et al., 2003). Complementary benthic "ground truthing" sampling methods such as core-sampling and underwater imaging (videography and photography) have to be used according to the size and the nature of the area to map (Kenny et al., 2003; Brown and Blondel, 2009; Brown et al., 2011; Holler et al., 2017).

MBES can be used to map the shape of the seafloor and physical properties of surficial sediments and video data complete the information to map the detailed habitats and communities as was done in the eastern Mediterranean on the Amon mud volcano (Dupre et al., 2007; Ritt et al., 2011).

The increasing use of modern robotic technology, such as remote operated vehicles (ROVs), will make deep-sea experimentation easier and more frequent. ROVs are used extensively by the offshore drilling industry to support deepwater activities, and are now being increasingly used by the international scientific community for enhanced deep-sea observation, fine scale mapping and sampling (Fabri et al., 2014; Lastras et al., 2016). Optical images can also be used for mapping especially on hard substrata that are very often highly undulating or uneven for MBES and on which Vulnerable Marine Ecosystems (VME) may have established.

In 2016 FAO edited a document, "*The Vulnerable Marine Ecosystems: Practices in the High Seas*", that catalogues progress made towards the identification and protection of VME (FAO, 2016b) and produced a FAO VME Portal and Database to support States and regional fisheries management organisations in protecting VMEs (www.fao.org/in-action/vulnerable-marine-ecosystems/en/). In 2017 FAO and GFCM, during their first working group on VME, decided to edit a list of Mediterranean habitat types and representative species that may contribute to form VMEs (GFCM, 2017).

Habitat suitability modelling is useful to predict distribution of species that are tightly-correlated with environmental parameters (e.g. slope, substrate type, temperature etc). Examples include cold water corals (Bargain et al., 2017; Fabri et al., 2017) as well as other benthic communities (Giusti et al., 2014; Lauria et al., 2016; Giusti et al., 2017; Lauria et al., 2017). Recent developments have been achieved in predictive models of seafloor habitats and benthic biotopes at the scale of large areas (Martin et al., 2014a). These habitats or biotope models seem highly suitable to monitor long-term variation in seafloor attributes, and make increasing use of MBES backscatter imagery (Brown and Blondel, 2009).

Sampling adds some more information to remote techniques (acoustic or optic). It allows comparison of the community and the population structures and the determination of the limit of the impacted area. An example off Malta shows how to determine the effects of fishing on the communities of fishing grounds (Dimech et al, 2012). This technique could be used to delimit the extent of the disturbance by looking at the community structure. Sampling is the only way to measure adverse impact on fauna living in sedimentary environment. A great list of indexes have been used in shallow water in the framework on the Marine Water Directive (2000/0/EC) but those indices have to be adapted in order to fulfil the MSFD objectives and to evaluate the ecological status of sedimentary bottoms and not the quality of the water (Diaz et al., 2004; Borja et al., 2009; Pinto et al., 2009; Martinez-Crego et al., 2010).

6.4 Knowledge On Threshold

Because of the lack of data on the response of deep-sea communities to anthropogenic stress, no threshold are presently available.

6.5 CONCLUSIONS

Three classes of physical impacts have been identified in the north-East Atlantic (Benn et al., 2010) and should be evaluated in the Mediterranean Sea : (1) low physical impact (non-fisheries scientific research, submarine telecommunication cables and waste disposal); (2) moderate impact (oil and gas activities and fisheries scientific research); (3) impact an order of magnitude greater than all the other activities (bottom trawling). However, waste disposal may cause long-term biotic impact and thus may not be classified as having low physical impact.

Since biological processes in the deep sea are generally considered to be slow, owing to low temperatures and the scarcity of food, with the consequence that the recovery of benthic communities requires more time than in shallow water environments, (1) the effects of waste disposal or deep-sea mining on deep-sea communities should be studied, and (2) until studied the precautionary approach should be adopted (Ahnert and Borowski, 2000).

6.5.1 Gaps

The spatial extent of bottom trawling has been described to be orders of magnitude greater than that for the other activities (Benn et al., 2010), monitoring of the extent of trawling activities is therefore necessary. It would thus be relevant for detailed VMS and/or AIS data from the different Mediterranean countries to be made available (or purchased in the case of AIS data) in order to produce a map of the entire Mediterranean Sea showing the total area potentially affected by bottom trawling between depths of 200 and 1000 m.

The Vessel Monitoring System (VMS) provides information regarding vessel code, position, time, speed and direction of the boat to the fishing authorities. In the EU, from 2005 onwards, VMS applies to all large fishing vessels exceeding 15 m in length (EC Regulation No. 2244/2003). Since January 1st 2012, VMS has been extended to all vessels longer than 12 m (EC Regulation No. 1224/2009). Although VMS records are primarily for fisheries enforcements, they are currently employed as a unique and independent tool to spatially and temporally allocate the distribution patterns of fishing activity (Mills et al., 2007; Witt and Godley, 2007; Gerritsen and Lordan, 2011; Madurell et al., 2012).

The Automatic Identification System (AIS https://www.marinetraffic.com/) was introduced by the International Maritime Organisation (IMO) to improve maritime safety and avoid ship collisions (International Maritime Organisation, 2004). Whereas the VMS is usually based on point-to-point satellite communications between the ship and the ground centres and is limited to fishing vessels, AIS messages are broadcast omni-directionally by all kind of vessels and can be received by other ships in the neighbourhood using ground-based receivers and satellites. The AIS system allows ships to exchange, in near real time, state vector (position, speed, course, rate of turn, etc.), static (vessel identifiers, dimensions, ship-type, etc.) and voyage related information (destination, Estimated Time of Arrival, draught, etc.) (Iternational Telecommunication Union, 2014). The wealth of information that can be collected from the AIS is gradually making the system a cornerstone of maritime surveillance, not only for safety, but also for security and situational awareness applications (Pallotta et al., 2013). The AIS entered into force in 2002 for all vessels with a GT ≥ 300 tons. In 2012, the system became compulsory for EU fishing vessels longer than 24 m LOA, and over the next two years it was progressively extended to medium-large fishing vessels and in May 2014 became compulsory for all fishing vessels longer than 15 m LOA (European Parliament, 2011; Fabi et al., 2017).

A considerable amount of munitions have been disposed of into the deep ocean for many decades and continues (Ahnert and Borowski, 2000). Exact location of dumpsites, as well as the quality and quantity of what was dumped, have not often been accurately recorded and much of existing information is still not available to the public (Ahnert and Borowski, 2000). The available information has been summarised by UNEP/MAP (UNEP MAP, 2009).

6.5.2   Recommendations

Submarine canyons are of major concern in the deep seas as they provides services to human population and they act as nutrient supply to the deep-ocean ecosystems (Fernandez-Arcaya et al., 2017). Therefore they deserve coordinated management and monitoring that will take into account the cumulative effects of multiple marine activities and stressors (Markus et al., 2015). Human activities that disturb the seafloor are not yet subjected to integrated strategic management and planning with full consideration of their regional and long distance effects (Markus et al., 2015). An ecosystem-based approach strategy for the deep ocean should be developed with international agreement (Danovaro et al., 2017).

Data availability should be facilitated including on telecommunication cables routes (cable width, buried cables), oil and gas industry structures and infrastructure (wellheads, platforms, cutting piles, weights, anchors, rock dumps, pipelines) and VMS and/or AIS data should be made easily accessible for monitoring and research purposes.

As the impact of bottom trawling is so damaging for deep-sea ecosystems, further regulation of bottom trawling should be considered.

We recommend long-term monitoring plans of areas where previous activities with an impact on the integrity of the deep-sea bottom have ceased in order to assess the rate and trajectory of eventual recovery of the seafloor and subseafloor communities.

7   Permanent alteration of hydrographical conditions

Permanent alteration of hydrographical condition can have two main causes: climate change factors combined with natural variability, and large scale human activities implemented by major infrastructures. The former are global in scale and may develop into a range of local effects. The latter would involve building large infrastructures, potentially having an effect on deep-sea environment, such as oceanic dams, detached ports or transcontinental and inter-islands bridges. Other major marine infrastructures such as offshore wind farms are usually installed in shallow water.

Concerning the definition of "permanency" the revision of the MSFD highlighted that "*Physical loss shall be understood as a permanent change to the seabed which has lasted or is expected to last for a period of two reporting cycles (12 years) or more.*"

7.1   *State Of The Art*

Surface water from the Atlantic enters the Mediterranean Sea through the Gibraltar Strait and, while circulating eastwards undergoes evaporation and depletion of nutrients, sinking in certain areas and transforming into the salty Mediterranean water later returning to the Atlantic as a deep flow again through the Strait of Gibraltar.

The Mediterranean Sea has a negative water budget, i.e., evaporation exceeds the sum of precipitation and river runoff. The loss of water is compensated at the Strait of Gibraltar by an inflow of surface Atlantic water (AW). AW is fresher than the residing waters (schematically the Mediterranean waters: MWs). Being less dense, AW constitutes the surface circulation, which describes a counter-clockwise circuit along the continental slopes through both basins. During winter, intense episodes of dry and cold northerly winds occur on the northern parts of both basins, causing dense water formation in some specific areas of the basin: severe cooling and evaporation of the surface water in the Levantine Basin increase the density of AW, which sinks to intermediate levels (about 300‑400 m) to form a distinct intermediate water mass (Levantine Intermediate Water: LIW) (Fig. 7.1b). However, recently Millot (2013, 2014) argued that the LIW in the Western Mediterranean Basin represents all intermediate waters formed in all zones of dense water formation in the Eastern Basin, and should be renamed Eastern Intermediate Water (EIW; Millot, 2013). During the most intense winters, mixing involves AW and the underlying MWs (Fig. 7.1c): the convection reaches deep layers, and the water masses that are formed can reach the bottom. More specifically, dense water formation is recurrently observed in the Rhodes gyre, the Northern Aegean, the Southern Adriatic, and the northern part of the Western Basin (mainly the Gulf of Lion) (Durrieu de Madron et al., 2011).

Like the global ocean, Mediterranean Deep Waters then feed a basin-scale thermohaline circulation. The presence of the Sicilian Strait (at about 400 m depth) prevents deep-water exchange, decoupling the deep circulation of the Eastern and Western Mediterranean basins (see Fig. 7.1c). Consequently, only intermediate waters formed in the Levantine Basin (Lascaratos et al., 1999) can flow westward across the Sicilian sill. Part of these Mediterranean deep and intermediate waters formed in both basins ultimately outflow at Gibraltar and cascades into the Atlantic (Millot, 2009, 2013, 2014).

As a result, the waters that remain trapped in the deepest parts of the (sub-) basins until newly formed denser waters arrive have longer residence times compared to the overlying waters. The new deep water then uplifts the older water masses, allowing them to overflow either through the Sicily Channel towards the Western Basin (cascading in the Tyrrhenian sub-basin) or through the Strait of Gibraltar towards the Atlantic Ocean, where there is also a sill at about 300 m only (Durrieu de Madron et al., 2011).

The areas of dense shelf water formation play a pivotal role in the functioning of the Mediterranean Sea (Fig. 7.1) (Estournel et al., 2005; Trincardi et al., 2007; Canals et al., 2009; Durrieu de Madron et al., 2013; Benetazzo et al., 2014).
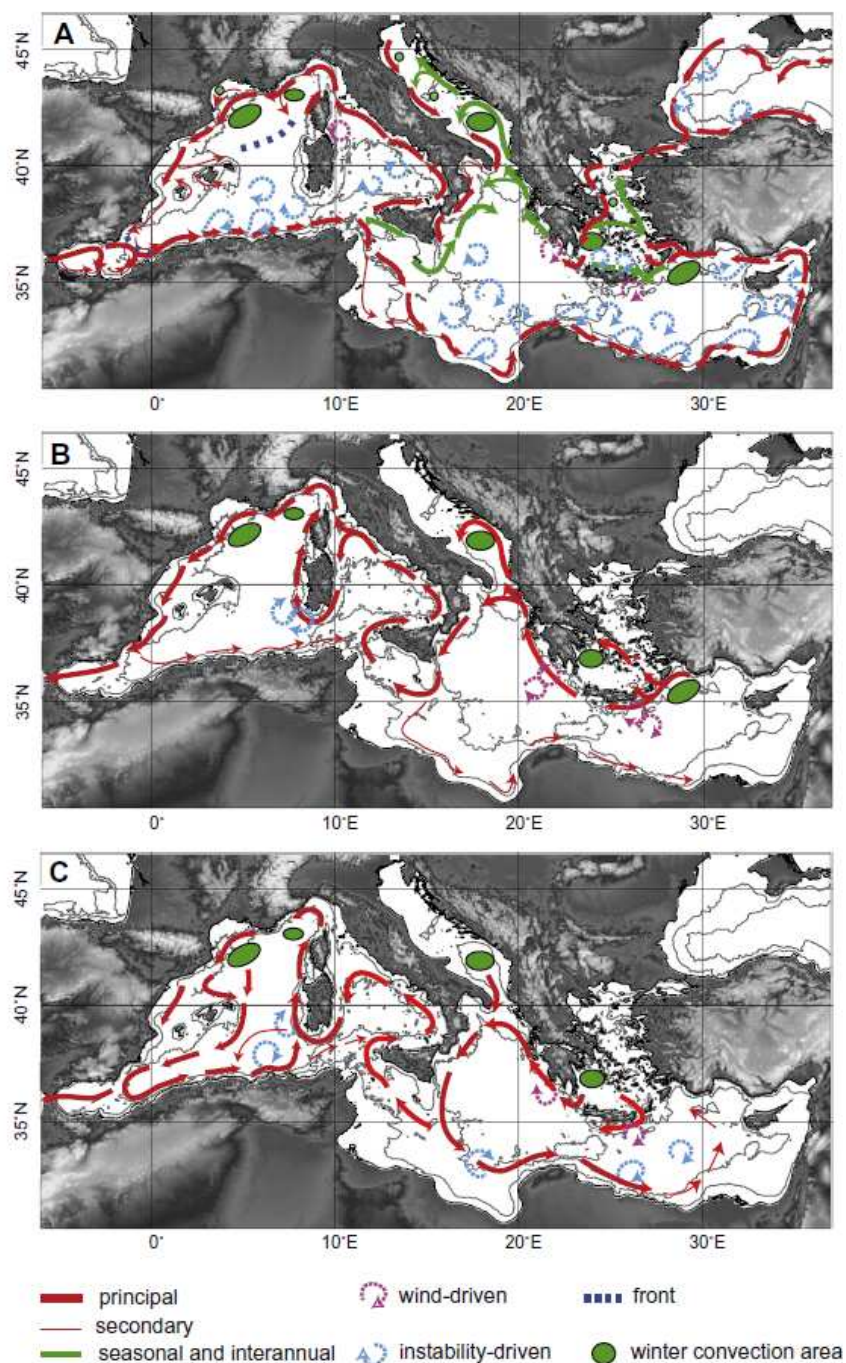
Figure 7.1 Circulation of surface (A), intermediate (B) and deep (C) water masses and areas of deep water formation (winter convection areas). The thin lines represent the 1000-m and 2000-m isobaths, from Durrieu de Madron et al. (2011).

The Western Mediterranean Deep Water (WMDW) is characterised by a temperature of 12.7°C and a salinity of 38.4 psu (Fig. 7.2). It is formed within the cyclonic gyre of the Gulf of Lion through open sea convection during extreme meteorological conditions in late winter and then spreads southward to fill the bottom layer of the Algerian-Provençal Basin (Lascaratos et al., 1999). The rate of formation and properties of the WMDW have been shown to be regulated by the amount and characteristics of the LIW (EIW; Millot, 2013) reaching the formation site. In the mid-2000s, a major deep-water renewal event occurred, with a dramatic increase of S and T of the deep water, filling the entire Western Mediterranean Basin as a result of dense shelf water formation and cascading, and offshore convection in the Gulf of Lion (Lopez-Jurado et al., 2005; Canals et al., 2006; Puig et al., 2013). This event is known has been named by some the Western Mediterranean Transition (WMT, see below) (Tanhua et al., 2013).

The Eastern Mediterranean Deep Water (EMDW) is significantly more saline and warmer than the deep water of the Western Basin (13.6°C, 38.7 psu) (Fig. 7.2). The Adriatic Sea is the principal deep water formation area in the Eastern Mediterranean Sea (Tanhua et al., 2013). Dense water then spreads through the Otranto Strait to fill the deepest parts of the Ionian and Levantine basins. In the early 1990s the Aegean Sea became the main deep water formation area, in an event known as the Eastern Mediterranean Transient (EMT, see below) (Roether et al., 1996; Tanhua et al., 2013).
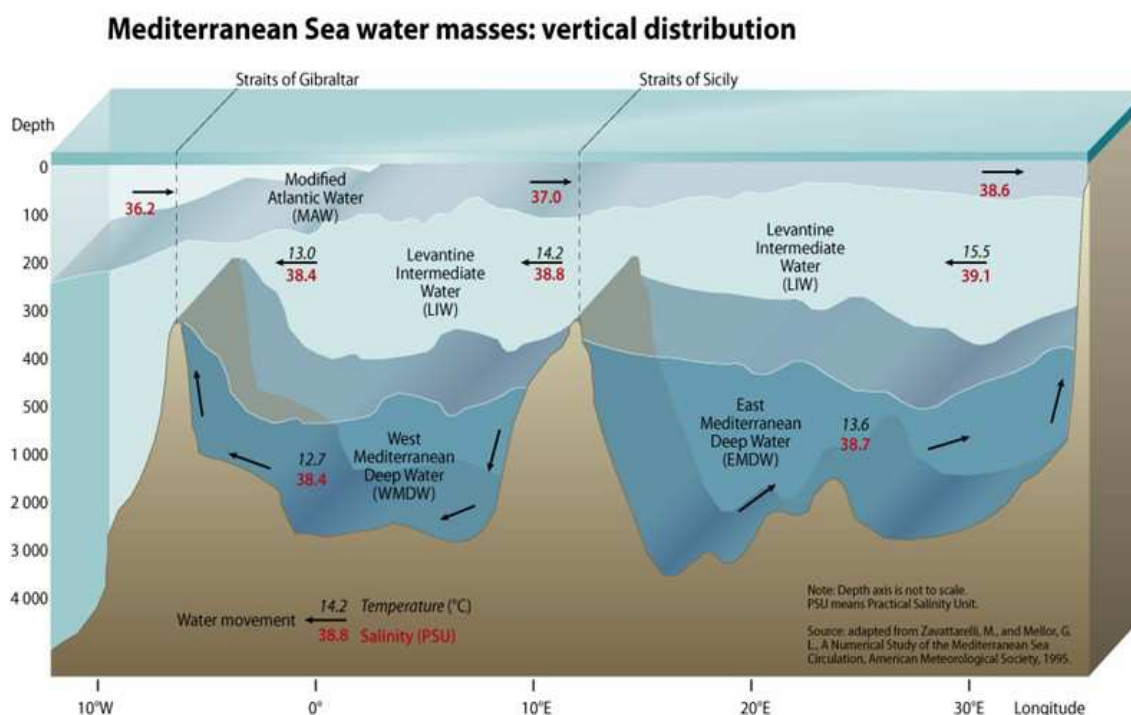
Figure 7.2. Vertical distribution of the water masses in the Mediterranean Sea, adapted from Zavatarelli and Mellor (1995).

7.1.1   The Eastern Mediterranean sea

During the last decades, deep Mediterranean waters have undergone drastic changes resulting in an alteration of the stratification associated to abrupt temperature and salinity increases (Schroeder et al., 2009). At the end of the 1980s, a 'regime shift' occurred, strongly influenced by northern hemisphere climate, producing changes in hydrographic properties, surface circulation and deep-water convection throughout the Mediterranean Basin (Conversi et al., 2010). In that period measurements of the deep layer of the Eastern Basin revealed a shift of the main site of deep-water formation from its usual source in the southern Adriatic to a new source in the Aegean sub-basins. This event, called the 'Eastern Mediterranean Transient' (EMT), started after 1987, peaked in 1992-94 and began to decline at the end the 1990s. It was generated from Aegean Sea forming and discharging massive amounts of unusually dense and saline waters(Roether et al., 1996; Schroeder et al., 2009). The outflow subsided strongly thereafter and only reached depths of 1,500– 2,000 m (Theocharis et al., 2002). It was accompanied by a vast rearrangement of the entire water column circulation as a result of shallower Aegean outflow and mixing of mid-depth water, lifting several hundred metres as a consequence of Aegean water addition deeper down. The effects altered also biogeochemical parameters, such as nutrients, induced a rise in oxygen consumption (Roether and Well, 2001; Klein et al., 2003) and, in the eastern Ionian, the lifting of the nutricline, by about 150 m (Klein et al., 1999). Ozer et al. (2017) stated that "while the East Mediterranean is fed by several external sources of nutrients (atmosphere, rivers, terrestrial runoff and submarine groundwater discharges)" it is reasonable that " the thermohaline flux variations attributed to the Adriatic-Ionian Bimodal Oscillating System (BiOS) mechanism have the most immediate and significant impact in magnitude on the available nutrients and the dynamics of the Eastern Basin primary productivity" (see also Mosetti (2016)).

Several factors determined the EMT. In the eastern Levantine Basin exceptionally saline waters, possibly assisted by eddies south of Crete, blocked the inflow of lower-salinity waters from the Ionian Sea (Malanotte-Rizzoli et al., 1999). Reduced precipitation influx of freshwater from the Black Sea and lessened inputs from shelf areas also contributed to the extra salinity. Kress et al. (2014) showed, on the basis of historical data, a cyclic a pattern in salinity (lower salinities at the beginning of the 1980s and 2000s and higher salinities at the beginning of the 90s and since 2006).

Furthermore, additional cyclic circulation changes of potential relevance took place. The upper-layer circulation Ionian gyre reversed from anticyclonic to cyclonic by mid 1997, disrupting the flow of modified AW from the Ionian Sea into the Levantine Basin (Borzelli et al., 2009) and causing a large net increase of salt in the overall budget of the Eastern Mediterranean Basin. Moreover, intermediate water formation in the southern Aegean Sea, or Cretan Sea (i.e. Cretan Intermediate Water, CIW) replaced to some point formation in the Rhodes Gyre (i.e. Levantine Intermediate Water, LIW) that was dominating previously.

Overall, the EMT affected the thermohaline and biochemical properties of Mediterranean waters, leading to substantial modifications in the exchanges between the Western and the Eastern basins, and the Adriatic and Aegean seas. However, the precise influence of the EMT on the Western Mediterranean Sea is unknown quantitatively.

7.1.2 Western Mediterranean Sea

Beyond the EMT in the Eastern Basin in the 1980's, abrupt hydrological changes have also been noticed in the Western Mediterranean Sea.. The main alarm clock for those changes was the so-called WMT event in winter-early spring 2004/05 in the Gulf of Lion and the North Catalan seas, which took place again in -early spring 2005/06 when it also reached the Ligurian Sea. Colder and denser new deep waters formed over the continental shelf by cooling and evaporation of the surface layer. In both years, these newly formed dense waters cascaded downslope until reaching their equilibrium depth (Fig. 7.3) (Canals et al., 2006; Palanques et al., 2006; Schroeder et al., 2009). Furthermore, intense open sea convection developed beyond the continental shelf in the open Gulf of Lion. The anomalously high volumes of newly formed deep water generated during intense cascading and convection events induced dramatic changes in the hydrological structure of the basin involving complete destratification of the water column and massive transfer of heat and salt to the deep layers, which where considerably renewed (Fig. 7.3). This caused the mixed depth layer reaching the bottom of the basin and the old resident deep waters were gradually uplifted by several hundreds of meters and replaced by the denser new deep water underneath (Fig. 7.3) (Canals et al., 2006; Schroeder et al., 2009; Martin et al., 2010). The 2004/05 event was the first of a series of similar events that occurred, with varying intensities, in some of the following years, namely in 2009/10, 2011/12 and 2012/13, which led to major changes in the structure of the intermediate and, especially, the deep layers in the Western Basin (Durrieu de Madron et al., 2013; Rumin-Caparrós et al., 2018). Subsequent evidence of reinforced thermohaline variability resulting from such dense water formation events producing large amounts of denser water masses than ever before was reported in the deep Western Basin (Schroeder *et al.*, 2016) (see Fig. 7.4). Such events also led to the transfer of huge amounts of sediment, organic matter and, eventually, pollutants from the coastal and shallow ocean to the deep and, therefore, likely impacted the biotic components of the deep ecosystem (Canals et al., 2006; Palanques et al., 2006; Salvadó et al., 2012b; Salvadó et al., 2017).

Hydrographic preconditioning (heat and salt content and structure of the water column before the onset of convection), and atmospheric forcing (heat, freshwater and buoyancy fluxes) triggered the exceptional formation of deep water. Also the progressive increase of heat and salt content in the intermediate layer, advected from east to west (LIW, related to the EMT) favoured the formation of the new dense water.

The thermohaline anomaly spread throughout the Western Mediterranean Sea, filling its deeper part below 1,500-2,000 m depth, and significantly accelerating the ventilation of the deep layers. Anomalous θ/S distribution (the so called "hook") appeared in the WMDW, resulting from the interaction between deep open sea convection (forming warmer and saltier deep water related to a saltier and warmer LIW) and dense shelf cascading water (colder and fresher), which reached the base-of-slope and bathyal plain mostly through canyons.
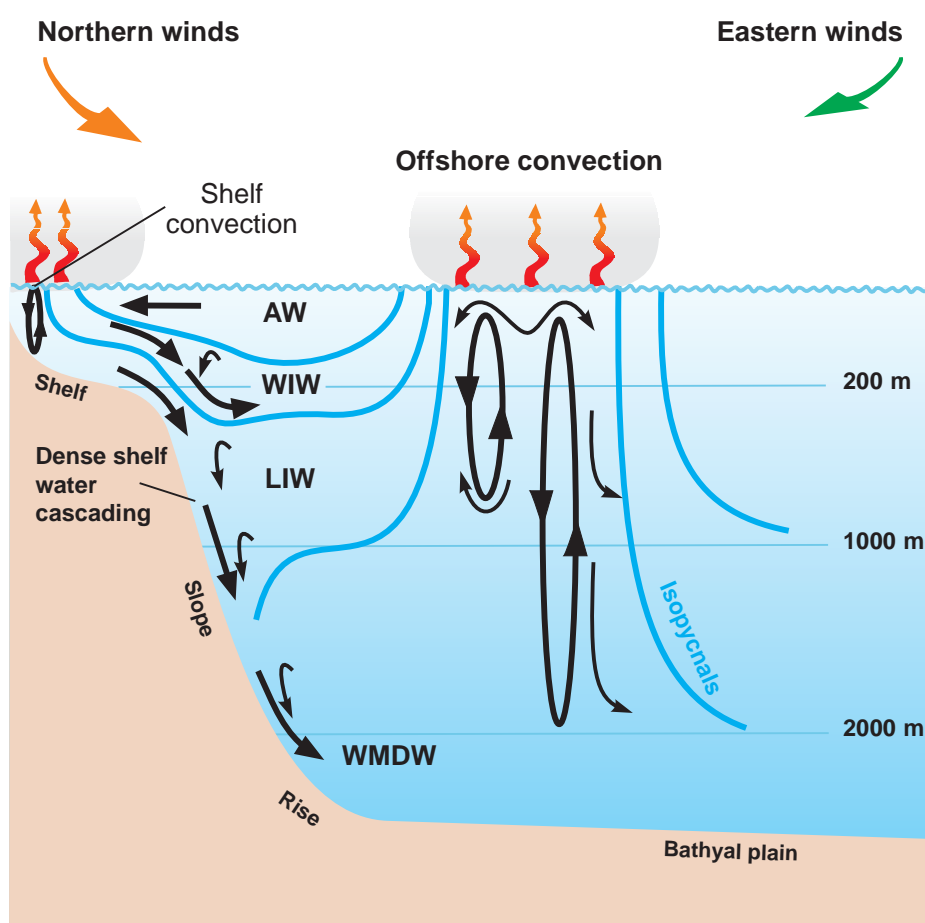
Figure 7.3. Conceptual sketch of the forcings leading to dense shelf water formation and cascading, and to offshore (open sea) convection in the Gulf of Lion and adjacent areas. Cold, dry and persistent northern winds (orange arrow, left upper corner) lead to cooling and evaporation at the sea surface (sinuous reddish arrows atop of the sea surface) resulting in the formation of dense shelf water that cascades down the over continental slope floor (black thick arrows). High intensity open sea convection could mix the entire water column (oval-shaped closed black arrows) where chimney-like structures form. Deformation of the isopycnals resulting from water column destratification is also shown. Thin black arrows indicate entrainment of ambience waters and secondary recirculations. Eastern humid winds leading to severe coastal storms (not discussed in the text) are also indicated (green arrow, right upper corner). AW: Atlantic Water. WIW: Western Intermediate Water. LIW: Levantine Intermediate Water. WMDW: Western Mediterranean Deep Water. Horizontal blue lines indicate reference depths, from Canals (2017).
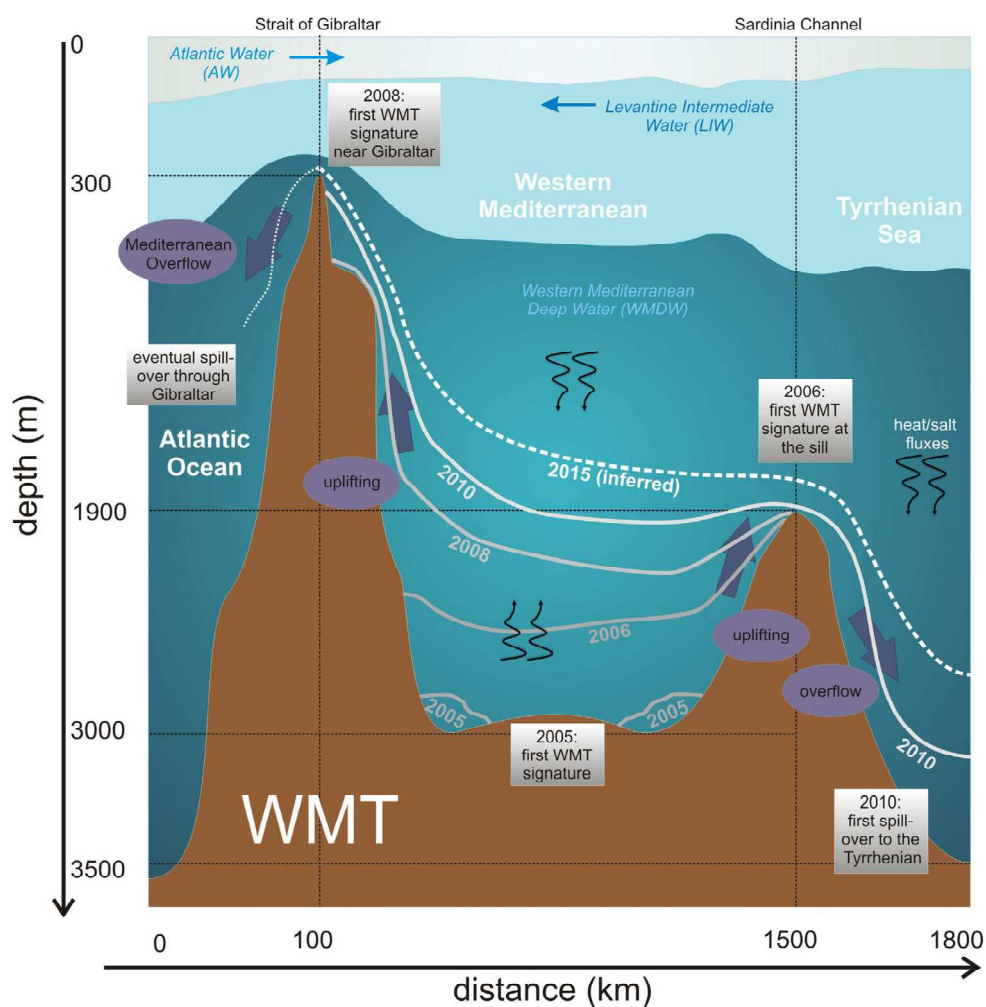
Figure 7.4: Temporal and geographic evolution of the processes associated to the WMT. The lines denoted by the years indicate the upper interface of the new WMDW. For 2015 a mean uplifting of this interface has been inferred from single stations, rather than from a whole transect. The dotted line in the Atlantic is a speculation about an eventual spill-over through Gibraltar, which will happen if the process continues (Schroeder et al., 2016).

Sediment-loaded dense shelf waters cascades have a direct effect on WMDW thermohaline properties and are responsible for the formation of thick and persistent bottom nepheloid layers (BNL) (i.e. layers of water that contains significant amounts of suspended sediment) over the slope, base of slope and basin (Chronis et al., 2000; Durrieu de Madron et al., 2005; Canals et al., 2006). Such layers could extend basin-wide and reach up to 1,5 km in thickness as reported by Puig et al. (2013).

Dense shelf water formation commonly occurs during the late winter-spring planktonic bloom in the Gulf of Lion and adjacent areas. Hence, during cascading events significant quantities of nutrients and organic matter, including fresh phyto- and zooplankton, are transported to intermediate and deep-water layers (Canals et al., 2006; Sanchez-Vidal et al., 2008) subsequently fueling the deep ecosystem (Danovaro et al., 1999; Company et al., 2008).

A comprehensive hydrographic dataset has pointed out a series of heat and salt content anomalies for the 1950-2000 period () in the Eastern and Western basins, and in the entire Mediterranean Sea (Rixen et al., 2005). On the whole, increases of temperature and salinity have involved the entire Mediterranean during the period 1950–2000. Whereas atmospheric forcing is a background driver for those changes, in the Western Basin the shifts seem chiefly related to environmental changes (e.g. decreases in precipitation since the 1940s and reduction of the freshwater inflow) while in the Eastern Basin they are mainly due to the pervasive influence of the EMT (Rixen et al., 2005; Kress et al., 2014).

The progressive warming and salinization of the Mediterranean Sea, which is particularly evident in intermediate and deep layers, is in agreement with the changes in the properties of the water masses resulting from or influenced by convection processes, and also shows noticeably variation according to formation regions and hydrographic water pre-conditioning prior to deep water formation itself (Tanhua et al., 2013).

7.2 Where Is The Knowledge

The Mediterranean Sea has been the subject of an intensive research during the last decades. The multidisciplinary Mediterranean Targeted Projects MTP-I and MTP-II/MATER, 1993-96 and 1996-00 respectively, launched by the Marine Science and Technology (MAST) Programme of the European Commission (EU), constituted highly significant milestones in that research effort. MTP-I combined ten different projects, involving 200 scientists from 70 institutions from around Europe. The aim of MTP-I was to examine the functioning of the Mediterranean Sea in all its aspects, with a strong multidisciplinary approach considering physical, geochemical and biological processes at the same time. MTP-II/MATER represented a step forward as instead of a federation of individual projects, like MTP-I, it was a single project with an integrated approach of the entire Mediterranean Basin, in its physical, sedimentological, chemical and biological aspects (Monaco and Peruzzi, 2002). MTP-II/MATER gathered 58 research groups from 10 EU member states and 3 non-EU states (Maillard et al., 2002) and still is the largest multidisciplinary research project ever supported by the European Commission for the comprehensive study of the Mediterranean Sea.

The historical data collected within these large research projects were made openly available through the MEDAR/MEDATLAS data base (Fichaut et al., 2003). From this platform it was possible to download specific data, mostly temperature, salinity and pressure with less datasets on other hydrographic parameters, such as oxygen, silicate, nitrates or phosphates.

Recently other portals, such as **EMODnet Physics** (http://www.emodnet-physics.eu), make available many hydrographic data from the Mediterranean Sea. EMODnet Physics is an operational service where near real time and historical validated marine data is made interoperable and freely available. It is built in cooperation and coordination with EuroGOOS (European Global Observing System) and specifically **MOONGOOS** for the Mediterranean, and other European marine integrative infrastructures (i.e. **SeaDataNet**, https://www.seadatanet.org/, a network of National Oceanographic Data Centres).

SeaDataNet shows that the two key basic parameters in Physical Oceanography, temperature and salinity, present a rather good coverage in the Western Mediterranean Basin, mainly offshore France and parts off Spain and Italy, while both coverage is insufficient in the Eastern Mediterranean Basin, where many areas have few and sparse data, such as those off Tunisia, Libya, Croatia and Turkey (Fig. 7.6). The main concentration of data in the Eastern part of the Basin is along the Israeli and the Greek coasts (Simoncelli et al., 2015).

The data coverage and density maps of figure 7.6 clearly shows that offshore areas are far less sampled than coastal areas. In addition, even if sampled, the deeper layers may not have been reached.
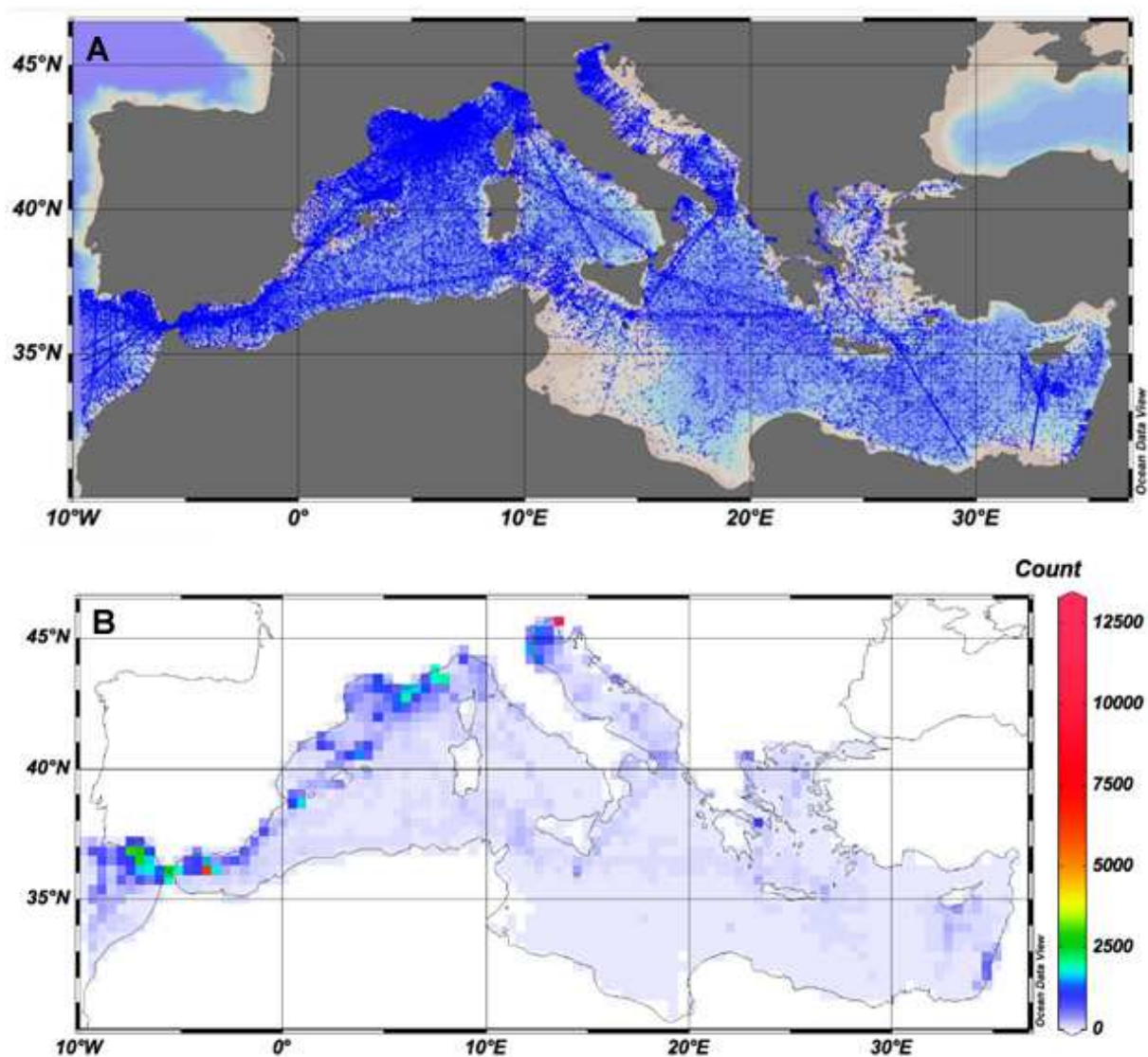
Figure 7.6. Temperature and salinity data collection for the Mediterranean Sea for the time period 1900-2014.

A: Data distribution (coverage) map. B: Data density map - from Simoncelli et al (2015).

7.3 How are hydrographical conditions measured

7.3.1 Methodological sampling aspects

During the late 1990s and the beginning of the twenty first century, several monitoring programs have been implemented in the Mediterranean Sea. Each of these programs is based on a different strategy and methodology (Vargas-Yanez et al., 2017).

The sustained **long-term monitoring** of basic hydrological parameters (temperature and salinity), collected as time series with adequate temporal resolution (i.e. with a sampling interval allowing to resolve all significant timescales) in key places of the Mediterranean Sea (e.g. straits and channels, zones of dense water formation and spreading, deep basins), constitute a priority in the context of global change (Schroeder et al., 2013) The Hydrochanges network is based on the mooring of **CTDs** at key places for the monitoring of the temperature and salinity variability of the water masses within the Mediterranean Sea (Schroeder et al., 2013). Time series collected under the Hydrochanges umbrella have already revealed shofts in the composition of the Mediterranean outflow through the Strait of Gibraltar (Millot, 2009).

Beyond Hydrochanges, a number of **mooring lines** continuously monitor sets of specific parameters including temperature and salinity. These mooring lines are not standardized and are generally maintained by research programs and individual labs and groups. DYFAMED and LION deep-water stations in the Northwestern Mediterranean Sea are good examples of long-term moorings provided valuable series of critical data (de Fommervault *et al.*, 2015; Houpert *et al.*, 2016). These mooring lines, equipped with instruments along the water column, are part of the Mediterranean Ocean Observing System (http://www.moose-network.fr/). Other deep-water moorings at key locations associated to research programs or maintained by individual research labs and groups are, for instance, the long mooring line linked to the ANTARES neutrino telescope (Tamburini et al., 2013), and the ones in Lacaze-Duthiers and Cap de Creus canyons, both at 1000 m depth, all of them in the Gulf of Lion. Long time series provided by these mooring stations have been pivotal in critical findings on the deep dynamics of the Mediterranean Sea during the last years.

The MEDARGO program maintains an array of **profiling floats** in the Mediterranean Sea (Poulain et al., 2007). These data have been very useful in the description of deep water formation (Smith et al., 2008b). Argo floats are autonomous profiling floats that drift at a given parking depth for a given time period. At the end of their drifting time, they dive to 2000 m and collect a profile of temperature and salinity during the upcast. The collected data are sent in real time to a data center after which they return to their parking depth.

In Spain, the RADMED program is devoted to the implementation and maintenance of a monitoring system around the continental shelf and slope, including some deep stations, around the Spanish Mediterranean (Lopez-Jurado et al., 2015). This observing system is based on periodic multidisciplinary cruises covering coastal, continental shelf and slope waters and also some deeper stations (> 2000 m) from the westernmost Alboran Sea to Barcelona in the Catalan Sea, also encompassing the Balearic Islands (Vargas-Yanez et al., 2017). The data set provided by the RADMED project has been merged with historical data from the MEDAR/MEDATLAS database for the calculation of temperature and salinity trends from 1900 to 2015. The analysis of these time series shows that the intermediate and deep layers of the Western Mediterranean Sea have increased their temperature and salinity with an acceleration of the warming and salting trends from 1943 onwards (Vargas-Yanez et al., 2017). Furthermore, Kersting et al. (2016) and Lavin et al. (2017) have recently compiled basic information on monitoring stations, either moored or drifting, in Spanish waters and/or by Spanish institutions and research groups.

In the last decade, the **glider technology** has been quite intensively used mainly in the Northwestern Mediterranean Sea for repeated cross-basin transects. Gliders are steerable autonomous platforms that

sample the ocean along saw-tooth trajectories between the surface and a maximum depth of 1000 m. The very first glider deployments in the Northwestern Mediterranean Sea were carried out in 2006. From 2010 on, gliders have been deployed on a regular basis in the framework of the MOOSE initiative. After MTP-I and MTP-II/MATER an intensive effort of collection of in situ observations has been carried out in the Northwestern Mediterranean Sea thanks to gliders, profiling floats, regular cruises and mooring lines. This integrated observing system enabled a year-to-year monitoring of the Western Intemediate Water (WIW) and new WMDW formation in the Gulf of Lion during four consecutive winters (2010‑2013) (Figs. 7.3 and 7.4) (Bosse et al., 2016).

Those data are used in **numerical models** implemented at regional or local scale to elucidate water mass formation and spreading, and the overall hydrological dynamics of the basin (Bonaldo et al., 2015; Estournel et al., 2016).

7.3.2 Spatial extent and distribution of permanent alteration of hydrographical conditions to the seabed and water column (D7C1) - Secondary

*Hydrographical changes to the seabed and water column shall be assessed as the spatial extent and distribution of permanent alteration of hydrographical conditions (e.g. changes in **wave action, currents, salinity, temperature**) to the seabed and water column, associated in particular with physical loss of the natural seabed.*

Physical loss shall be understood as a permanent change to the seabed which has lasted or is expected to last for a period of two reporting cycles (12 years) or more. Monitoring shall focus on changes associated with infrastructure developments, either on the coast or offshore. This criterion is assessed in relation to total natural extent of all habitats in the assessment area. The extent of the assessment area hydrographically altered shall be in square kilometres (km²).

The main changes detected in the deep Mediterranean Sea are related to the abrupt shift of the deep water formation sites and rates in the Eastern Mediterranean Basin in the late 1980's and early 1990's (Vargas-Yanez et al., 2017). This event, commonly known as the Eastern Mediterranean Transient (EMT), occurred at some moment between 1987 and 1995 and led to a dramatic increase of the temperature and salinity of the new Eastern Mediterranean Deep Water (EMDW), which replaced and uplifted the old EMDW (Roether et al., 2007). The changes observed in the Eastern Mediterranean Sea have been transmitted to the Western Mediterranean Sea as saltier and warmer intermediate waters flowed through the Sicily channel after the EMT (Gasparini et al., 2005; Millot, 2008). The arrival of these saltier intermediate waters and some exceptional atmospheric conditions during winters 2005 and 2006 in the Western Mediterranean Sea would have been responsible for the appearance of a distinct new Western Mediterranean Deep Water (WMDW). This water mass is warmer, saltier and denser than the older WMDW and has occupied the bottom layer of the basin. Subsequent intense events of dense water formation in the Western Basin occurred in 2009/10, 2011/12 and 2012/13.

7.3.3 Spatial extent of each benthic habitats types adversely affected due to permanent alteration of hydrographical conditions (D7C2) - Secondary

Benthic broad habitats types or other habitat types, as used for descriptor 1 and 6, shall be considered. The spatial extent of each benthic habitat type adversely affected (physical and hydrographical characteristics and associated biological communities) due to permanent alteration of hydrographical conditions shall be assessed.

Member States shall establish threshold values for the adverse effects of permanent alterations of hydrographical conditions, through regional or subregional cooperation.

Environmental impact assessment hydrodynamic models, where required, which are validated with ground-truth measurements, or other suitable sources of information, shall be used to assess the extent of effects from each infrastructure development. This criterion is assessed in relation to total natural extent of each benthic habitat type assessed. The extent of each habitat type adversely affected shall be in square kilometres (km²) or as a proportion (percentage) of the total natural extent of the habitat in the assessment area.

The availability of semi-labile Dissolved Organic Carbon (DOC) at depth represents an important input of energy for the deep-water microbial loop. The export of DOC during deep water formation plays an important role in the carbon cycle by linking Mediterranean atmospheric and surface dynamics with deep-water microbial ecosystems, with possible consequences on larger organisms feeding on the microbial communities. Furthermore, changes in biological activity at the surface producing large settling particles may have an impact on the organisms that rely on vertical fluxes to subsist.

The Mediterranean Basin displays the largest concentrations of anthropogenic CO_2 in any marine or oceanic basin, which could be tentatively attributed to the transfer efficiency to the basin interior of the above mentioned dense shelf water cascading and open sea convection processes (Fig. 7.3) (Hassoun et al., 2015; Canals, 2017). It is to be expected that such an injection of anthropogenic CO_2 results in an increased acidification of Mediterranean intermediate and deep water masses, with so far unknown effects on sensitive groups of organisms.

Because of their relatively high abundance, mesopelagic fishes play a relevant role in the food web (Olivar et al., 1998; Somarakis et al., 2002; Cuttitta et al., 2004). In addition, they are sensitive to environmental changes and are influenced by circulation patterns and hydrographic features. However, there are very few studies about them in the Mediterranean Sea, except in the Ligurian Sea. Because fish larvae concentrate in the epipelagic layer (Sabates and Maso, 1990; Sabates and Olivar, 1996) and are highly sensitive to changes in their environment, studying their assemblages can be a proxy for understanding the importance of climate change on the offshore and deep communities. In addition, the monitoring of larval diet and survival will indicate how mesopelagic fishes recruitment faces environmental changes that occur in the epipelagic zone. Furthermore, changes in currents and salinity can influence the spreading pattern of larvae and breeding and spawning areas.

7.4 Knowledge On Threshold

No threshold can be addressed or this descriptor.

7.5 CONCLUSIONS

One obvious difficulty is to discriminate between hydrographic changes directly derived from large-scale human activities and natural variability eventually linked to climate change (Touratier and Goyet, 2011).

This descriptor is meant to address mainly infrastructures or activities that can produce significant impacts on hydrographical conditions at a large scale. At present, it does not look as if such changes have an effect on deep-sea hydrographical characteristics. Only hypothetical future actions, such as the injection of CO_2 in the deep water layers or damming of Gibraltar or other straits would have a significant permanent impact (Gower, 2015). However, exactly because of their impacts, this kind of actions seems quite unrealistic, at least for the years to come. Thus for this descriptor in the deep Mediterranean Sea, shifts in temperature and salinity or increase of episodic events such as cascading, should be monitored as priorities (Zampoukas et al., 2014a).

7.5.1 Gaps

The coverage of observations is highly inhomogeneous, with large data voids in both space and time. Although there is a view that the "most interesting" areas and processes are rather well covered, such a feeling could prove to be wrong. There is no reason to think that most phenomena that are worth of study are located in the northern, European regions of the Mediterranean Sea. In order to partially overcome the unevenness in data distribution, density (Fig. 7.6) and likely quality, gridded products are generated through the objective analysis of the available observations. Products generated from databases for instance are very useful and hence widely used to analyse variability at different scales. However, even if gridded products have a homogeneous spatio-temporal distribution, the problems derived from the paucity and inhomogeneities of observations are still present. Some discrepancies exist between different works that could be a consequence of the scarcity of data and their irregular distribution making results very sensitive to data analysis methodology (Vargas-Yanez et al., 2009; Vargas-Yanez et al., 2012). Differences between the results obtained when analyzing temperature and salinity time series from different databases and monitoring programs have been evidenced by Jorda and Gomis (2013) and Lasses et al. (2015).

7.5.2 Recommendations

Even though climate change is considered to be part of the prevailing background environmental conditions and therefore not explicitly addressed through the MSFD, for the interpretation of monitoring data, the effects of climate change need to be taken into account. For this reason the existence of an adequate monitoring programme able to describe these background large-scale changes together with long time series dataset is an implicit requirement for this descriptor and for the MSFD as a whole (Zampoukas et al., 2014a). In that respect an enlarged coordination and integration scheme on deep water observing stations mostly consisting of instrumented moorings beyond MOOSE would be highly beneficial in the short, mid and long term. A widened, strengthened MOOSE could eventually make it. It's a kind of initiative for which the EC umbrella would be particularly well suited.

According to the recommendation provided by the MSFD Expert Network on MSFD descriptor 7 (Gonzalez et al., 2015), the monitoring of this descriptor should provide, on one hand, background information at different spatial (from sub-region to local) and temporal scales on variations of hydrographical conditions, which might not be connected (at least not directly) to human activities. On

the other hand specific monitoring for D7, cross-cutting with D6, should assess the extent of the area affected by alterations and impacts with a focus on the list of possible areas where alterations could be expected due to activities (mostly extraction) or new developments of deep-sea infrastructures.

Also important is that the monitoring of the effects of hydrographical changes should not aim primarily at field based measurements in the affected area, but concentrate on modelling of the changes in currents, waves and bottom shear stress due to human activities in the area, using appropriately calibrated models, validated with in situ datasets. This will make it possible to determine the extent of any parameter changes including how large the change will be in a certain area. From this starting point the effect on marine ecosystems can be determined. Field measurements will be necessary in areas where the changes are large enough to have significant effects on the marine ecosystem at which point ground truthing will be considered appropriate. In such a situation on-going monitoring of changes in benthic or pelagic fauna could be used to indicate any effects of permanent hydrographical alterations. Even when there is no clear indication that an activity will cause an important hydrographical alteration, some minimum field measurements will be needed to confirm the prediction of the models (Zampoukas et al., 2014a).

More and better distributed, both in space and time, long-term data series are needed to carefully assess warming rates, especially in the Eastern Basin where data are currently lacking, as well as to clarify the reasons for such a warming. This increase in temperature is highly susceptible of having severe impacts on the role of the Mediterranean Sea in the global carbon cycle. Moreover, changes in species distributions and modifications of Mediterranean habitats are presently occurring and will undoubtedly occur in the next decades with or without permanent hydrographic changes (Durrieu de Madron et al., 2011). Disentangling cause and effects, and identifying feedbacks amongst different stressors is a crucial step.

8   Descriptor 8 : Concentrations of contaminants

Descriptor 8 of the MSFD requires that "*concentrations of contaminants are at levels not giving rise to pollution effects*". Relevant pressure to consider for the MSFD is the input of other substances (e.g. synthetic substances, non-synthetic substances, radionuclides).

The Mediterranean is surrounded by continents with intense human activities, which constitute sources of chemical contamination and may cause degradation and serious damage for the coastal and marine zones. These coastal pollution inputs may be transferred to the open deep ocean by several processes including the recently discovered dense shelf water cascading (DSWC) through submarine canyons. Atmospheric deposition is also a relevant source of pollutants for thre open deep waters. Bioaccumulation and trophic transfer increase the concentrations of many pollutants of low water solubility.

Anthropogenic inputs of chemicals are one of the main threats currently affecting the global ocean (Halpern et al., 2008). Once introduced into the sea, contaminants can be redistributed or transported throughout the environment by human activity and natural physical and biochemical processes. Contaminants remain in the water and especially in the sediment, from which they can be resuspended. Many substances can also accumulate in biota and thus in the food web.

The oligotrophic nature of the open Mediterranean (and its possible changes) makes it more sensitive to the bioaccumulation processes because the "biodilution" of contaminants by organic carbon in the water column is reduced. In addition, the rapid turnover at the base of the food webs may also be a key factor for contaminant biomagnification.

8.1   State of the Art

Like many seas in the world, the Mediterranean is affected by inputs of various chemical contaminants, including trace elements, artificial radionuclides and organic substances. Our knowledge of concentration levels, fluxes, behavior within the water and sediment columns or toxicological impacts for the ecosystem is very different, depending on the group of contaminants. Some, such as cesium (^{137}Cs) or mercury (Hg), have been studied in different research projects and locations, but for most of them there are relatively few studies, from which it is difficult to infer the extent and consequences of the contamination (Durrieu de Madron et al., 2011). Furthermore, the deep sea is the environment from which pollutant information is lacking the most.

8.1.1   Trace elements

Atmospheric inputs constitute one major driver of the biogeochemical cycling of trace elements (TEs) in the Mediterranean Sea (Migon et al., 2002), which are characterized by a European background signature (natural and anthropogenic) upon which Saharan dusts are superimposed (Guerzoni et al., 1999). Both the magnitude and the mineralogical composition of atmospheric dust inputs indicate that eolian deposition may be an important (50%) or sometimes dominant (80%) contribution to sediments in the offshore waters of the entire Mediterranean basin (Guerzoni et al., 1999).

In water

The dissolved TE distributions in the Mediterranean Sea are atypical compared to other areas of the world ocean, as the distributions of metals such as Cd, Cu and Ni (as well as Cr) are dominated by lateral advection and vertical mixing rather than by biogeochemical cycling (Morley et al., 1997).

The case of mercury (Hg) is very particular since the Mediterranean waters export this metal to the North Atlantic Ocean by means of the Western Mediterranean Deep Water (WMDW) that is advected westward through the Gibraltar Strait (Cossa et al., 2017). Thus, the average concentrations of total Hg in unfiltered water near the Gulf of Lion are 1.11 ± 0.06 pmol L^{-1} and in the deep profiles near the foot of the continental slope are 1.29 ± 0.17 pmol L^{-1}. These concentrations are much higher than those in the Atlantic waters entering through the Gibraltar Straight, 0.2-0.3 pmol L^{-1} (Knoery et al., 2015). Indeed, even if the atmospheric Hg deposition onto the Mediterranean Sea is substantial, the low Hg concentrations found in upper water layers where removal processes (biological uptake and photo-induced reduction) are active (Cossa and Coquery, 2005) contrast with the deepest water layers. Atmospheric deposition, deep-sediment resuspension and shelf or mobilization process at the top of the benthic boundary are probable sources of Hg enrichment but more research is needed to elucidate what is the origin of the Hg excess in the Mediterranean Sea. Few studies have reported data on mercury in deep waters (Cossa et al., 1997; Horvat et al., 2003; Kotnik et al., 2007; Cinnirella et al., 2013; Kotnik et al., 2014; Kotnik et al., 2015; Kotnik et al., 2017) (Fig. 8.1).

Tributyltin (TBT) contamination levels in deep waters far from pollutant sources have been studied in the open sea in the northwestern part of the Mediterranean Sea (between Corsica and France) by Michel and Averty (1999). The presence of TBT in deep waters was attributed to the circulation of water masses during winter.

In sediment

Trace element concentrations and distributions in the sediments of the Mediterranean Sea have been reported in many papers on the contamination of coastal areas, but there are very few studies on deep-sea areas. Some TEs are involved in organic matter synthesis through deep remineralization (Durrieu de Madron et al., 2011).

The Hg concentrations in some coastal areas have been studied but data on Hg concentrations in Mediterranean deep-sea cores are still lacking. Mercury transfer and transformation in the abyssal nepheloid layer need to be further explored. Two studies reported on few station in the deep bottoms (Cossa and Coquery, 2005; Ogrinc et al., 2007), one on the Var canyon (Heimbürger et al., 2012) and one in the Adriatic Sea (Kotnik et al., 2015).

In organisms

The concentrations of Hg in Mediterranean organisms (methylmercury being the bioamplified and toxic form of this metal) are higher than in the same species of other oceans for a comparable seawater concentration. This difference is the most identifiable feature of the "Mediterranean mercury anomaly" (Cossa and Coquery, 2005; Cossa et al., 2012). The contrast is even higher for deep-sea fish, as the Hg concentration in their tissues is commonly high (Cresson et al., 2014; Chouvelon et al., 2018). Few other studies have focused on methylmercury in deep-sea organisms (Hornung et al., 1993; Kress et al., 1998; Koenig et al., 2013c; Storelli and Barone, 2013; Storelli et al., 2013; Cresson et al., 2014; Llull et al., 2017; Chouvelon et al., 2018) (see descriptor 9).

Triphenyltin (including TBT) is the predominant organotin component in deep-sea fish showing transport capacity far from point sources associated to particulate matter and its higher resistance to degradation (Borghi and Porte, 2002).
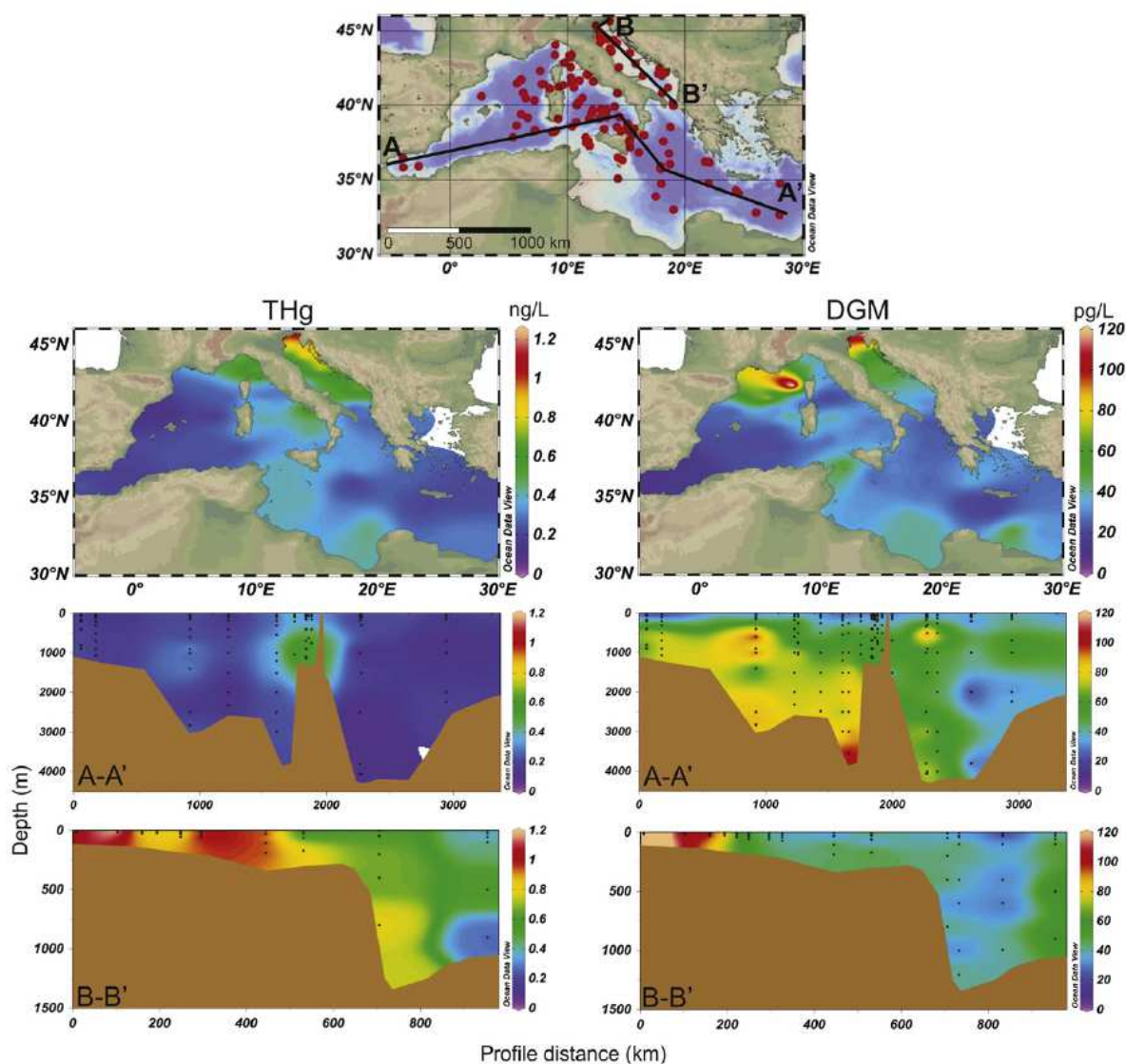
Fig. 8.1: Spatial and vertical distribution of Total Hg (THg) and Dissolved Gas Mercury (DGM) in the Mediterranean Sea (A-A' profile) and in the Adtriatic Sea (B-B' pofile). All performed cruises were included. Surface layer was sampled at depth pf approx. 2 m. Red and black dots represent sampling locations. DGM concentrations are in pg/L. Map from Kotnik et al. (2017).

8.1.2 Radionuclides

A variety of practices and activities introduced radionuclides into the marine environment, e.g. military activities, nuclear fuel cycle operations and the use of radioisotopes by research centers, hospitals and industry. The main sources of man-made radionuclides into the Mediterranean Sea are the atmospheric fallout arising from the nuclear industry and both past above ground nuclear bomb testing and the Chernobyl accident, the wash-out of the river catchment basins contaminated by this fallout (Durrieu de Madron et al., 2011).

<u>In water</u>

The isotope ^{137}Cs can be considered as a conservative parameter in marine waters because its distribution is mainly controlled by physical processes such as water mass advection and convection. In contrast, the distribution of plutonium isotopes is also governed by its chemical speciation and its strong association with particles, which causes it to be more effectively removed from the water column.

Both nuclides have decreased over time in Mediterranean surface waters (Papucci et al., 1996; Leon Vintro et al., 1999; Fowler et al., 2000). In the case of ^{137}Cs, it tends to increase with time in deep waters due to its conservative behavior. Papucci et al. (1996) thus observed an increase below a depth of 1000 m from an average of 1 mBq l_1 from 1970–1982 to a mean value of 2 mBq l_1 in 1986–1992 in the western basin. For $^{239+340}$Pu, Fowler et al. (2000) reported a 62% decrease in concentrations in surface waters of the northwestern Mediterranean Sea between 1990 and 1999. Residence time estimates for plutonium in the western Mediterranean Sea range from 2.5 year in the Lacaze-Duthiers canyon in the Gulf of Lion to 15–30 year in the open water (Fowler et al., 1990; Papucci et al., 1996; Fowler et al., 2000). In the case of the more particle-reactive transuranic nuclide, ^{241}A ^{241}Am, it has a muchlower residence time (5–10 year) in open Mediterranean surface waters, which is likely due to its greater binding affinity than plutonium to aluminosilicate particles that frequently enter these waters via Saharan dust events (Fowler et al., 2000).

A basin-wide distribution of ^{236}U/^{238}U atom ratios(Uranium) and ^{129}I (Iode) has just been produced by Castrillejo et al. (2017). The results show that radionuclide-poor Atlantic Water is entering at the surface through the Strait of Gibraltar whereas comparably radionuclideenriched Levantine Intermediate Water is sinking in the Eastern Basin and flowing westward at intermediate depths (Fig. 8.2). Low radionuclide levels were found in the oldest water masses at about 1000-2000 m depth in the Eastern Basin (Castrillejo et al., 2017). The inventories of ^{236}U and ^{129}I cannot be explained only by global fallout from atmospheric nuclear bomb testings carried out in the 1950s and 1960s. We estimate that the liquid input of ^{236}U from the nuclear reprocessing facility of Marcoule (France), via the Rhône river, was of the same order of magnitude than the contribution from global fallout, whereas liquid and gaseous releases of 129I from Marcoule were up to two orders of magnitude higher than global fallout. For both radionuclides, the contribution from the Chernobyl accident is found to be minor (Castrillejo et al., 2017).

<u>In sediment</u>

Concentrations of ^{137}Cs and $^{239+340}$Pu have been measured in the sediments of various parts of the Mediterranean Sea, including deep basins, and they have been found in the uppermost centimeters of the sediment (Garcia-Orellana et al., 2009). Their concentrations and inventories in coastal environments are generally higher because, in specific places, the contribution of land-based sources can exceed atmospheric inputs (Durrieu de Madron et al., 2011).

In organisms

Studies on anthropogenic radionuclides in marine organisms in the Mediterranean Sea also underscore that the radionuclide levels are constantly decreasing due to modifications of the inputs.

Very little work has been done to examine the trophic transfers of man-made radionuclides. Recent studies conducted on the ^{137}Cs content in hake (*Merluccius merluccius*) and their prey in the Gulf of Lion have revealed that the ^{137}Cs content shows a clear trend for an increase with hake size (Harmelin-Vivien et al., 2012). The content of ^{137}Cs in hake and their prey also shows a tendency to increase with d15N, which can be considered as a proxy for trophic level, and the observed bioamplification of ^{137}Cs between prey and hake remains less than a factor of 5.
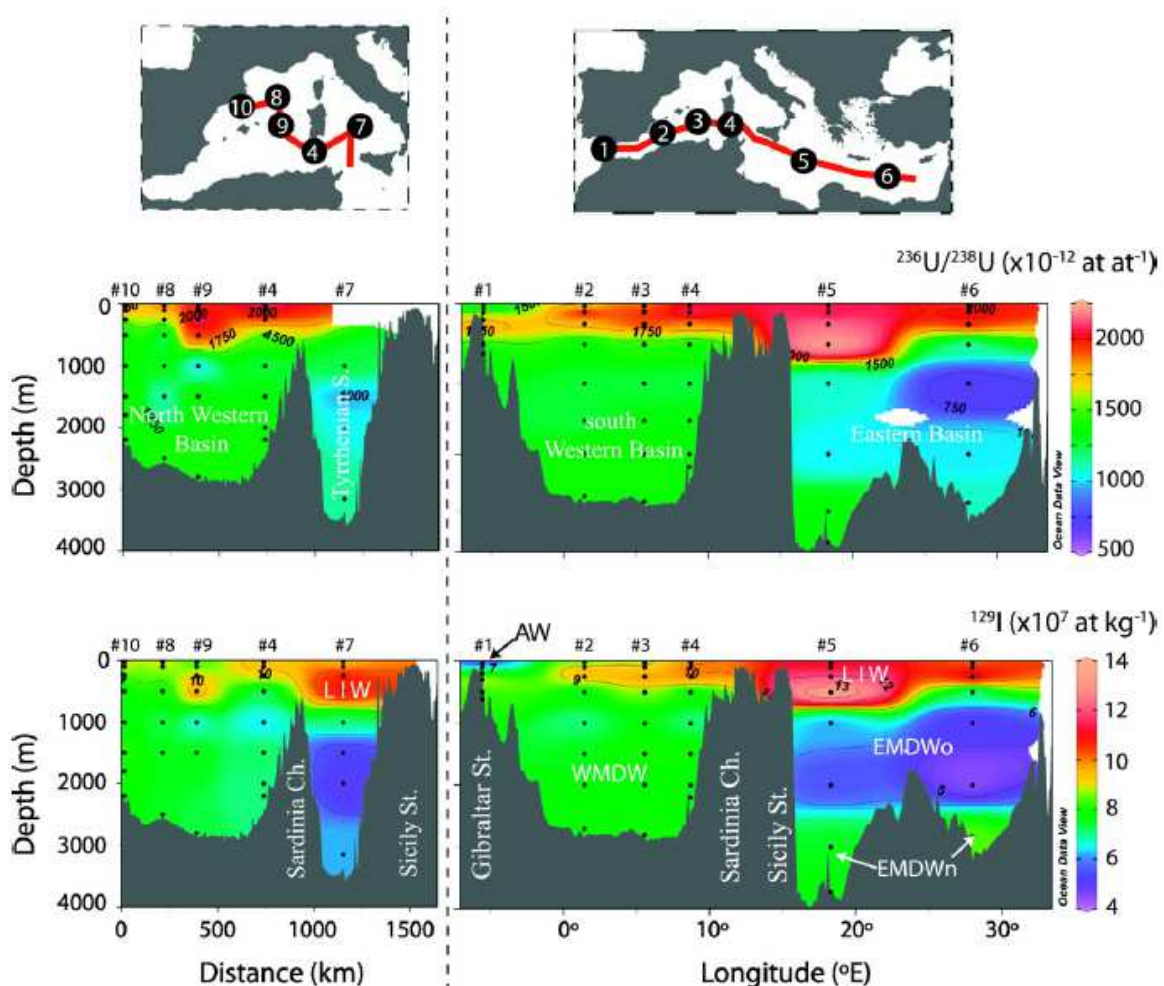
Fig. 8.2. Depth distribution of the $^{236}U/^{238}U$ atom ratios (top panels) and the ^{129}I concentrations (bottom panels) along two sections of the Mediterranean Sea: 1) distance section crossing the northern Alguero-Balear region and the Tyrrhenian Sea in theWMS (left), and 2) longitudinal section crossing the Southwestern and Eastern Basins, fromthe Strait of Gibraltar to the Levantine Basin (right). Station numbers (#) are indicated. Main water masses are represented in bottom panels: Atlantic water (AW), Levantine Intermediate Water (LIW), Eastern Mediterranean DeepWater (EMDW) andWestern Mediterranean DeepWater (WMDW). n: new; o: old. Map from Castrillejo, et al. (2017).

8.1.3   Organic contaminants

Organochlorine compounds (OCs), such as polychlorobiphenyls (PCBs) and chlorinated pesticides, constitute a group of persistent organic pollutants of worldwide concern due to their toxic effects. Their high lipophilicity, hydrophobicity, chemical stability and resistance to biological degradation have led to their accumulation in biological tissues and biomagnification through the food chain.

Environmental contamination by PCBs was recognised more than forty years ago (Jensen et al., 1969). Since then, numerous studies have described the presence of PCBs and organochlorine pesticides in nearly all ecosystems and humans from all countries (Kalmaz and Kalmaz, 1979). This widespread occurrence is observed despite the discontinuation in the use of these compounds in most world areas as consequence of national or international banning regulations, e.g. the Stockholm Convention. Their presence in nearly all ecosystems is due to their high chemical stability and properties such as semi-volatility, intermediate air-water distribution coefficients (log Kaw between -2 and 0), and high hydrophobicity (octanol-water distribution coefficients; log Kow > 6.0), which gives to these compounds capacity for long-range transport and accumulation in organisms and ecosystems, even those located far away from the pollution sites (Iwata et al., 1994). Other compounds belonging to this group are more water soluble (logKaw between -2 and -4) and have lower bioaccumulation potential.

OCs enter into the marine environment by effluent discharges, atmospheric deposition, runoff and other means (Iwata et al., 1993). Once in the water column association to particulate matter, transport and settling play an important role in their transfer from surface to deep waters and sediments (Buesseler, 1998). Particle settling is also favored by biological processes involving incorporation into the food web, organism migration, vertical mixing, formation of large particles such as fecal pellets, organic matter aggregation, and others. Besides these mechanisms, other hydrodynamic processes induce lateral (cross-slope) transport from continental shelves to the adjoining environments (Heussner et al., 2006; Martin et al., 2006; Zuñiga et al., 2009). Storms, water advection, internal waves, sediment instability, current intensification, dense shelf water cascading and deep water currents (Pont et al., 2002; Canals et al., 2006; Sanchez-Vidal et al., 2008) may potentially involve significant postdepositional transport after discharge from continental water sources.

Polycyclic aromatic hydrocarbons (PAHs) are pollutants of priority concern due to their toxicity and their continuous release into the environment as consequence of human activities (Mesquita et al., 2016), e.g. forest fires, petroleum production and fossil fuel combustion (Broman et al., 1988; Lipiatou et al., 1993; Bouloubassi et al., 2006). Atmospheric deposition and/or sorption on biotic and abiotic seawater particles are two major processes for PAH incorporation into deep marine environments in open sea areas (Ko et al., 2003; Lin et al., 2016).

In water

Reviews on organochlorine and organobromine compounds (Fowler et al., 1990; Dachs et al., 1996; Tolosa et al., 1997; Albaigés, 2005; Salvadó et al., 2012a; Salvadó et al., 2012b) and on PAHs (Lipiatou et al., 1993; Dachs et al., 1996; Tolosa et al., 1996; Lipiatou et al., 1997; Bouloubassi et al., 2006; Salvadó et al., 2017) in the Mediterranean Sea have been published. The understanding on the fates and sinks of organic contaminants in the Mediterranean Sea is still hindered by the lack of temporal surveys of their concentrations in surface and deep particles.

Recently, it has been observed that besides the regular particle sedimentation processes, the DSWC pulses have capacity of transport of organochlorinated compounds or PAHs over several tens of kilometers in offshore deep waters in a few days, peak fortnightly-averaged settling fluxes of 960 ng·m^{-2}·d^{-1}, 630 ng·m^{-2}·d^{-1}, 340 ng·m^{-2}·d^{-1} and 180 ng·m^{-2}·d^{-1} for PCBs, DDTs, CBzs and lindane, respectively, have been measured (Salvadó et al., 2012b; Salvadó et al., 2017) (Fig. 8.3).

The overall significance of the enhanced flux deposition due to DSWC episodes can be assessed by calculation of the inventories by integration of the average fluxes during the time intervals in which DSWC and common sedimentation were predominant. The inventories of organochlorine compounds and PAHs show that deposition during DSWC is more important than during common sedimentation (Salvadó et al., 2012b; Salvadó et al., 2017).
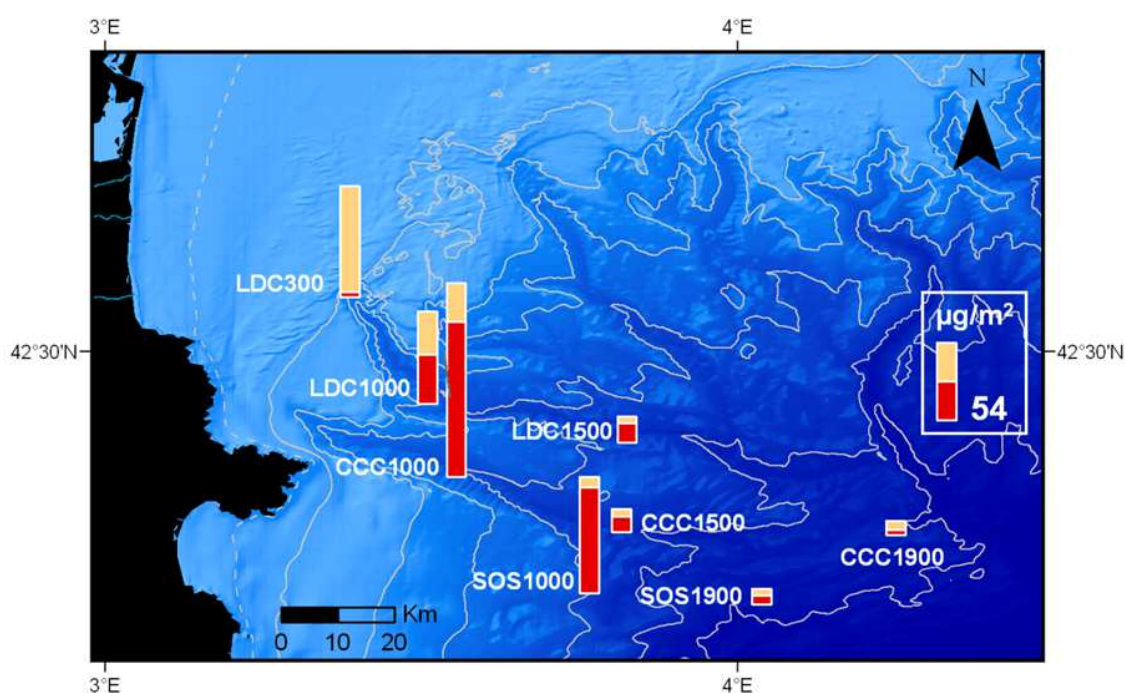
Figure. 8.3. Inventories (µg·m^{-2}) of settling organochlorine compounds (sum of PCBs, DDTs, CBzs and lindane) during DSWC (red) and during common sedimentation (in orange). The acronyms refer to Cap de Creus (CCC) and Lacaze-Duthiers (LDC) canyons and Southern Open Slope (SOS) sites. Numbers besides the acronyms refer to water column depth (Salvadó et al., 2012b).

In sediment

The contents of organochlorine compounds and PAHs in the Mediterranean Sea have been the subject of many studies, most of them dealing with coastal regions, especially in France, Spain and Italy. According to Gomez-Gutierrez et al. (2007), who present a review of sediment contamination, the chemical contamination by POPs in the Mediterranean area is associated with urban/industrial activity and river discharges and particularly affects harbors and coastal lagoons. The northern coast is the main area of concern for POP pollution. A general decline in concentrations has been observed over time, more evident for DDTs than for PCBs. Hydrophobic organic compounds, such as PAHs and PCBs, bind strongly to sediments. They can thus serve as a long-term source of contaminants long after the original source has been removed.

All large-scale surveys have demonstrated that the northern Mediterranean is subject to much higher anthropogenic inputs; therefore, much higher PCB levels are present in these sediments (Albaigés, 2005). Few studies deal with sediments in deep oceanic environments (Elder et al., 1976; Arnoux, 1981; Villeneuve, 1981; Arnoux, 1983; Sanchez-Pardo and Rovira, 1985; Burns and Villeneuve, 1987; RNO, 1987; Abd‑Allah, 1992; Tolosa et al., 1995; Salvadó et al., 2013) (Fig. 8.4).
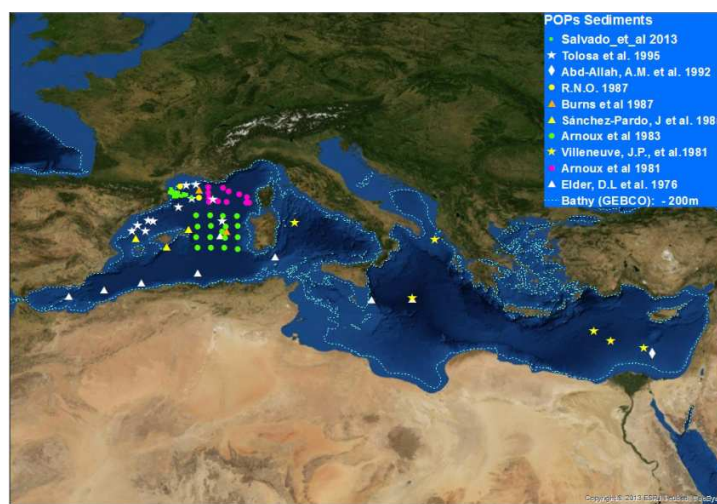
Figure 8.4. Deep-sea sediment sampling stations from the Mediterranean Sea dedicated to PCB, DDT and HCB analyses. Compiled and updated by J.O. Grimalt and J.F. Lopez from Gomez-Gutierrez et al. (2007).

Concerning PAHs, coastal areas, and continental shelves and slopes are dominated by petrogenic PAHs, whereas the deep basins of the northwestern Mediterranean Sea are characterized by high amounts of pyrogenic PAHs. Furthermore, the similar distributions of PAHS in deep-sea sediments account for the prevailing dominance of atmospheric inputs (Tolosa et al., 1996). In the Gulf of Lion, off-shelf export of PAH through submarine canyons toward the slope and open sea has been shown to be significant (ca. 21% of the total input) (Bouloubassi et al., 2012). Two other studies reported on PAH in deep sediments around Ustica island (off Sicily) (Berto et al., 2009), and in the Sicily channel (Mzoughi and Chouba, 2011) (Fig. 8.5).

Figure 8.5: Deep-sea sediment sampling stations from the Mediterranean Sea dedicated to HAP analyses. Compiled by J.O. Grimalt and J.F. Lopez

<u>In organisms</u>

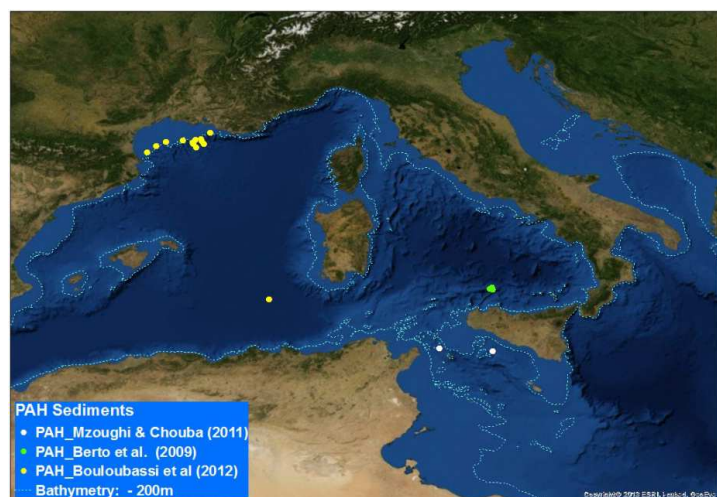Studies of organic chemical contaminants in marine biota may involve different species. Within the limited number of available studies on deep-water environments fish have generally been the organisms of choice. Muscle and liver have been investigated at higher extent. Both types of fish samples provide complementary information. The results from one and the other tissue cannot be evaluated under the same critreria. See descriptor 9 for a more complete review.

Studies of organic pollutants in fish muscle have been developed on the most abundant megafaunal species. In 2008-2009, sample collection in the NW Mediterranean encompassed three fish species belonging to different phylogenetic families, namely *Alepocephalus rostratus* (Alepocephalidae), *Coelorinchus mediterraneus* (Macrouridae) and *Lepidion lepidion* (Moridae), and the red-shrimp *Aristeus antennatus* (Koenig et al., 2013b). Previous studies on organic contaminants in muscle of deep-sea fish were developed in 1996 and involved *Mora moro* (Solé et al., 2001). Other studies have been conducted on organisms from the Gulf of Lion: three fish and two sharks (*Helicolenus dactylopterus*, *Phycis blennoides*, *Lepidorhombus boscii, Scyliorhinus canicula* and *Galeus melastomus*) (Cresson et al., 2016).

Liver samples studied in deep-sea fish encompassed *Chimaera monstrosa*, *Raje* spp (Storelli et al., 2004b), *Lophius budegassa* (Storelli et al., 2004a), *Trachyrincus trachyrincus* (Storelli et al., 2009), *Coelorhinchus caelorhincus* (Storelli et al., 2007; Storelli et al., 2009), *Nezumia sclerorhynchus* (Storelli et al., 2007) and *Mora moro* (Solé et al., 2001).

8.2   Where is the knowledge

The Barcelona Convention for the Protection of the Mediterranean Sea, including the Mediterranean Action Plan (MAP) and the Mediterranean Marine Pollution Monitoring and Research Program (MED POL), encouraged the implementation of monitoring programs for evaluating the health status of this water body. Recently, United Nations Environment Program (UNEP) produced a regionally based assessment of sources, environmental levels, transport pathways and effects of persistent toxic substances in the environment, considering the Mediterranean Sea as one of the regions for the study (UNEP, 2002, 2003). In this renewed political context, the monitoring of mercury, cadmium, PCBs, DDTs, HCB, PBDEs, perfluoroacids and PAHs in the deep-sea environment of the Mediterranean basin remains of crucial importance.

These compounds have sufficient chemical stability to reach the deep-sea environments during transport, e.g. settling, DSWC, and are introduced into the environment in large amounts which results in significant accumulation in these environments according to the studies available. The physical-chemical properties of all of them except perfluoroacids enhance their adsorption to water particles and bioaccumulation into marine organisms. Thus, these compounds have been found in deep water fish and seafood. However, perfluoroacids have also been found in deep water fish, which is consistent with their widespread environmental occurrence.

Data on the concentrations of these pollutants in the Mediterranean environments will be useful not only to fill knowledge gaps on the state of chemical contamination, but also to produce baseline data for taking decisions regarding future issues of environmental conservation.

8.3   How are contaminants measured

8.3.1   The concentration of contaminants in case of <u>chronic pollution</u> (D8C1) - Primary

For each contaminant, the concentration shall be expressed in the matrix used (water, sediment, biota), whether the threshold values set have been achieved, and the proportion of contaminants assessed which have achieved the threshold values, including indicating separately substances behaving like ubiquitous persistent, bioaccumulative and toxic substances (uPBTs), as referred to in Article 8a(1)(a) of Directive 2008/105/EC.

<u>Contaminants</u>

One pollutant of high significance in this context is mercury (Hg). The concentrations of this metal in Mediterranean organisms are higher than in the same species of other oceans, despite the rather similar Hg concentrations in waters of the Mediterranean Sea and other marine ecosystems.

Organochlorine compounds such as polychlorobiphenyls (PCBs) and chlorinated pesticides, constitute a group of persistent organic pollutants of worldwide concern due to their toxic effects. This widespread occurrence is observed despite the discontinuation in the use of these compounds in most world areas as consequence of national or international ban regulations, e.g. the Stockholm Convention.

Polybromodiphenyl ethers are new compounds which accumulate in the deep sea because of their hydrophobicity and chemical stability.

Perfluorinated acids should also be investigated in view of their widespread occurrence.

PAHs are pollutants of priority concern due to their toxicity and continuous release into the environment as consequence of human activities, e.g. forest fires, petroleum production and fossil fuel combustion. Atmospheric deposition and/or sorption on biotic and abiotic particles in seawater are two major processes for PAH incorporation into deep marine environments in open sea areas.

<u>Sites</u>

The information on the concentrations of the different pollutant classes in the deep Mediterranean Sea is very limited. Most studies refer to the NW area and even in this case the amount of information is too short to identify which are the main processes determining the transport and accumulation of diverse pollutant classes into these deep environments and what are the deleterious toxic effects in organisms. In this area, the role of DSWC has been identified. Full understanding of the transport dynamics of organic pollutants and metals in this area would provide reference information for other Mediterranean zones.

The zones of sea water exchange between major water bodies, e.g. Gibraltar Straight, Sicily Channel and mouth of the Adriatic Sea, should also be considered for a good understanding of the dynamics of pollutants in the Mediterranean.

Another important zone is the Mediterranean region under the influence of the Nile discharges.

The Aegean and Adriatic Seas are also important areas with specific oceanographic characteristics to be considered.

<u>Monitoring times and frequency</u>

Collection of sediment cores without lose of the top layer, e.g. with multicorer devices, would be very important if these cores are dated for reconstruction of the temporal trends during the industrial revolution.

Deployment of sediment traps and collection of monthly samples, or even forthnigthly samples (better), would also be very adequate.

Sample collection of water at different depths and analysis of the dissolved and particulate matter would also be important. These samplings could be performed on a seasonal basis taking into account the sampling difficulties.

8.3.2 The health of species and the condition of habitats adversely affected in case of <u>chronic pollution</u> (D8C2) - Secondary

Member States shall establish a list of species and habitats which are at risk from contaminants, and relevant tissues to be assessed, and habitats, through regional or subregional cooperation. Adverse effects on the health of species and the condition of habitats (such as their species composition and relative abundance at locations of chronic pollution) due to contaminants including cumulative and synergetic effects (and their threshold) shall be established.

Abundance of population or estimate of the extent of habitat adversely affected by chronic pollution shall be assessed. The use of criterion D8C2 shall be agreed at regional or subregional level. The outcomes of the assessment of criterion D8C2 shall contribute to assessments under descriptors 1 and 6, where appropriate.

Within the limited number of available studies on deep-water environments fish have generally been shown to be useful sentinel organisms for the pollution levels. Muscle and liver have been investigated at higher extent. They provide complementary information.

Specific attention should be paid to the most abundant megafaunal species, e.g. *Alepocephalus rostratus* (Alepocephalidae), *Coelorinchus mediterraneus*, *Coelorhinchus caelorhincus, Trachyrincus trachyrincus* and *Nezumia sclerorhynchus* (Macrouridae), *Chimaera monstrosa* (Chimaeridae), *Lophius budegassa* (Lophiidae), *Raje* spp (Rajidae), and *Lepidion lepidion* and *Mora moro* (Moridae). In addition, the red-shrimp *Aristeus antennatus* is also a good sentinel organism providing information over shorter time intervals.

8.3.3 The spatial extent and duration of <u>significant acute pollution events</u> (D8C3) - Primary

An estimate of the total spatial extent and total duration of significant acute pollution events shall be assessed for each year.

Significant pollution transfers have been observed in association to DSWC. The main processes to be monitored seem to be related with these events.

8.3.4 The health of species and the condition of habitats in case of <u>significant acute pollution event</u> (D8C4) - Secondary

The adverse effects of significant acute pollution events on the health of species and on the condition of habitats (such as their species composition and relative abundance) shall be assessed and, where possible, eliminated.

Where the cumulative spatial and temporal effects are significant, criterion D8C4 shall contribute to the assessments under descriptors 1 and 6 by providing an estimate of the abundance of each species (or the extent of each broad habitat type) that is adversely affected.

Deep-sea organisms have adapted to particular environmental conditions (high pressure, low temperature and absence of light), and they may respond differently than coastal species to pollution exposure. In the deep sea Mediterranean environment is mostly oxic. The main stress pollution markers to monitor should be those related to oxidative stress.

The cytochrome P450 system and glutathione-S-transferases (GSTs) play a key role in the biotransformation (monooxygenation and conjugation) of lipophilic foreign chemicals, such as PAHs and polychlorinated biphenyls (PCBs) (Koenig et al., 2012). Substantial differences in monooxygenase or transferase activities and number of isoenzymes have been reported in marine organisms, depending on habitat, pollutants load, etc. Metabolic rates of deep-sea fish are known to decrease with depth as a result of several interacting factors, such as low temperatures, low food availability and poor locomotor capabilities. Strong differences from coastal species in terms of xenobiotic metabolising enzymes could be anticipated.

An increase in oxidative stress can also result in the induction of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPX) and superoxide-dismutase (SOD) as a protection mechanism against the generation of oxidative radicals. However, when this protective mechanism is overwhelmed, lipid peroxidation (LP) (i.e. the oxidation of polyunsaturated fatty acids) can occur.

8.4   Knowledge on Threshold?

In general, the degree of information on the deep sea environments is very limited. Only in the NW Mediterranean some processes such as those related to DSWC have been identified. No information is available on other deep-sea Mediterranean areas. In this respect, the accumulation of pollutants in the sediments and deep-sea organisms and the analysis of this compounds in deep waters would provide substantial information to get progress into the understanding of the distribution patterns of these pollutants. In this context, pollutant transfer to deep sea areas in association to DSWC is a novel phenomenon not previously described that will provide substantial knowledge on the incorporation of chemically stable pollutants to deep waters. Furthermore, the elucidation of the pollution effects into marine species inhabiting deep water environments will be needed to elucidate what stresses hamper the reproduction and development of these organisms and what are effects for the deep water ecosystems.

8.5   CONCLUSIONS

The present level of understanding of the chemical pollution stress into the Mediterranean deep water environments is very limited. The NW Mediterranean is the only area in which information from several studies is available and even in this case the current knowledge is clearly insufficient for a comprehensive description of the main pollutants arriving to these areas, sources, loads, transport mechanisms and depositional fluxes. In the rest of the Mediterranean there is virtually no information.

The studies in the NW Mediterranean have recently shown that DSWC through canyons may funnel large volumes of sediment and pollutants from the continental shelf to the deep margin and basin in addition to the processes related with atmospheric deposition and coastal discharges and subsequent lateral transport. This DSWC mechanism is in contradiction with the paradigm of retention of river and urban residues near the discharge zone by precipitation/flocculation of sediment particles and organic matter. The DSWC method of transport from coastal to deep water environments may be very relevant in many areas of the Mediterranean Sea considering the hydrodynamics of this land-enclosed marine environment. An inventory of the main discharge areas and their impact into the deep water environments is urgently needed, particularly in a marine system where most of the coastal areas are under strong anthropogenic influence.

Furthermore, the relevance of pollutant atmospheric deposition in comparison to coastal discharges has to be assessed. This aspect is urgently needed to get progress into the understanding of the "Mediterranean mercury anomaly" but also to balance the influence of fallout vs coastal discharges for many other pollutants. Adequate understanding of the sources and transport mechanisms of the main pollutants discharged into the Mediterranean Sea will allow to design adequate remediation strategies with efficient results/economic cost ratios

Constructive information on the chemical contamination should be obtained from harmonised monitoring programs based on international expertise. Experience should come from coastal areas for which data sets are very difficult to discuss on a large-scale for two reasons: most of the studies refer to local anthropogenic sources, and the parameters necessary to compare concentrations between various sites (e.g., grain size or organic matter content) are generally not reported.

9 Descriptor 9: Contaminants in fish and other seafood for human comsumption

Descriptor 9 considers the presence of hazardous substances (i.e. chemical elements and compounds) or groups of substances that are toxic, persistent and liable to bio-accumulate, and other substances or groups of substances which give rise to an equivalent level of concern in wild caught organisms (i.e., fish, sharks, crustaceans, molluscs) in the different deep-sea (sub) regions for human consumption.

9.1 State of the Art

9.1.1 Pressures

Release of chemicals is one of the major threats for marine environment and ecosystem functioning because chemicals are detected at all organization levels, from individual to ecosystem (Halpern et al., 2008; Tartu et al., 2013). This concern is high in the Mediterranean Sea, a sea rounded by industrialized and highly-populated countries (Durrieu de Madron et al., 2011).

Anthropogenically-induced chemical contamination has now reached a global dimension and at present there are no more pristine ecosystems. This applies also to the deep sea, one of the most remote systems from human impact (Garcia et al., 2000; Solé et al., 2001; Storelli and Perrone, 2010; Koenig et al., 2013a; Koenig et al., 2013b; Cresson et al., 2014). The deep Mediterranean Sea is not an exception, since it is characterized by extensive urban and industrial wastewater discharges from neighboring countries, leading to an accumulation of contaminants and debris in deep-sea sediments of different sectors of the Mediterranean Sea (Galil et al., 1995; Galgani et al., 1996; Sarda and Bozzano, 2001).

Deep-sea species are commonly considered as long-lived and slow-growing, which increases potential exposure time to contamination (Drazen and Haedrich, 2012; Koenig et al., 2013a; Koenig et al., 2013b; Koenig et al., 2013c). Several studies have demonstrated that fish living in association with sediments are particularly exposed to and accumulate contaminants. Some deep-sea species have a high commercial interest and the high contamination level found increase the associated risk for human health (Rotllant et al. (2006) and references therein).

Contaminants in fish and other seafood for human consumption might arise from numerous anthropogenic sources such as land-based industrial activity, discharge, nuclear accidents, heavy shipping lines, mining activities, but natural oceanographic and geological factors including geothermal activity might also be responsible for elevated levels of contaminants (especially metals) in deep-sea organisms (Halpern et al., 2008).

A number of contaminants in marine environment gave rise to concern both from an environmental and public health point of view. Regulatory levels have been laid down for lead, cadmium, mercury, polycyclic aromatic hydrocarbons, dioxins and dioxin-like PCBs and radionuclides (Commission Regulation (EC) No 1881/2006). Other substances of concern are arsenic, non-dioxin like PCBs, phthalates, organochlorine pesticides, organotin compounds, brominated flame retardants and polyfluorinated compounds.

Chemical contamination in fish and seafood is a complex process resulting from a balance between inputs of contaminants, mostly through diet, and their excretion (Solé et al., 2001; Trudel and Rasmussen, 2001; Cresson et al., 2014). Investigating contamination levels in fish and seafood require first understanding what organic matter sources and associated contaminants fuel the trophic webs, and

what metabolic processes are involved in detoxification. Several biological and environmental factors can affect dietary input and excretion of contaminants, potentially originating high inter- and intra-specific variability (Cresson et al., 2016).

9.1.2   Possible Impacts

The presence of contaminants in fish and other seafood for human consumption at levels above the regulatory levels established by community legislation can have a negative impact both on the health of the consumers and on the use of marine resources (Swartenbroux et al., 2010).

Fish represents an important component of the human diet mainly due to its high nutritional value, but it could represent a serious threat for human health when contaminated.

Long term consumption of foodstuff contaminated with toxic metals may lead to their accumulation in several vital organs. This accumulation may cause liver, kidney, cardiovascular, nervous and bone disorders. Increasing anthropogenic emissions and growing public awareness of the potential health impacts of mercury have led to the establishment of advisories and consumption limits for the general population and particularly for sensitive subgroups (e.g. pregnant women and young children; Koenig et al. (2013c)).

Persistent organic pollutants (POPs) are of particular concern due to their high hydrophobicity, toxicity and persistence (Scheringer et al., 2004). Because of their hydrophobic nature, POPs present in the aquatic systems have a high affinity to bind to suspended particles. Previous findings have suggested a long-term vertical transport of organic contaminants from surface waters to the deep-sea floor (Dachs et al., 2002; Wania and Daly, 2002; Scheringer et al., 2004; Bouloubassi et al., 2006).

Regarding dioxin compounds, present evidence suggests that polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) may persist for many years in marine sediments and in marine biota even in the most remote parts of the world, such as the open oceans and the Antarctic and Artic Oceans (Oh, 2000). In the Mediterranean Sea, persistent organochlorinated compounds have been reported in the tissues of different deep-water fish (Escartin and Porte, 1999; Porte et al., 2000; Solé et al., 2001). Although the deep-sea fishes analysed in those studies (i.e. *Coryphaenoides guentheri*, *Lepidion lepidion*, *Mora moro*, *Bathypterois mediterraneus*, and *Alepocephalus rostratus*) are not commonly consumed in the Mediterranean region, the high level of contaminants there found are indicative of a general contamination of the deep-sea environment and in turn of the prey these apical predators feed on, such as cephalopods, crustaceans and other fishes. Exposure to polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) can cause several endocrine, reproductive and developmental problems in animals, including human beings (Van den Berg et al., 1998). In 1997, the IARC declared 2,3,7,8-TCDD carcinogenic for humans (WHO, 1997). It is well established that human diet constitutes the main source of total exposure to PCDD/Fs, with over 90% of this total burden relating to food of animal origin, whereas intake associated with the consumption of vegetables and fruits could be considered negligible (Beck et al., 1989).

Long-lived artificial radionuclides, particularly, ^{90}Sr, ^{137}Cs, and plutonium isotopes, are released into the atmosphere after nuclear incident and led to the contamination of seawater, sediments and seafood. Consequences of the fallout for human and animal health depending on the quantities of released radioactivity and their atmospheric and oceanic dissipation (Buesseler et al., 2011; Buesseler et al., 2012). In general the biological effects of any contaminant are dependent on the dose received. In the case of ionizing radiation, dose is linked to the energy absorbed in the body of living organisms from two pathways. One source is external irradiation from the surrounding contamination such as in sediment or water, mainly from gamma rays, but also beta radiation for small organisms (of sizes <1 cm). A second source is internal irradiation due to internalization of radionuclides, whatever the physiological process involved. Improper calculation of dose is one of the main factors reducing the scientific validity, and thus acceptance, of many studies on biota inhabiting Chernobyl (Smith, 2008;

Beresford and Copplestone, 2011), and more recently Fukushima (Garnier-Laplace et al., 2011; Beresford et al., 2012). However, very few studies examined the effects of artificial radionuclides on deep-sea species (Harmelin-Vivien et al., 2012). A study carried out on pacific blue fin tuna and some deep-sea fishes off Japan after Fukushima accident showed similar values of contamination in all the species analysed (Fisher et al., 2013).

9.2   Where is the knowledge

It has been often reported that Mediterranean Sea is heavily contaminated, as a consequence of its particular hydrographical characteristics and high anthropogenic pressure (Meadows, 1992; Kütting, 1994; Borrell et al., 1997). Efforts have been devoted to reduce anthropogenic inputs (i.e. by increasing the wastewater treatment plants) and to protect this ecosystem. The Barcelona Convention for the Protection of the Mediterranean Sea, including the Mediterranean Action Plan (MAP) and the Mediterranean Marine Pollution Monitoring and Research Program (MEDPOL), has encouraged the implementation of monitoring programs for evaluating the health status of Mediterranean Sea.

Despite the relevance of pollutant concentrations in deep-sea organisms for human and wildlife health, only a limited number of studies have investigated the concentrations of contaminants of the Mediterranean deep-sea fauna exploited for human consumption (Storelli et al., 2004b; Storelli et al., 2007; Harmelin-Vivien et al., 2009; Harmelin-Vivien et al., 2012; Koenig et al., 2012; Koenig et al., 2013a; Koenig et al., 2013b; Koenig et al., 2013c; Cresson et al., 2014; Cresson et al., 2016; Junque et al., 2017; Llull et al., 2017; Chouvelon et al., 2018).

Metal concentrations detected in fish tissue vary widely depending on different factors such as the metal typology, the trophic guilds, the age/length of the species and the habitat characteristics. Certain species tend to accumulate metals at higher rates than others and it has been established in numerous studies that species with benthic behavior or bottom dwellers, such as angler fish and frostfish, concentrate contaminants to a high degree (Marcotrigiano and Storelli, 2003). It has been hypothesized that species relying on pelagic food web, such as the blue whiting *M. poutassou*, which feeds on krill *Meganyctiphanes norvegica* or lampfish *Notoscopelus elongatus* (Macpherson, 1981), may be less influenced by the Hg methylated in the thermohalocline zone, and thus displayed lower Hg values compared with other bathydemersal species (Cresson et al., 2014; Chouvelon et al., 2018). Recent studies pointed out a potential risk for human health from the consumption of deep-sea organisms contaminated by heavy metals. Thus, a study on deep-sea fish captured for commercialization and human consumption showed that 100% of the specimens of dusky grouper (*Epinephelus marginatus*), 38% of common sole (*Solea solea*), 26% of European hake (*Merluccius merluccius*), 25% of common sea bream (*Pagrus pagrus*) and small-spotted catshark (*Scyliorhinus canicula*) and 11% of white sea bream (*Diplodus sargus*) exceeded the EU recommended limit, 0.5 µg/g ww (Junque et al., 2017; Llull et al., 2017). In addition, 15% of angler (*Lophius piscatorius*) exceeded the targeted EU limit of 1 µg/g ww for this species (Junque et al., 2017; Llull et al., 2017). Furthermore, for the red shrimp *Aristeus antennatus*, which is one of the most valuable resources of the Mediterranean, mercury levels considerably exceeded the recommended 0.5 µg/g w.w. limit and should be consumed with caution (Koenig et al., 2013c).

Despite its major importance, actual bioaccumulation of PCB in deep-sea fish and crustaceans for human consumption is not as documented as in other marine ecosystems, highlighting the need for a better assessment of the factors influencing bioaccumulation mechanisms and variability of PCB contamination. Few studies have been published so far regarding the PCB accumulation patterns observed in the decapod crustacean *A. antennatus* and some deep-sea fishes and sharks (Koenig et al., 2012; Koenig et al., 2013b; Cresson et al., 2016).

Rotllant et al. (2006) have reported for the first time the presence of dioxin compounds in deep-sea organisms dwelling at depths below 600 m. Individuals of *A. antennatus* were characterized by concentrations of PCDD/Fs of the same order of magnitude, or slightly higher, as those found in shallow-water species (*Melicertus kerathurus*) with respect to land-generated contamination. This highlights the widespread distribution of these pollutants and the potential threat posed to the biodiversity of fragile and vulnerable ecosystems such as the deep sea.

Few information has been published so far about radionuclide concentration in marine organisms for human consumption. ^{137}Cs, being the main anthropogenic contributor to radioactive dose to humans from seafood, is the only radionuclide systematically measured by the different countries through their national networks for monitoring environmental radioactivity (Atwood, 2010). Recently, Harmelin-Vivien et al. (2012) revealed that the ^{137}Cs content in hake muscle (*Merluccius merluccius*) sampled in the Gulf of Lion, showed a clear trend with the increase of the hake size.

Regulatory levels for contaminants are set on the basis of scientific advice provided for by the European Food Safety Authority (EFSA) considering their toxicity as well as their potential prevalence in the food chain. Although established regulatory levels are adequate for the management of public health protection, they are generally too high to be used as an indicator of the pollution of the marine environment. Nevertheless, a study on fish commercialized for human consumption showed that the provisional tolerable weekly intake percentages recommended by FAO/WHO for methylmercury in adults and children would be exceeded by 150% and 190%, respectively, in the case that the whole fish consumption would be of Mediterranean origin (Llull et al., 2017). Indeed, thresholds for assessing pollution effects in the marine environment are usually lower. Furthermore, there rarely is a well-defined established simple quantitative link between levels of contaminants in marine environment and levels in fish and other seafood. Current approach for monitoring fish and other seafood for compliance with levels set for public health protection are very different from monitoring of biota for environmental purposes. Moreover, existing monitoring programs for fish and sea food for public health reasons generally focus on estimating consumer exposure rather than assessing environmental status.

In order to assess the environmental status of the marine environment, major efforts should be devoted regarding design of the sampling plans, sampling procedures, selected tissues analysis and traceability to the location of catching or harvesting. With respect to mercury, for instance, the Mediterranean Sea is characterized by variations in mercury distribution, with extremely high mercury concentrations zones. Deep-sea Mediterranean fishes, such as tuna and swordfish, tend to exhibit higher levels of metal accumulation than those of populations inhabiting other areas such as the Atlantic Ocean (Damiano et al., 2011).

An alternative approach such as assessments using environmental quality standards (EQSs) and environmental assessment criteria (EACs) or levels of biological effects response, however, fits more readily within descriptor 8. Since rarely there is a link between levels set for public health protection and GES, the question whether it is actually possible to use these levels set for public health protection for quantifying GES remains an open issue (Swartenbroux et al., 2010).

9.3 How are contaminants in fish and other seafood for human consumption measured in seafood

9.3.1 Methodological sampling aspects

Assessment of the indicators should at least consider the actual levels, the frequency that levels exceed the regulatory levels, the number of contaminants for which exceeding levels have been detected and the source of the contamination. It is also recommended to take into account in the assessment the importance in the human diet of the species showing exceeding levels. Strictly spoken, Good Environmental Status (GES) would be achieved if all contaminants are at levels below those established for human consumption or showing a downward trend (for the substances for which monitoring is ongoing but for which levels have not yet been set). However, it is generally felt that GES for descriptor 9 must be judged in view out the monitoring of descriptor 8, also dealing with contaminants in marine environment. It is recognized that while descriptor 9 requires analysis of edible portion (usually muscle tissue of fish), liver may be a preferred matrix for trend detection for many substances (see descriptor 8) (Swartenbroux et al., 2010).

Monitoring should at least consider the following contaminants for which regulatory levels have been set: i) Heavy metals: Lead, Cadmium and Mercury; ii) Polycyclic aromatic hydrocarbons; iii) Dioxins (including dioxin like PCBs). In addition to these, artificial radionuclides should be monitored in case of nuclear accidents or in any other case of radioactive emergency, which is likely to lead or has led to significant radioactive contamination of food.

Additionally, the following contaminants of relevance should be monitored: i) Arsenic; ii) Non dioxin like PCBs; iii) Phthalate; iv) Organochlorine pesticides; v) Organotin compounds; vi) Brominated flame retardants; vii) Polyfluorinated compounds.

9.3.2 Contaminants listed in Regulation (EC) No 1881/2006. (D9C1)

Heavy metals: lead, cadmium and mercury

As for shallow-water organisms, for deep-sea species it is highly recommended to follow specific sample preparation procedures for lead, cadmium and mercury analysis. The analyst shall ensure that samples do not become contaminated during sample preparation. Wherever possible, apparatus and equipment coming into contact with the sample shall not contain those metals to be determined and be made of inert materials e.g. plastics such as polypropylene, polytetrafluoroethylene (PTFE) etc. These should be acid cleaned to minimise the risk of contamination. High quality stainless steel may be used for cutting edges.

There are many satisfactory specific sample preparation procedures which should be applied. Those described in the European Committee for Standardisation (CEN) Standard EN 13804 "Foodstuffs – Determination of trace elements – Performance criteria, general considerations and sample preparation" have been found to be satisfactory but others may be equally valid.

For the analysis of heavy metals, the following deep-sea species have been analysed in previous research: small-spotted catshark *Scyliorhinus canicula* and blackmouth catshark *Galeus melastomus* among sharks, blackbelly rosefish *Helicolenus dactylopterus*, blue whiting *Micromesistius poutassou*, greater forkbeard *Phycis blennoides,* European hake *Merluccius merluccius and Lepidorhombus bosci* and *Mora moro* among teleosts*)* and Norway lobster *Nephrops norvegicus* and the red-shrimp *A. Antennatus* among crustaceans (Perugini et al., 2009; Cossa et al., 2012; Koenig et al., 2013c; Cresson et al., 2014; Perugini et al., 2014; Llull et al., 2017).

Polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyls (PCBs)

Regulatory levels for polycyclic aromatic hydrocarbons set in community legislation for public health reasons currently consider only benzo(a)pyrene, since benzo(a)pyrene is used as a marker for the presence of the whole class of PAHs.

Specific sample preparation procedures should be followed. The analyst shall ensure that samples do not become contaminated during sample preparation. Containers shall be rinsed with high purity acetone or hexane before use to minimise the risk of contamination. Wherever possible, apparatus and equipment coming into contact with the sample shall be made of inert materials such as aluminium, glass or polished stainless steel. Plastics such as polypropylene or PTFE shall be avoided because the contaminants can adsorb onto these materials.

Information on benzo(a)pyrene contamination on edible deep-sea organisms of the Mediterranean Sea is practically non existent. In previous research on PCB accumulation in deep-sea species, fishes such as blackbelly rosefish *Helicolenus dactylopterus*, greater forkbeard *Phycis blennoides* and shark species such as the catfish *Scyliorhinus canicula* were targeted on the basis of their trophic position, their abundance and their importance in slope and canyons community functioning (Carrasson and Cartes, 2002; Fanelli et al., 2013b; Goujart et al., 2013; Papiol et al., 2013; Tecchio et al., 2013a; Cresson et al., 2016).

Dioxins and dioxin-like PCBs

Regulatory levels for dioxins and dioxin-like PCBs set in community legislation for public health reasons consider sum of dioxins and sum of dioxins and dioxin-like PCBs (WHO, 1997).

As for shallow-water species, in the case of deep-sea fish, the skin has to be removed as the maximum level applies to muscle meat without skin. However, it is necessary that all remaining rests of muscle meat and fat tissue at the inner side of the skin are carefully and completely scraped off from the skin and that these rests of muscle meat and fat tissue are added to the sample to be analyzed.

The samples must be stored and transported in glass, aluminum, polypropylene or polyethylene containers. Traces of paper dust must be removed from the sample container. Glassware shall be rinsed with solvents, certified to be free from dioxins or previously controlled for the presence of dioxins.

Analysis should be performed using high-resolution gas chromatography/mass spectrometry (HRGC/HRMS) methods.

For the analysis of dioxin compounds in deep-sea species, previous studies focused on decapod crustaceans *A. antennatus* (Rotllant et al., 2006) and *Pasiphaea multidentata* (Castro-Jimenez et al., 2013).

Radionuclides

The analysis of radionuclide concentrations should be carried out immediately following a nuclear accident or any other case of radiological emergency which is likely to lead or has led to significant radioactive contamination of fish or seafood.

Once released to the environment, radionuclides can be rapidly incorporated into marine organisms either by uptake from seawater or by food ingestion (Fowler and Fisher, 2005). For ^{134}Cs, ^{137}Cs, ^{40}K, ^{210}Pb and ^{210}Po analyses, the protocol used for the determination of these radionuclides in mussels described by Baumann et al. (2013), can be followed. Cesium is most often measured in the edible part of fish, because Cs, like K, is enriched in fish flesh. Because the flesh represents the largest component of fish body weight, these analyses provide a good estimate of total Cs content and are the most relevant for human consumers (Buesseler et al., 2017). The ability of an organism to accumulate radionuclide is expressed through the use of becquerels per kilogram (Bq/kg).

9.4 Knowledge on Threshold

Knowledge on contaminant threshold of deep-sea Mediterranean species exploited for human consumption is extremely limited and mostly referred to mercury. There is evidence that red shrimp *A. antennatus* which is one of the most valuable fishing resource of the Mediterranean Sea can contain mercury concentrations above the threshold level defined by the Regulation (EC) No 1881/2006 (Koenig et al., 2013b). This applies also to commercially exploited fish *Phycis blennoides* and to other deep-sea fish and decapod crustacean species (Cresson et al., 2014). Preliminary results indicate also that the tissue of *Acanthephyra eximia* (a deep-sea decapod crustacean), which occupies an intermediate trophic position in deep-sea food webs (Fanelli et al., 2013b; Papiol et al., 2013) can have Cd concentrations above the threshold defined by EU for human consumption (Kress et al., 1998). Thus, there is an urgent need to systematically analyze the contaminant concentrations in edible deep-sea species taking into account at least the list of the contaminants present in the Regulation (EC) No 1881/2006.

9.5 CONCLUSIONS

9.5.1 Gaps

Most of the studies aimed at analyzing the levels of contaminants in fish and seafood for human consumption have been performed for shallow-water species. Very few researches have been conducted on deep-sea species and in most of the cases they were not of commercial interest.

Assessment of heavy metals concentrations is mainly performed in shallow Mediterranean species of commercial or cultural interest. Numerous studies have investigated trophic position and mercury concentration in species such as hake, mullet, tuna or marine mammals (Harmelin-Vivien et al., 2009; Cossa et al., 2012; Storelli and Barone, 2013; Llull et al., 2017). Fewer data are available regarding mercury concentrations for species living in deep Mediterranean waters. However, there is considerable concern regarding such metal for these species (Harmelin-Vivien et al., 2009; Koenig et al., 2013c; Cresson et al., 2014; Llull et al., 2017; Chouvelon et al., 2018).

In addition, despite the relevance of pollutant concentrations in deep-sea organism for human and wildlife health, only a limited number of studies have thus far investigated the levels of POP contamination of the Mediterranean deep-sea fauna (Harmelin-Vivien et al., 2012; Koenig et al., 2013a; Koenig et al., 2013b; Cresson et al., 2016; Junque et al., 2017).

Regarding dioxin compounds, PCDD/Fs have been reported in shallow crab, lobster and prawn tissue (Rappe et al., 1991; Abad et al., 2003; Bodin et al., 2004). However, few studies have been performed on deep-sea species in the Mediterranean Sea (Rotllant et al., 2006; Storelli et al., 2007).

Thus, there is an urgent need to perform further studies to analyse the level of concentrations of the different contaminants in deep-sea species with a special focus on those of commercial interest.

9.5.2 Recommendations

Results from monitoring of contaminants under descriptor 8 ("Concentrations of contaminants are at levels not giving rise to pollution effects") and descriptor 9 should be integrated. Data acquired from monitoring under descriptor 8 are an important element in selecting contaminants for descriptor 9.

Biological factors can influence concentrations of contaminants in fish, such as age, sex and reproductive stage. To avoid obscuring real environmental trends, monitoring programs planned to detect spatial and/or temporal trends should take account of these factors during sampling design and assessment.

Different factors such as historical and present ecosystem health status and local anthropogenic activities, should be taken into account when designing a monitoring plan. The assessment of the specific situation in the (sub) region shall determine the substances to be analyzed in monitoring programs, or on additional substances to be included. In addition, levels of contaminants do vary between (sub) regions, and some contaminants are more important than others for a specific (sub) region, due to differences in activities and inputs. Due to important differences in the presence for some contaminants, care should be taken when comparing their levels in fish and other seafood between different deep-sea (sub) regions.

In order to make monitoring results more comparable between deep-sea (sub) regions, it would be advisable to select a limited number of target species from the most consumed species of fish and other seafood. General criteria for the selection of the species to be used for monitoring include species more prone to bio-magnify/bio-accumulate specific classes of contaminants, species representative of different trophic levels or habitats, species representative of the specific deep-sea (sub) region.

10 Descriptor 10 : Marine Litter

Marine litter, originating from many unspecified sources, is one of the most serious, rapidly developing and worsening global environmental problems. In 2012, the global production of waste had reached 3.4 billion tons and this figure is expected to double by 2025(Jambeck et al., 2015), while about half of this amount concerns non-biodegradable materials (i.e. plastics and metals). Plastics are ubiquitous in the marine environment, in vast quantities and are present even on the most remote areas of the planet. This is evident in certain areas of the globe for which plastics can be found in excess, often more than 80% of the recorded marine litter items. Periodic assessments of the state of the marine environment, monitoring and the formulation of environmental targets are perceived as part of the continuous management process within the MSFD. Of the 11 descriptors listed in Annex I of the MSFD for determining GES, descriptor 10 has been defined as "Properties and quantities of marine litter do not cause harm to the coastal and marine environment".

The present chapter reports on the literature review on marine litter in deep Mediterranean Sea to assess the current state of MSFD implementation. It also provides information about priorities and recommendations to better support MSFD implementation.

10.1 State of the Art

Recent reviews on marine litter have described the distribution, fate, degradation, transport, impacts and monitoring of marine litter, in the Mediterranean Sea also addressing gaps for research, and management (MSFD Technical Subgroup on Marine Litter, 2013; CIESM, 2014; Suaria and Aliani, 2014; Galgani et al., 2015; Tubau et al., 2015; UNEP, 2015; Ioakeimidis et al., 2017).Our review has then focused on the deep sea environment, the scope of IDEM.

Because of (i) large cities, rivers and shore uses, (ii) some of the largest amounts of Municipal Solid Waste that are generated annually per person (208 – 760 kg/Year) and more than 700 tons of plastics entering the basin every day (Jambeck et al., 2015), (iii) extensive tourism and recreational boating, (iv) fisheries, (v) 30% of the world's maritime traffic, and (vi) a closed basin, the Mediterranean has been described as one of the most affected areas by marine litter in the world. Researchers predicted that, without management measures, the amount of plastic dumped will rise by a factor of ten in the next decade and by a factor of 2.17 between 2010 and 2025 in the Mediterranean Sea. Current knowledge of the quantities of litter in the Mediterranean Sea, the degradation and fate of litter in the marine environment and its potentially harmful biological, physical and chemical impacts remains limited. Research recently demonstrated (i) the importance of hydrodynamics, (ii) the impact of plastic that include entanglement, physical damage and ingestion, the release of chemicals, the transport of species and the alteration of benthic community structures, and (iii) social and economic harm (MSFD Technical Subgroup on Marine Litter, 2016).

Sources of marine litter are traditionally classified as either land-based or sea-based, depending on where the litter enters the water. Studies on land-based sources of pollution indicated inputs at a level of 50 billons particles every year only for the Po river (Vianello et al., 2015) and another study suggested that 677 tons of microplastics were entering the Mediterranean Sea every year(Tweehuysen, 2015). Uncontrolled discharges also act as main sources of litter in the Mediterranean Sea because not all coastal cities are controlling their waste discharges in adapted structures. Ocean-based sources of

marine litter include merchant shipping, cruise liners, fishing vessels, military fleets, and offshore installations. It is estimated that garbage dumped by merchant ships to the Mediterranean may be in the range of 0.5 million tons (UNEP, 2015).

Circulation is the primary driver of marine litter transport. Semi-enclosed seas that are surrounded by human settlements, such as the Mediterranean Sea, are likely to have particularly high concentration of marine debris. In this basin, studies have already documented the transport of litter and its accumulation on the seafloor, concluding that coastal submarine canyons act as conduits for the transport of marine debris into the deep sea areas (UNEP, 2015).

Most litter comprises high-density materials and hence sinks. Even low-density synthetic polymers, such as polyethylene and polypropylene, may sink under the added weight of fouling growth or additives. General strategies for the investigation of seabed debris are similar to those used to assess the abundance and type of benthic species. More than 50 studies have been conducted worldwide between 2000 and 2015, but, until recently, very few covered extensive geographic areas or considerable depths.

Only few studies have focused on debris located at depths deeper than 500 m in the Mediterranean (Galgani et al., 1995; Galil et al., 1995; Galgani et al., 1996; Galgani et al., 2000; Galgani, 2011; Mifsud et al., 2013; Ramirez-Llodra et al., 2013; Sanchez et al., 2013; Fabri et al., 2014; Pham et al., 2014; Tubau et al., 2015) (see Table 10.5). Galgani et al. (2000) did not observe trends in deep sea pollution over time off the European coast, with extremely variable distribution and debris aggregation in submarine canyons. Higher bottom densities are also found in particular areas, such as around rocks and wrecks, and in depressions and channels. In some areas, local water movements carry debris away from the coast to accumulate in high sedimentation zones. The distal deltas of rivers in deep sea environments may also transport riverine debris into deeper waters, creating high accumulation areas.

A wide variety of human activities, such as fishing, urban development and tourism, contribute to these patterns of deep seabed debris distribution. Fishing debris, including ghost nets, prevails in commercial fishing zones and can constitute high percentages of total litter(Ioakeimidis et al., 2017). More generally, accumulation trends in the deep sea are of particular concern, as plastic longevity increases in deep waters and most polymers degrade slowly in areas devoid of light and with lower oxygen content.

As marine litter affects different ecological compartments, the study of its impact on marine biota of all trophic levels on the same temporal and spatial scale is of increasing importance. So far, 79 studies have investigated the interactions of marine biota with marine litter (mainly plastics) in the Mediterranean basin (Deudero and Alomar, 2014, 2015). These studies cover a wide range of depths (0 m to 850 m) and a large temporal scale (1986 to 2014), unveiling a vast array of species that are affected by litter, ranging from invertebrates (polychaetes, ascidians, bryozoans, sponges …), fish, and reptiles to cetaceans.

For ingestion, highly affected species may include deep sea fish (Anastasopoulou et al., 2013) and invertebrates (Murray and Cowie, 2011; SPA/RAC, 2017), especially detritivours and filter feeders. More important for the deep sea, organisms have been shown to utilize the debris items in oceans as habitats to hide in, adhere to, settle on, and move into new territories. By sinking, debris may have an impact on the deep sea environment, providing solid substrates and new habitats, impacting the distribution of benthic species even in remote areas (Katsanevakis et al., 2007; Ramirez-Llodra et al., 2013; Pham et al., 2014; Tubau et al., 2015; Taviani et al., 2017).

In recent years, secondary pollution from the leaching of pollutants from litter has been extensively studied(UNEP, 2015). There is however no information about contamination of the deep seafloor through marine litter.

Litter on the sea floor also gives rise to a wide range of economic and social impacts. Despite the bans on bottom trawling beyond *1000m* and on driftnet *fishing* are legally binding for all countries bordering the Mediterranean, economic impacts are most often described as including the impacts "from fishing" and "on fishing" when ecosystem degradation is an extremely complex cost to evaluate.

10.1.1 Pressures

The abundance of plastic debris is very location-dependent, with mean values ranging from 0 to over 7,700 items per km² (UNEP, 2015). Mediterranean sites tend to show the highest densities, due to the combination of a populated coastline, coastal shipping, limited tidal flows and a closed basin, with exchanges of water that are limited to Gibraltar. In general, bottom debris tends to become trapped in areas with low circulation, where sediments accumulate.

Counts from 7 surveys and 295 samples in the Mediterranean Sea and Black Sea (2,500,000 km², worldatlas.com) indicate an average density of 179 plastic items/ km2 for all compartments, including shelves, slopes, canyons and deep sea plains (Galgani et al., 2015), in line with trawl data on 3 sites described by Pham et al. (2014). On the basis of these data, we can assume that # 0.5 billion litter items are currently lying on the Mediterranean seafloor. In his finding, the study also indicated the importance of plastic, at 62.7 % +/- 5.47 of total debris . An analysis of types of litter from data collected from regular monitoring of litter on the seafloor in the gulf of Lion confirmed these results (Table 10.1).

Table 10.1: Typology of marine Litter collected between 30 and 800 m in the Gulf of Lion, France (Medits Cruises, 2013 and 2014, IFREMER, MSFD/Medits protocol, unpublished results). Note that pieces of coal are also found on ancient shipping lines.

Items	2013	2014	% (2013 + 2014)
Plastic (bags and pieces)	283	543	35,33
Plastic bottles	43	38	3,46
Food wrappers	184	95	11,93
Plastic sheets	342	90	18,48
Other plastics	39	33	3,08
Fishing nets	26	82	4,62
Fishing lines	20	98	5,05
Other fishing items (pots, etc.)	9	59	2,91
Synthetic ropes	29	45	3,17
Tires	2	1	0,13
Other fishing items (gloves, etc.)	3	6	0,38
Metallic cans	17	13	1,28

Items	2013	2014	% (2013 + 2014)
Other metallic objects	7	3	0,43
Containers	11	8	0,81
Cables	0	2	0,09
Hooks	0	0	0,00
Glass objects (bottles, etc.)	35	43	3,34
Pieces of glass	3	1	0,17
Ceramics	9	1	0,43
Clothes	17	34	2,18
Large pieces of tissus	11	1	0,51
Natural ropes	5	4	0,38
Sanitary objects	0	7	0,30
Man made wood	0	6	0,26
Paper/Cardboard	1	4	0,21
Other identified items	2	14	0,68
Non identified items	4	5	0,38

On slopes, the dominant litter items recovered was fishing gear (59%), followed by plastic (31%). In some areas, fishing gears may account for the largest part of debris, depending on fishing activity. As an example dominant type of debris (89%) consisting of fishing gear were found on rocky banks in Sicily and Campania (Angiolillo et al., 2015) (LIFE BaℏAR for N2K project, unpublished data). Deep analysis finally detected that the "Distance to the coast" variable accounted for less than 20% of the variance in the distribution of litter between canyons (UNEP, 2015).

In the Gulf of Lion, only small amounts of debris were collected on the continental shelf. Most of the debris was found in canyons descending from the continental slope and in the bathyal plain, with high amounts occurring to a depth of more than 500 m (Table 10.2).

Table 10.2: Distribution of debris in the Gulf of Lion in relation to the depth (After UNEP, 2015)

Depth (m)	Tows	Total area (km²)	Total debris	Plastics	Debris (km^{-2})
<200	57	3.03	337	229 (68%)	111.2
200-1000	21	0.816	568	483 (85%)	696
>1000	10	0.17	631	537 (85%)	3712

In the Mediterranean, static gear is an important part of ghost fishing and fishing can continue for years. Estimated ghost catches are generally believed to be well under 1% of landed catches and was estimated annually, for hake, between 0.27% and 0.54% of the total commercial landings.

Mapping the litter on the seafloor enables to estimate the accumulation areas. In a study on 67 sites conducted in the Adriatic Sea using commercial trawl (Strafella et al., 2015), analysis of marine litter, sorted and classified in major categories confirmed that plastic is dominant in terms of weight followed by metal. The highest concentration of litter was found close to the coast likely as a consequence of high coastal urbanization, river inflow and extensive navigation. Recently, benthic marine litter was investigated in 4 study areas from the Eastern Mediterranean (Saronikos, Patras and Echinades Gulfs;

Limassol Gulf) (Ioakeimidis et al., 2014). Densities ranged from 24 to 1211 items/km2, with the Saronikos Gulf being the most affected area. Plastics were predominant in all study areas ranging from 45.2% to 95%. Metals and Glass/Ceramics reached maximum values of 21.9% and of 22.4%. In another example, the distribution and abundance of large marine debris were investigated on the continental slope and bathyal plain of the northwestern Mediterranean Sea during annual cruises undertaken between 1994 and 2009 (Galgani, 2011). Different types of debris were enumerated, particularly pieces of plastic, plastic and glass bottles, metallic objects, glass and diverse materials including fishing gear. The results showed considerable geographical variation, with concentrations ranging from 0 to 176 pieces of debris/ha. In most stations sampled, plastic bags accounted for a very high percentage (more than 70%) of total debris.

In 2005, benthic anthropogenic debris was quantified for the first time around the Maltese Islands (central Mediterranean). 357 items were sampled from 3.5 km2 of swept area (Figure 1D10) with plastic (47%), metal and glass (13% each) as most prevalent types of litter by number. An attempt was made to correlate litter abundance and environmental variables, including fishing activities, but no interpretable correlations were found (Figure 1D10).
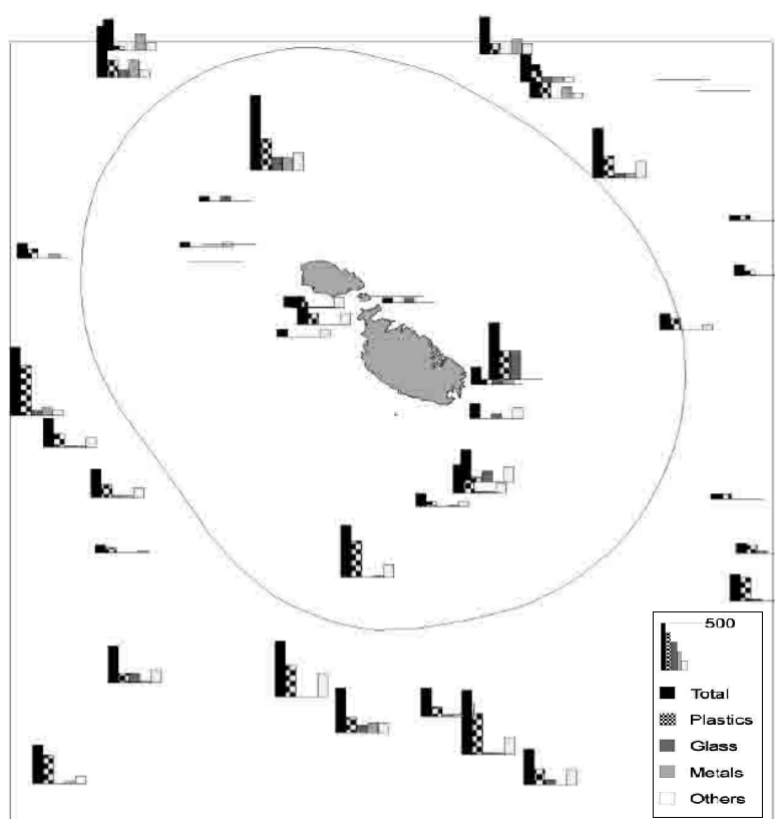

Figure 10.1: Litter densities on the seafloor around the Maltese islands in 2005, from Misfud et al. (2013).

10.1.2 Microplastics

In addition to large marine debris, there is growing concern with regards to microplastics (items < 5mm). Small plastic particles can directly enter the ocean as cosmetic abrasives (i.e. microbeads), preproduction plastic pellets or textile fibres entering wastewater circuits, commonly referred to as primary plastics. Additionally, by the combined mechanical, biological, photic and thermal actions, large plastic objects floating on the sea surface can break down into numerous small fragments, referred as secondary microplastics.

Depending on the density of the polymer, microplastics may float and sink only when negative buoyancy is reached due to ballast effect, strongly by e.g. colonization by organisms, adherence to phytoplankton and/or aggregation with organic debris (for example polyethylene and polypropylene), or may sink and behave as very fine grained sediments (for example polyester) (Woodall et al., 2014).

Global models consistently predict some of the highest concentrations of floating plastics in the world to occur in the Mediterranean Sea (Eriksen et al., 2014; Cozar et al., 2015; Suaria et al., 2016), with concentrations above those found in the inner accumulation regions of the great Subtropical Gyres. Reasons behind that are the limited outflow of surface waters, a densely populated coastline and intensive fishing, shipping, touristic and industrial activities. According to the most recent global models the Mediterranean Sea retains between 21% and 54% of global microplastic particles ($3.2-28.2 \times 10^{12}$ particles), equivalent to between 5% and 10% of global mass ($4.8-30.3 \times 10^{3}$ tonnes), mostly because of small average particle size (van Sebille et al., 2015).

Floating microplastics is the source for deep sea microplastics. While recent large scale surveys of microplastics floating in Mediterranean waters have provided extensive characterization of their abundance, geographical distribution, size and polymer type (Cozar et al., 2015; Suaria et al., 2016), the link with sea floor microplastics is not well defined. Different types of plastic items (pellets, films, fishing threads, foam, fragments, the latter accounting for up to 87%) have been described at the surface when fibers are the most abundant in sea bed sediments. The average microplastic concentration in surface waters was 423 g km^{-2} (or 243,853 items km^{2}). Floating microplastics are low-density polymers such as polyethylene and polypropylene, followed by polyamides, plastic-based paints, polyvinyl chloride, polystyrene and polyvinyl alcohol (Suaria et al., 2016) and most of them are not expected to sink. The highest abundances expressed in terms of items and mass concentrations are always recorded in the western Mediterranean and the Adriatic Sea. However, the spatial distribution is irregular and there is no clear association with main sea surface circulation or distance to the coast (Suaria et al., 2016).

Abundant concentrations of microplastics have also been reported from remote and presumably unspoiled environments such as the deep seafloor (Van Cauwenberghe et al., 2013; Woodall et al., 2014), which has evidenced that the deep sea could be considered a major sink for microplastics (Woodall et al., 2014). Plastic microfiber abundance in the sediments ranged from 1 particle / 25cm3 to 10- 35 fibres per 50 ml of sediment (average 17.5 fibres per 50 ml of sediment). The large amounts of fibers in one study and the only one microplastic in the other, raise however the question of a possible contamination during sample processes and analysis, a very common scheme when considering low sized fibers as a main part of total microplastics (GESAMP, 2016). With two studies only, a more complete assessment of microplastics in the Mediterranean seafloor is still needed.

Identification by FT-IR spectroscopy confirmed that fibres were plastic, with a predominance of polyester followed by polyamides, acetate, and acrylic (Woodall et al., 2014). Most of these polymers

are negatively buoyant, which may account for the difference in microfiber composition between microplastics in the seabed (mainly polyester, with a density of 1.37 g cm^{-3}) and those floating in surface waters (mainly polyethylene and polypropylene, with a density of 0.90-0.95 g cm^{-3}).

Colonization by organisms, adherence to phytoplankton and aggregation with organic debris and small particles in the form of marine snow, or the occurrence of oceanographic events that transfer particle loaded waters from shallow ocean regions to the deep sea, will eventually enhance the arrival of microplastics to deeper areas (Woodall et al., 2014). This mechanism is however not so well understood as grazing of organic matter and live settled animals may refloat the microplastics.

10.1.3   Impacts

As marine litter affects different ecological compartments, the study of its impact on marine biota of all trophic levels on the same temporal and spatial scale is of increasing importance. Effects from the studies were classified into entanglement, ingestion, and colonization and rafting (Gregory, 2009). Entanglement has been described for many species. As recent work has shown, lost gear or litter in general can also harm benthic organisms and habitats, including deep sea Mediterranean species like sponges, gorgonians, or certain cold water corals (Fabri et al., 2014; Pham et al., 2014; Tubau et al., 2015). In the Mediterranean, static gear is an important part of ghost fishing. Losses are then often a combination of rough bottom (rocks, wrecks) and strong currents complicating the retrieval of the gears and giving very variable results (pieces of netting and/or ropes, bundles of nets, etc.). On a daily basis ghost catches are assumed to decline quickly with ghost catches at 5% of the active catches after 90 days (Brown and Macfadyen, 2007). FANTARED (FANTARED 2, 2003) showed however that gear lost/discarded in deep water with little tidal/current activity can continue to fish for years rather than months (Brown and Macfadyen, 2007; Macfayden et al., 2009). On rocky bottoms, gillnet catch rates can be near zero over an 8–11-month period (Ayaz et al., 2006; Ayaz et al., 2010) while in almost all bottom conditions, ghost catches initially showing a high percentage of fish before switching progressively to catches dominated by crustaceans.

For ingestion, highly affected species may include deep sea fish (Anastasopoulou et al., 2013) and invertebrates (Murray and Cowie, 2011; SPA/RAC, 2017), especially detritivorous or filter feeders. More important for the deep sea, organisms are shown to utilize the debris items in oceans as habitats to hide in, adhere to, settle on, and move into new territories. By sinking, debris may have an impact on the deep-sea environment, providing solid substrates and new habitats, impacting the distribution of benthic species even in remote areas (Katsanevakis et al., 2007; Ramirez-Llodra et al., 2013; Pham et al., 2014; Tubau et al., 2015).

In recent years, secondary pollution from the leaching of pollutants from litter has been extensively studied. There is however no information about contamination of the deep seafloor through marine litter.

Litter in the marine environment gives rise to a wide range of economic and social impacts. Value for the closed Mediterranean Sea may be more important due to the population in the region, fishing, maritime traffic, and tourism. In this basin, there is little or no reliable data on what the exact costs are. For the deep seafloor, economic impacts are most often described as including the impacts "from fishing" and "on fishing" when Ecosystem degradation is an extremely complex cost to evaluate.

Bottom gillnet fisheries are very common throughout the Mediterranean basin, with more than 20,000 boats involved (http://firms.fao.org/firms/fishery/761/en) (UNEP, 2015). Target species are largely represented by demersal and bentho-pelagic fish, crustaceans and locally cephalopods. Although few entrapments in bottom gillnets have been documented (see Table 10.5), this may be in part due to under-reporting as a result from the reluctance of fishers to report such incidents.

The causes of gear loss are important not only in terms of impacts but for developing appropriate management measures. Conflict with other activities, working in deep water or bad weather conditions or very hard grounds, working very long nets or more gears than permitted but also 'discarded/ abandoned' gears are the most important reasons of losses

10.2 Where is the knowledge

In the Mediterranean Sea, activities on marine debris involve or are related to (i) International Agreements and initiative, (ii) International bodies, institutions, (iii) Stakeholders, (iv) NGos, foundations and associations, and (v) Research institutions.

Amongst existing projects on marine Litter, some are dedicated to specific aspects of MSFD (beaches, floating, impacts on large organisms, etc.) that are not of direct importance when addressing deep sea issues. The following tables (Tables 10.3 and 10.4) summarize the existing projects of importance for marine litter in the deep sea and relevant for IDEM.

Thanks to the Alfred Wegener Institute (AWI), an almost complete list of available Information on litter in the Mediterranean sea can be obtained at the following link http://litterbase.awi.de/litter_detail and the location of studies with available data is available at http://litterbase.awi.de/litter (Tekman et al., 2017).

Available information for deep sea Litter is listed in Table 10.5. There is not so much difference within the main Mediterranean basins when densities are most often related to the local sources. Canyons that are located in the vicinity of large towns are the most affected when distal areas are cleaner. Locally, holes wreck , depressions are accumulating litter. Then, large amounts of fishing gears are found in fishing grounds (North Adriatic or Sicily for example).

Deep sea Microplastics in the Mediterranean have been reported on in only two studies, describing variable concentrations (Van Cauwenberghe et al., 2013; Woodall et al., 2014).

Table 10.3: Available sources of information from institutions, other than academic, for deep-sea litter.

Institution	Marine litter relevance	MEDPOL relevance	Monitoring relevance	Countries involved
UNEP/ MEDPOL	UNEP Regional Plan Marine Litter	Medpol activities	ECAP monitoring plan (see UNEP, 2013 and 2014)	UNEP contracting Parties http://www.unepmap.org
CIESM	marine Litter Topic addressed by the Biogeo-chemistry committee (III)	Supporting science	Reports on scientific aspects of Marine litter in the MED	www.ciesm.org http://www.ciesm.org/people/ board/index.htm
GFCM	- Recommendations on Marine Litter, report of the 13th meeting, Rome 2013, - GCFM also Address the issue of ghost fishing	Collaborate in initiatives that mitigate major impacts such as those related to reduce amount of fishing gear as litter, etc.	Information available on derelict fishing gears http://www.gfcm.org/fi/we bsite/GFCMMultiQueryAc tion.do	http://www.gfcm.org/gfcm/ about/en#Org-OrgsInvolved
RAMOGE	Ad hoc group on ICZM / marine Litter	RAMOGE is a sub-regional institution of the Barcelona convention.	Pilot experiments in the deep sea (2016 and 2017)	Italy (Liguria) , Monaco, France (South east area) http://www.ramoge.org/fr/defau lt.aspx
DG ENV	Various analysis/report on marine litter. GES Technical group Marine Litter (69 experts), technical support for the implementation of MSFD on D10	Support to European parties (technical, GES, Targets, reduction)	Guidance and protocols provided to EU countries	EU countries http://ec.europa.eu/ environment/marine/ good-environmental-status/ descriptor-10/index_en.htm
WASTE ATLAS	*Crowdsourcing free access map that visualizes municipal solid waste management data across the world*	Data on waste (generation, treatment, landfills, etc.) by countries, Map of dumping sites		164 Countries worldwide, data on Med countries http://www.atlas.d-waste.com

Table 10.4: Available sources of information from relevant research projects in the Mediterranean Sea.

Project name	Region	Objective
HERMIONE - Hotspot Ecosystem Research and Man's Impact On European Seas http://www.eu-hermione.net/, April 2009 - Sept 2012	Mediterranean Sea and North-East Atlantic deep-waters	One of the aims of HERMIONE was to provide stakeholders and policy-makers with scientific knowledge to support deep-sea governance aimed at the sustainable management of resources and the conservation of ecosystems.
PERSEUS - Policy-oriented marine Environmental Research for the Southern European Seashttp://www.perseus-net.eu/site/content.php	Mediterranean & Black Seas	Design of an effective and innovative research governance framework, which will provide the basis for policymakers to turn back the tide on marine life degradation.
DeFishGear ADRIATIC IPA, 2013-2016, http://defishgear.net/	Countries around the Adriatic sea	Marine litter and dereliction of fishing gear in the Adriatic Sea.
MEDITS (http://www.sibm.it/SITO%20MEDITS/principaleprogramme.htm),	Mediterranean countries	MEDITS (International bottom trawl survey in the Mediterranean, CFC funded) does include assessments of marine litter on the seafloor, on regular basis.
GHOST Life, 2013-2015 , http://www.life-ghost.eu/index.php/en/	North Adriatic	Promotes concrete measures to preserve and improve the ecological status of the rocky habitats (*Tegnùe*) in the north Adriatic sea. Includes the impacts of abandoned, lost or discarded fishing gears (*ALDFG*) on marine biodiversity.
MERMAID Sea's ERA, 2013-2015	Greece, France Turkey	Marine environmental targets linked to regional management schemes based on indicators developed for the Mediterranean. Include marine litter (pressure/ impacts links).
GES TG marine Litter	European member states	Technical group to support the implementation of MSFD. Produces recommendations, reports, and started recently on baselines
INDICIT	South OSPAR and Mediterranean SEA	An EC/DG ENV/ second cycle project to support the implementation of criteria D10C3 and D10C4 within MSFD
MEDCIS	European parties from the Med Sea	An EC/DG ENV/ second cycle project to support the implementation of MSFD, addressing specific Mediterranean issues.
EMODNET / chemistry-litter (DG MARE) http://www.emodnet-chemistry.eu	All European countries	An EC/DG MARE/ project to support the collection and storage of data on marine Litter at the EU scale.

Two additional projects (Act4litter and MEDsealitter) must be cited despite non considering directly the deep Mediterranean Sea.

Table 10.5: Marine litter concentration on deep seafloor (>200m), updated from (Galgani et al., 2015).

Location	Environmental compartment	Date	Sampling	Depth	Density (min-max)	% plastics	References
Malta	Sedimentary bottom	2005	Trawl (44 hauls, 20 mm mesh)	50-700	97 +/- 78/km2	47	(Mifsud et al., 2013)
Montenegro	Shelf/ slopes	2009	trawling	48 - 746	6-59% of total catches	NA	Petrovic & marcovic, 2013 in UNEP, 2015
France-Mediterranean	Seabed, slopes	2009	17 canyons, 101 ROV dives	80-700	3.01 /km survey (0-12)	12 (0-100)	(Fabri et al., 2014)
Mediterranean sea	Seabed, Bathyal / abyssal	2007-2010	292 tows, Otter/agasiz trawl, 12 mm mesh	900-3000	0.02- 3264.6 kg/·km² (including clinkers)	nd	(Ramirez-Llodra et al., 2013)
Turkey/ Levantine basin,	Seabed, Bottom/Bathyal	2012	32 hauls (trawl, 24 mm mesh)	200-800	290 litter (3264.6 kg·) /km²	81.1	(Güven et al., 2013)
Mediterranean, Southern France	Shelves & canyons	1994-2009 (16 years study)	90 sites (trawls, 0.045 km2/tow)	0-800	76-146/ km² (0-2540)	29.5 -74	(Galgani et al., 2000) & unpublished data
Greece (Saronikos Gulf)	Seabed (fishing ground)	2013	69 hauls (50mm mesh)	50-350	1211±594 items/km2	95,0±11,9	(Ioakeimidis et al., 2014)
Levantine basin (Cyprus)	Seabed (fishing ground)	2013	9 hauls (50mm mesh)	60-420	24±28 items/km2	67,4±7,7	(Ioakeimidis et al., 2014)
Italy (North Thyrrenian)	Shelf	2010-2011	69 dives (26 areas, 6.03 km2)	30-300	90 debris items/ km² (0- 160)	92% (89% from fishing)	(Angiolillo et al., 2015)
Italy (Tyrrhenian)	Fishing Grounds (Rocky banks)	2010-2011	ROV observations	70-280	0.0029 km2	-	(Bo et al., 2014a)
Italy, North-western Adriatic Sea	Seabed	2011-2012	67 hauls,	1 – 260	85 721 kg/km²	34%	(Bo et al., 2014a)
Catalan margin	Submarine canyons, slope	2011	ROV observations (26 dives)	156-1731	540 items km-2 (0-167)	72%	(Tubau et al., 2015)
Malta	Sedimentary bottom	2005	Trawl (45 hauls, 20 mm mesh)	45-700	8 limestone slabs / km2	n/a	(Pace et al., 2007)

10.3 How is Marine Litter measured

10.3.1 Methodological sampling aspects

The use of observation tools, i.e. Remote Operated Vehicles (ROVs) and Submersible Vehicles, is a possible approach for deep-sea environments. Unfortunately, these methods require considerable resources but are of great use for areas not accessible from the surface. Such cruises using submersible vehicles have to be optimized for the monitoring of several Descriptors under the MSFD (i.e. D1 biodiversity or D6 Seafloor integrity). They would be useful to assess marine litter far beyond the commonly used fishing grounds (soft bottoms) and the continental shelf and extend the assessment of marine litter in bathyal and abyssal environments. Moreover, the Mediterranean Sea is a special case, as its shelves are not extensive and its deep-sea environments can be influenced by the presence of coastal canyons. Continental shelves are proven accumulation zones, but they often gather smaller concentrations of debris than canyons without any specific measures taken until now

Marine debris monitoring in the deep Sea consists of at-sea surveys, estimates and impacts measurements. In the deep Mediterranean Sea data is scarce.

Protocols for the evaluation of deep sea litter are available for both video analysis and trawling (MSFD Technical Subgroup on Marine Litter, 2013). In the deep sea environment, monitoring of marine litter is to focus on specific aspects: D10C1 sea-bed litter, D10C2 microplastics in sediments, D10C3 microplastics ingested by deep-sea species and D10C4 benthic species entangled/covered by litter, possibly inventoried through videos from surveys with ROVs/ submersibles. Sampling has been is mostly opportunistic. The challenges are also to deal with the lack of standardization and compatibility between methods. For this, the institutional MEDITS survey programme (International Bottom Trawl Survey in the Mediterranean) is of real interest for european member states and has a real potential for monitoring (Bertrand et al., 2002). It is planned to sample benthic and demersal species between 80 and 800 m, through systematic bottom trawl surveys and with a common standardized sampling methodology and protocols at a Euro-Mediterranean scale. The recent collection of seafloor litter data on a regular basis will provide assessments at the basin scale, with a potential of 1280 sampling stations to be considered also from non-European countries. This monitoring scheme will be also of interest for regular sampling of deep sea demersal fishes for evaluating ingested microplastics, once methods of analysis will be agreed by the scientific community. Monitoring remains yet to be implemented for D10C4 (SPA/RAC, 2017), probably through sub regional experiments first, with reinforced coordination, capacity building, quality assurance, and harmonization.

10.3.2 Composition, amount and spatial distribution of litter (D10C1) - Primary

Litter (excluding micro-litter) shall be classified in the following categories: 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.

The MSFD requires that 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 subregional specificities.

Unit: amount of litter per category in number of items per square kilometre (km²) for surface layer of the water column and for seabed.

For this criteria and the sea floor, data is available through an opportunistic approach, using ROV or submersibles video. It may not be available on regular basis but will provide consistent and harmonized data, enabling a good evaluation of GES.

10.3.3 Composition, amount and spatial distribution of micro-litter (D10C2) - Primary

Micro-litter (particles < 5mm) shall be classified in the categories 'artificial polymer materials' and 'other'.

The MSFD requires that the composition, amount and spatial distribution of micro-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 subregional specificities.

Member States shall establish threshold values for these levels through cooperation at Union level, taking into account regional or subregional specificities.

Unit: amount of micro-litter per category in number of items and weight in grams (g) per kilogram (dry weight) (kg) of sediment for the coastline and for seabed.

With available and relevant protocols for large microplastics (>300µm) sampling of sediment will permit to assess the levels of microplastics a various depths and on regular basis, depending on ongoing cruises. Lower sized microparticles will need however some more complex analytical methods and improvements to avoid contamination and permit chemical characterization before implementation.

10.3.4 Amount of litter and micro-litter ingested by marine animals (D10C3) - Secondary

Litter and micro-litter shall be 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 subregional cooperation.

The MSFD requires that 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 subregional cooperation.

Unit : amount of litter and micro-litter per category 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.

The outcomes of criterion D10C3 shall also contribute to assessments under descriptor 1, where appropriate.

The monitoring may be based on incidental occurrences (e.g. stranding of dead animals, entangled animals or affected individuals per survey).

This criterion will be difficult to measure in the deep sea. Only microplastics ingested by deep sea fishes may provide sufficient data, but this will need to better define protocols, their limits and extensive surveys prior to provide relevant scientific and technical basis for monitoring.

10.3.5 Number of individuals of each species adversely affected (D10C4) - Secondary

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 subregional cooperation.

The MSFD requires that 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 subregional cooperation.

Unit: for each species, an estimate of the number of individuals in the assessment area that have been adversely affected (lethal; sub-lethal) per species.

The outcomes of this criterion shall also contribute to assessments under descriptor 1, where appropriate.

The monitoring may be based on incidental occurrences (e.g. stranding of dead animals, entangled animals or affected individuals per survey).

This criterion will need some research before the implementation of monitoring. Assessment of interactions between litter and marine organisms (entanglement, injuries, etc.) using video from ROVs or submersibles appears to have a good potential to support monitoring of benthic habitats.

10.4 CONCLUSIONS

10.4.1 Baselines and thresholds

The Mediterranean (or subregions in the sea) lack a baseline against which to measure progress. It is clear that baselines and targets for monitoring have to be proposed and adjusted to the specific constraints of the deep-sea environment. Due to the differences between the Mediterranean sub-regions in terms of litter densities in the deep, the unequal spread of available datasets, and some countries belonging to two or more sub-regions (Italy, Greece), it will be important to agree a process to define baselines and targets, either considering baselines for the various litter indicators at the level of the entire basin (Mediterranean Sea) or at the sub-regional level. UNEP/MedPol has already defined common baselines for some criteria/indicators that will have to be refined in the context of the deep-sea environment (Table 10.6).

Table 10.6: Proposed general baselines for monitoring marine litter found in the deep seafloor in the Mediterranean Sea (UNEP, 2015)

Indicator	minimum value	maximum value	mean value	Proposed baseline
17 seafloor (items/km^2)	0	7700	179	130-230

Clearly, these baseline values have been defined on the basis of the existing published literature and will need to be refined and specifically address the categories targeted by specific reductions measures.

Environmental targets are qualitative or quantitative statements that are important for management as they will enable regions to (i) link the aim of achieving objectives such as Good Environmental Status (GES) to the measures and effort needed, (ii) measure progress towards achieving the objective by means of associated indicator(s), and (iii) assess the success or failure of measures enacted to prevent marine litter from entering the seas and to support management and stakeholder awareness. Our current lack of knowledge with regards to metrics to be used is such that absolute targets are difficult to set; Moreover, it will be difficult to link baselines and targets to reduction measures since litter may be transported long distances, may accumulate for long periods and may degrade. Targets will then have to be considered in the special context of the deep sea where accumulation is a permanent process, collection is not possible, except in the case of fishing gears in limited shallower areas, and the only way out is the degradation with time that is not known and has not been addressed in the deep sea environments. The best strategy will define most relevant locations, locating hot spots areas (large cities, canyons, deep fishing grounds, converging zones, etc.), then focus on most important items by nature (plastic) or sources (fishing gears). For strategic and operational objectives, the marine litter MEDPOL regional plan listed a series of prevention and remediation measures that should be partly considered for the deep sea environment. In this compartment, quantitative reduction targets should nevertheless be considered. However, due to a large set of factors affecting the quantities and distribution of marine litter in a certain area, it can be very challenging to detect clear reduction trends in the deep sea that can be associated to the implementation of measures taken locally.

In regards to the coordinated monitoring strategy in the Mediterranean Sea and technical or scientific considerations, accessible targets were proposed (Table 10.7) considering baselines that may

be optimized after the 2015/2016 first results from monitoring (UNEP, 2015). Targets may focus on the total amount of marine litter first, with some specific targets on individual items after impacts of reduction measures can be evaluated. For seafloor litter and microplastics, a significant decrease in amount requires overcoming the constraints of diffuses and uncontrolled sources

Table 10.7: Operational targets of interest for the D10 in the Mediterranean Sea as proposed within the UNEP/MAP Marine Litter Regional Action Plan (UNEP, 2015)

ECAP Indicators	Type of target	Minimum	Maximum	Recommendation	Remark
SEAFLOOR LITTER (EI 17)	% decrease	stable	10% in 5 years	Statistically Significant	Targeted on specific top items may be more relevant and feasable
MICROPLASTICS (EI 17)	% decrease	-	-	Statistically Significant	sources are difficult to control (trans border movements)
INGESTED LITTER (EI 18)	% decrease in the rate of affected animals and quantity/weight of ingested litter			Statistically Significant	Movements of litter and Animals to be considered

Harmonization of sampling protocols for the seafloor and for microplastics are highly recommended because the comparability of available data remains restricted, especially with respect to different size class categories, sampling procedures, analytical methods, and reference values. Organization of data collection is another important task to consider prior to the implementation of monitoring. For now, and for the deep-sea environment, data collection is to be organized within various projects such as the MEDPOL RAP or MEDITS for the seafloor litter, with a DG MARE/EMODNET Chemistry –litter project to technically support this. This latter project is also planning to organize the collection and storage of microplastics data, directly of interest for deep sea environments. Some gaps are still remaining, such as (i) the management of data on indicators D10C3 (microplastics in deep sea organisms) and DC4 (deep sea organisms entangled in marine litter, including ghost nets), and (ii) the strategy to organize this data collection and storage.

10.4.2 Setting priorities

The MSFD Technical Subgroup on Marine Litter (2013), CIESM (2014), UNEP (UNEP, 2015), and the European project STAGES (http://www.stagesproject.eu) recently reviewed the gaps and research needs of knowledge, monitoring, and management of marine litter. This requires scientific cooperation among the parties involved prior to reduction measures due to complexity of issues.

Typically, valuable and comparable data could be obtained by standardizing our approaches. In terms of distribution and quantities, identification (size, type, possible impact), evaluation of accumulation areas (canyons, and specific deep sea zones), and detection of pathways bringing litter to the bottom, prior to locating hotspots and to better understanding transport dynamics. Further development and improvement of modelling tools must be considered for the evaluation and identification of both the sources and fate of litter. A better understanding of rates of degradation and the development of appropriate methodologies to quantify degraded materials, including nanoparticles will be an important step in terms of research.

Pilot-scale monitoring has now become an important step towards monitoring litter and its impacts in terms of determining baselines and/or adapting the strategy to local areas. A better understanding of entanglement (lethal or sub lethal) and of how litter is ingested by marine organisms are key questions for the deep sea environment. A more precise definition of targets (GES) and the identification of parameters/biological constraints and possible bias sources to be considered when defining the good environmental status. Work on specific deep sea "sentinel" species (fishes and invertebrates) is also important, as it may provide additional protocols supporting the measurement of impacts. The identification of species that settle on deep marine litter, the nature of constraints for the colonization of artificial substrates in the deep and the possible translocation of species in relation to transport of litter are also key questions to consider for a better understanding of harm.

Knowledge about the extent of ghost fishing is still very limited due to the costs and practical difficulties of underwater survey work and partial knowledge about fish stocks losses. There are actually no overall estimates of the extent of the problem for the Mediterranean as a whole and this has become a priority, especially in the deep sea.

In conclusion, marine litter in the Mediterranean has become a critical issue. Management and reduction still need to be developed, implemented and coordinated. However, a number of key issues will have be considered in order to provide a scientific and technical background for a consistent monitoring, a better management system, and science based reduction measures. The following points are relevant for the near future, with a list of actions and research to be initiated in order to improve basic knowledge and to support both monitoring and management of the deep sea environment:

1. Identify pathways in order to follow the transport of marine litter to the bottom.
2. Map hot spots (accumulation areas, areas at risk) of marine litter in the deep,
3. Evaluate the quantity and localization of lost fishing gears.
4. Determine the sinks for marine litter (budgets, fluxes, etc.) and better understand degradation
5. Define a GIS platform to support the integration and the analysis of all monitoring data.
6. List (inventory) species (also biofilms) settled on litter in the Mediterranean deep sea environment; Develop a database on rafted species to better explain the risk of dispersion and the possible colonization of new areas
7. Evaluate the distribution and changes of micro plastics in the seafloor/deep seafloor and quantify ingested micro plastics in key species, in deep to demersal species.
8. Support the rationalization of monitoring (i.e. common and comparable monitoring approaches (standards/baselines, inter calibration, data management system and analysis/quality insurance).
9. Define specific baselines and targets for important litter categories found in deep seas, that may individually targeted by reduction plans or measures by the Mediterranean countries (plastic bags, fishing gears)
10. Identify new indicator species for assessment of impact (entanglement, ingestion, micro plastics, and rafted species), and define thresholds for harm
11. Evaluate the potential loss of fish stocks due to the main types of abandoned/lost fishing gears
12. Organize and harmonize ghost fish collection.

For the deep Sea, and within the context of MSFD, points 2, 4, 7 and 8 are more critical when point 11 will have to be considered in the plan for reduction measures

10.4.3 Recommendations:

An analysis of existing data, reports, syntheses and recommendations together with the consideration of specific constraints of the deep sea environment led us to consider the following necessary steps to support implementation of MSFD for descriptor D10 in the deep Sea:

- Because of the technical constraints and necessary means to access the deep sea environment, most activities will rely on opportunistic approaches.
- The harmonization of protocols, data collection frameworks (EMODNET, MEDITS; UNEP/MAP/MEDPOL), enabling a common management of data and the possible extension to non EU country is a priority and will have to be partially addressed within IDEM. The project will ensure an access to deep sea litter data (from international bottom trawl surveys and EMODNET data base on micro plastics under development).
- Locate accumulation areas, when possible, and define reasons why litter accumulates?
- For D10C2 (Micro plastics): A synthetic review of existing data for the deep sea and the identification of the most relevant protocols for their application to the deep sea will be the first step. This will be better coordinated with activities from the MEDCIS project, considering specific constraints for the deep sea environment (relevance of D10C3 indicators, way to collect samples and organize deep sea monitoring), for sampling and for protocols.
- Baselines: Linking the work with the one underway within GES TG marine litter, IDEM will analyze the specificity of top items (plastics, fishing gears, etc.), define specific baselines for this main litter types, at basin scale and evaluate the relevance of defining specific baselines for micro plastics in deep sea sediments
- IDEM will identify the possible targets settings when reduction cannot be by other means than normal degradation at sea that is a long term process. For deep seafloor, the focus will be on ghost nets and plastics/micro plastics.
- Linking with the project INDICIT to evaluate the potential of video analysis for D10C4, IDEM will contribute to the definition of specific protocols /guidance using video analysis to monitor entangled organisms (in fishing gears for fishes or covered by plastics for invertebrates).
- Organize the development of common and harmonized schemes to collect data using video systems
- Consider and possibly evaluate the acceptable levels of marine litter for the deep sea environment and initiate the development of indicators dedicated to economic impacts in the deep sea environment (ghost fishing, costs of litter to ecosystem services).
- Promote dissemination of protocols to the deep sea community
- A main deliverable will consist of a report addressing priorities and proposing specific deep sea baselines/targets for main litter items, when possible.

11 Descriptor 11 : Introduction of Energy

The eleventh descriptor (D11) deals with the introduction of energy in the marine environment by human activities. It states that the "*introduction, including underwater noise, must be at levels that do not adversely affect the environment*". In this regard, the MSFD recognizes underwater noise as a marine pollution.

The aim of the European Union's Marine Strategy (Directive, 2008/56/EC) is, in fact, to protect more effectively the marine environment across Europe from all the pressures derived by human activities. Article 3(8) of the Directive states that: " *'pollution' means the direct or indirect introduction into the marine environment, as a result of human activity, of substances or energy, including human-induced marine underwater noise, which results or is likely to result in deleterious effects such as harm to living resources and marine ecosystems*''. In addition, marine underwater noise appears also in ANNEX I (i.e. Qualitative descriptors for determining good environmental status) as the ''introduction of energy at levels that do not adversely affect the marine environment'' and is listed in ANNEX III (i.e. indicative lists of characteristics, pressures and impacts) where underwater noise (e.g. from shipping, underwater acoustic equipment) is defined as a ''physical disturbance''.

11.1 State Of The Art

Sound travels approximately four times faster in seawater than in air (Brekhovskikh and Lysanov, 2006; Au and Hastings, 2009; Ross, 2013), covering longer distances than in air (Nelms et al., 2016; Prideaux, 2017). Many marine organisms use sound to communicate, navigate and locate food. On the other hand, an array of human activities produces underwater noise, often at similar frequencies, which may have harmful effects on marine biodiversity.

11.1.1 Pressures

Ocean ambient noise is generated by a variety of sources, either from physical processes and marine organisms. In addition, the last hundred years have seen the introduction of many anthropogenic sources that are currently contributing to the general noise budget of the oceans. The extent to which noise in the sea impacts and affects marine ecosystems has become a topic of considerable concern to the scientific community (Wenz, 1962; National Research Council, 2003; Hildebrand, 2009).

Natural causes include geophysical events such as wind-generated waves, earthquakes, precipitation, and cracking ice, as well as biological phenomena such as whale songs, dolphin clicks, and fish vocalizations. Fish utilize sound for navigation and selection of habitat, mating, and communication (Simpson et al., 2005). Marine mammals use sound as a primary means for underwater communication and sensing (Wartzok and Ketten, 1999). Toothed whales have developed sophisticated echolocation systems to sense and track the presence of prey (Au, 1993). Baleen whales have developed long-range acoustic communication systems to facilitate mating and social interaction (Edds-Walton, 1997).

Anthropogenic sources of noise can affect marine organisms and include commercial shipping, oil and gas exploration, development and production (e.g. air-guns, ships, oil drilling); naval operations (e.g. military sonars, communications, and explosions); fishing (e.g. commercial/civilian sonars, acoustic deterrent, and harassment devices); research (e.g. air-guns, sonars, telemetry, communication, and navigation); and other activities such as construction, icebreaking, and recreational boating (Hildebrand, 2009). Anthropogenic sounds emitted from different human activities vary significantly in terms of the frequencies and intensities (Table 11.1) (Buck, 1995; Richardson et al., 1995; Gisiner et al., 1998; Conservation and Development Problem Solving Team, 2000).

Table 11.1. Examples of reported anthropogenic noise in the sea with various frequencies and intensity levels, from Peng et al. (2015).

Types of the Anthropogenic Sound	Frequency	Intensity Level	References
Bottom-founded oil drilling and mining	4–38 Hz	119–127 dB re 1 µPa	Richardson et al., 1995
Pile driving	30–40 Hz	131–135 dB re 1 µPa	Richardson et al., 1995
Drillship	20–1000 Hz	174–185 dB re 1 µPa	Richardson et al., 1995
Semisubmersible drilling vessel	10–4000 Hz	~154 dB re 1 µPa	Richardson et al., 1995
Seismic airguns	100–250 Hz	240–250 dB re 1 µPa	Richardson et al., 1995
The Acoustic Thermometry of Ocean Climate Project (ATOC)	~75 Hz	~195 dB re 1 µPa	Buck, 1995
Navy Sonar	100–500 Hz	~215 dB re 1 µPa	Conservation and development problem solving team, University of Maryland, 2000
High Frequency Marine Mammal Monitoring Sonar (HF/M3)	~3000 Hz	~220 dB re 1 µPa	Conservation and development problem solving team, University of Maryland, 2001
Supertanker & container ship	6.8–70 Hz	180–205 dB re 1 µPa	Richardson et al., 1995 Gisiner et al., 1998
Medium size ship (ferries)	~50 Hz	150–170 dB re 1 µPa	Richardson et al., 1995
Boats (<30 m in length)	<300 Hz	~175 dB re 1 µPa	Richardson et al., 1995
Small ship (support & supply ship)	20–1000 Hz	170–180 dB re 1 µPa	Richardsonet al., 1995

The sources of ocean ambient noise can be divided into 3 frequency bands: low (10 to 500 Hz), medium (500 Hz to 25 kHz) and high (> 25 kHz) (Hildebrand, 2009). In general, low-frequency sources experience little attenuation, thus allowing for long-range propagation, until the deep sea.

A generalized deep-water ocean ambient noise spectrum is presented in Fig. 11.1. This graph follows the one presented by Wenz in 1962, but it has been modified to reflect higher levels of low-frequency ambient noise than levels common in the 1960s, owing to increased anthropogenic activity (McDonald et al., 2006) and an improved understanding of low-frequency noise levels in the absence of anthropogenic sources (Kewley et al., 1990; Cato and McCauley, 2002).
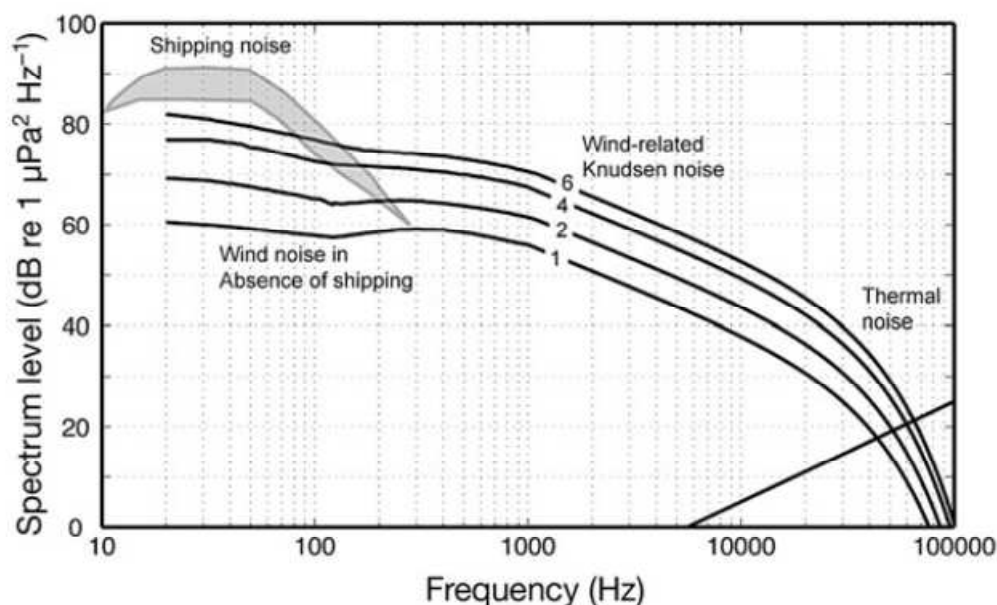
Figure 11.1. *Generalized ocean ambient noise spectral levels for a deep-water site with the receiver located at 1000 m depth. Four different noise mechanisms are denoted in the plot: thermal noise (Mellen, 1952); wind related noise (Knudsen et al., 1948), which is a function of sea state (numbered curves); wind noise at low frequency observed in the absence of shipping (Cato and McCauley, 2002); and modern shipping noise (grey shading) (McDonald et al., 2006), from Hildebrand (2009).*

The low-frequency band, that can propagate easily to the deep sea, is dominated by anthropogenic sources: primarily, commercial shipping and, secondarily, seismic exploration. Since the 1960s, the number of ships has nearly doubled, and the vessel size and propulsion power have generally increased, ambient noise levels have correspondingly increased over the same period.

In addition to shipping, during last decade, the noise caused by seismic surveys (i.e., airguns) has become a major concern since the number of surveys increased (especially in the south-eastern European part of the Mediterranean Sea). For geophysicists, seismologists and oceanographers, sound is the most powerful tool available to determine the geological structure of the seabed and to look for oil and gas reserves deep below the seafloor (André et al., 2011b; Frisk, 2012). A seismic survey is a form of geophysical survey widely used for oil and gas (hydrocarbon) exploration. It tries to deduce elastic properties of material by measuring their response to seismic (elastic) waves, produced by airguns (Fig. 11.2). The air guns produce high-intensity, low-frequency impulsive noise at regular intervals, mostly between 10 and 300 Hz (Carroll et al., 2017). The typical sound intensity level of each pulse of an air gun array is around 260-262 dB in water at 1m and surveys typically run continuously over many weeks (Prideaux, 2017).

Oil industry operations have traditionally been conducted in shallow water on the continental shelf, but, during the past few decades, exploration has moved to deeper waters (>500 m) along the continental slope. As oil exploration has moved into deeper water, the potential for long-range propagation of seismic signals has increased. Indeed, in deep-sea waters there is a greater potential for sound to propagate at great distances, crossing ocean basins, by coupling into the deep sound channel.
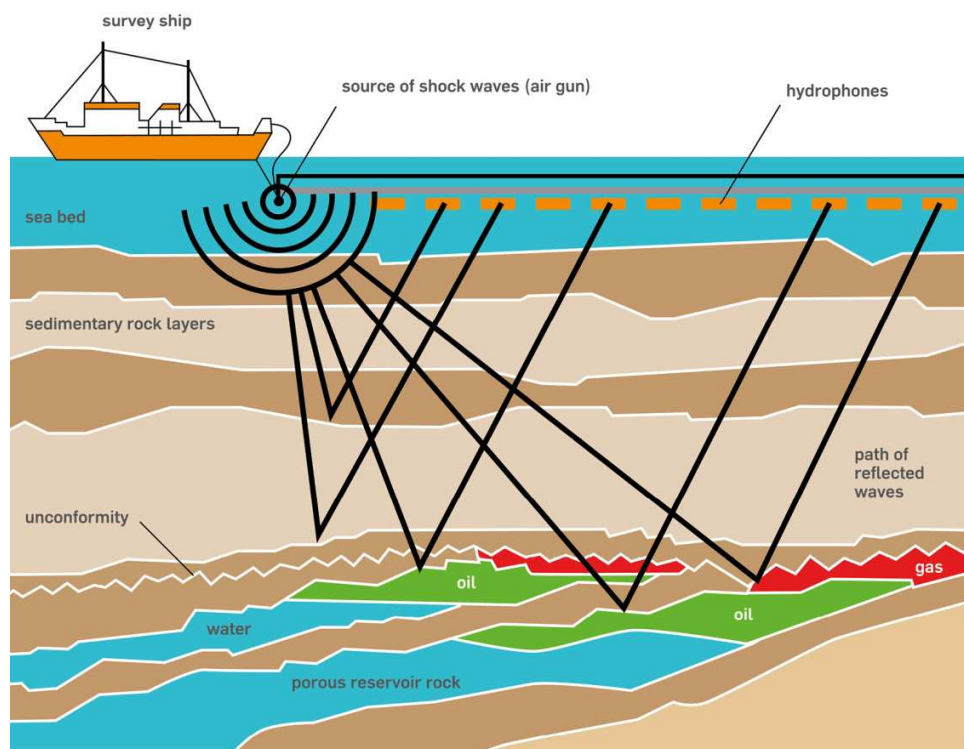
Figure 11.2. *Scheme of an offshore seismic survey*

During last ten years, the Mediterranean Sea, and especially its south-eastern portion (see also chapters on D6 and D7 on this point), has become an area of growing interest for exploration and exploitation of oil and gas. While the strait of Sicily and part of the Levantine Sea off Cyprus were surveyed since the early 2000's, in 2012 and 2013, seismic surveys extended to the areas of the Adriatic Sea and Aegean Sea (Maglio et al., 2016). As it stands now, all regional seas in the south-eastern Med, apart from the Aegean Sea, are to be subjects of future seismic surveys. In addition to the planned future surveys (Maglio et al., 2016), new developments are planned in the area of the southern Adriatic, off Montenegro near the border with Albania and south of Cyprus. Furthermore, in the spring of 2017, Turkey launched a vessel to carry out seismic surveys northwest of Cypriot island, in the EEZ (ENI, 2017). This fact stresses the transboundary feature of the seismic surveys issue and calls for cooperation among countries.

11.1.2 Possible Impacts

Marine mammals, sea turtles, and fish are known to be the most susceptible marine organisms to anthropogenic noise. The occurrence of low frequency noise in the deeper part of the basins will be particularly important for deep-diving marine mammals, such as beaked whales, since the ambient noise they use as a background for echolocation will decrease rapidly with depth.

Although long-term changes in ambient noise levels may have significant impact on marine mammal behavior (Foote et al., 2004), our understanding of noise level variations on decadal time scales is scarce (Tyack, 2008). Also our knowledge about the environmental risks of anthropogenic noise on marine

organisms is still scant (Dolman et al., 2011; Papanicolopulu, 2011). The question is whether human-generated noise may interfere with the normal use of sound by the marine animals (i.e. chronic effects that may affect the long-term ability of marine animals to develop their normal activities, reproduce, and maintain sustainable populations) or cause physical harm to them (i.e. acute effects that may compromise the short-term ability of these animals to survive).

Studies showed that anthropogenic noise can cause auditory masking, leading to cochlear damage, changes in individual and social behavior, altered metabolisms, hampered population recruitment, and can subsequently affect the health and service functions of marine ecosystems (Peng et al., 2015). Organisms that are exposed to elevated or prolonged anthropogenic noise may experience direct injury ranging from bruising to organ rupture and death (barotrauma). This damage can also include permanent or temporary auditory threshold shifts, compromising the animal's communication and ability to detect threats. Marine organisms can be also displaced from their habitats. Finally, noise can mask important natural sounds, such as the call of a mate, the sound made by prey or a predator. In addition, factors such as stress, distraction, confusion and panic, can affect reproduction, death and growth rates, in turn affecting the long-term welfare of populations of animals (Southall et al., 2000; Southall et al., 2008; Popper et al., 2014; Hawkins and Popper, 2016; Prideaux, 2017).

Despite a knowledge gap remains regarding impacts on deep-sea organisms, certain effects have been documented. Firstly, our knowledge about actual impacts of anthropogenic underwater noise, such as those from seismic surveys on marine mammals, has grown considerably in recent years. Seismic surveys can cause death, damage to body tissue, (e.g. internal hemorrhaging, disruption of gas-filled organs like the swim bladder), interruption of normal activities including feeding, schooling, spawning, migration, and displacement from favored areas.

Regarding sea turtles, they usually do not reach extreme depths, even if *Caretta caretta* can reach a maximum depth of 233 m depth (Lutcavage and Lutz, 1997). The effects of anthropogenic noise especially from seismic surveys (airgun: array peak source level 252 dB re 1 uPa) on sea turtles is much less researched than those on marine mammals. Research on sea turtles performed in the south-eastern Mediterranean region shows that they can detect sounds at low frequencies, which overlap with the low frequency of seismic airguns (Nelms et al., 2016). Similar to marine mammals, noise from airguns induced a dive response in loggerhead turtles, *Caretta caretta*, which indicated an induced avoidance response (DeRuiter and Larbi Doukara, 2012). They can potentially cause physical damage, hearing damage, behavioural change, chronic impacts, and stress (Popper et al., 2014). Besides sea turtles, also sharks and rays are poor studied in anthropogenic noise impact studies (Weilgart, 2017) and most of the research has been carried out outside of the Mediterranean region. Anthropogenic noise, both from shipping and seismic surveys, may have physical, behavioural, physiological, and catch rate effects on both fish and invertebrates (Carroll et al., 2017). Regarding fishes, their sensitivity to certain frequencies varies in the different species. For instance, the cartilaginous fish (sharks, rays), which lack gas-filled air bladders, are highly sensitive to low frequency sound (approximately 20 to 1,500 Hz) (Casper et al., 2013). Fish with swim bladders are more susceptible to physical injury such as barotrauma (Popper et al., 2014).

Regarding the effects on invertebrates, recent studies show that widely used marine seismic survey airgun operations negatively impact zooplankton (McCauley et al., 2017). In addition, it is also documented for shallow-water organisms that ship noise even affects DNA integrity of mussels. These impacts can cause reduced growth, reproduction, and immune response (Wale et al., 2016). Further studies are needed to increase our knowledge about the impacts of anthropogenic noise also on deep-sea marine organisms. Similarly, André et al. (2011a) demonstrated morphological and ultrastructural evidence of massive acoustic trauma in four cephalopod species (*Loligo vulgaris*, *Sepia*

officinalis, *Octopus vulgaris*, and *Illex coindetii, two of these, I. coindetii and L. vulgaris* can be found in waters deepr than 200 m, while the occurrence of *O. vulgaris* below 200 m, is scarce and occasional) subjected to low-frequency noise exposure, which caused permanent and substantial alterations of the sensory hair cells of the statocysts. Outside the Mediterranean, the noise generated by geophysical seismic surveys (peak sound pressure levels at 175 dB re 1 µPa) has been singled out as the cause for atypical mass strandings of giant squids (*Architeuthis dux*) as well (Guerra et al., 2011). The full extent of the impact of seismic surveys at the population level is mostly unknown, partially due to the lack of baseline knowledge about the abundance and distribution of species.

11.2 Where Is The Knowledge

The Agreement on the Conservation of Cetaceans in the Black Sea, the Mediterranean Sea and the contiguous Atlantic area (ACCOBAMS), in accordance with the Secretariat of the Mediterranean Action Plan of the United Nations Environment Program (UNEP/MAP), launched a study to develop a basin-wide strategy for underwater noise monitoring in the Mediterranean Sea.

To help managing the deep-sea marine ecosystem and mitigating adverse effects of anthropogenic noise on deep-sea organisms, there is a need to understand more about the role of sound production and reception in the behavior, physiology, and ecology of these species and to provide insights into important aspects of their biology. This understanding, in turn, previously requires the detection and identification of the sound sources of interest under real conditions.

The most generally accepted prediction of noise level trend at low frequencies is due to Ross (1987, 1993) who used noise levels from the 1950s and data published by Wenz from the mid-1960s to predict an increase of about 3 dB/decade or 0.55 dB/yr.

Although there is some evidence, as discussed above, about the effects of shipping and airgun noise on marine organisms, more data and observations are needed to understand the bioecological implications for deep Mediterranean Sea.

The development and broad use of passive acoustic monitoring techniques have the potential to help assessing the large-scale influence of artificial noise on marine organisms and ecosystems. Deep-sea observatories have the potential to play a key role in understanding these recent acoustic changes. LIDO (Listening to the Deep Ocean Environment) is an international program (http://www.listentothedeep.com/acoustics/index2.php?web=presentation&lang=en) that is allowing the real-time long-term monitoring of marine ambient noise as well as marine mammal sounds at cabled and standalone observatories (André et al., 2011b).

In addition, in 2012, the cabled deep-sea multidisciplinary observatory, "NEMO-SN1", was deployed in the Ionian Sea, at a depth of 2100 m, 25 km off Catania. NEMO-SN1 was equipped with a seismic hydrophone, that allowed to monitor fin whale and low frequency noise (10 Hz–1000 Hz). Both shipping noise and seismic airgun pulses highly contributed to the low frequency background noise. Average values and percentile distribution of noise were measured on all the acquired data and, separately, in a contour of each airgun pulse detected. Seismic airgun pulses were detected in 4 of the 10 analyzed months and they were presumably produced hundreds of km away from sensor's location. Airguns were also detected in presence of emitting fin whales (Sciacca et al., 2016).

A recent report by Maglio et al. (2016) describes the extension of the seismic survey areas in the Mediterranean Sea between 2005 and 2015. In the last 10 years, the Ionian Sea has been among the regions with the highest numbers of seismic exploration activities (considering both the number of surveys and the number of permits for explorations). In particular, between 2012 and 2013, an extended area off the coast of Greece was surveyed and results from Sciacca et al. (2016) seem to confirm the presence of seismic survey vessels located in this area.

Further studies on the impact of noise on the Mediterranean fin whale and on the other cetacean species living in or transiting the Ionian Sea (Caruso et al., 2015) are foreseen, taking advantage of the data provided by the acoustic antennas of the KM3NeT project (Viola et al., 2014; KM3NeT Collaboration, 2015). These arrays of acoustic sensors are going to be installed on the KM3NeT Infrastructure between 2500 and 3500 m depth, offshore Portopalo di Capo Passero, in South-East Sicily (Adrián-Martinez et al., 2016).

11.3 How is introduction of underwater noise measured

11.3.1 Methodological sampling aspects

Underwater noise can be classified as impulsive or continuous in terms of its effects on marine life. It is well known that high powered impulsive noise may cause direct acute effects such as hearing loss, tissue damages and death to individuals of sensitive species such as cetaceans. It may also cause the permanent displacement of organisms from the feeding area. Continuous noise entails a chronic exposure mainly associated with stress and behavioral changes potentially leading to negative effects at the population level over time.

Other than the impulsive or continuous nature of noise, its frequency spectrum is relevant for designating indicators, because sound propagation is frequency dependent. Also, marine species sensitivity to sound is frequency dependent. Therefore, on one hand, the frequency nature of noise should be considered as a determining factor, on the other hand key species (sensitive, emblematic, etc.) of the Mediterranean Sea should be considered while addressing monitoring guidance.

The descriptor 11 of the Marine Strategy Framework Directive of the European Union and therefore two separate criteria are used for impulsive noise and continuous noise (criteria D11C1 and D11C2, respectively). D11C1 addresses space-time distribution of impulsive noise sources, while D11C2 addresses levels of continuous noise through the use of measurements and models. The proposed strategy on noise monitoring recommends several adaptations for deep Mediterranean case. Particularly, both indicators are closely related to the acoustic biology of key deep-diving marine mammal species of the Mediterranean.

The main challenge is to understand the large-scale influence of artificial noise on marine organisms and ecosystems. The use of deep-sea observatories can be crucial in the assessment and monitoring of these natural and anthropogenic signals. In this sense all the deep-sea cabled observatories of the Mediterranean (i.e. NEMOS-SN1 in the Western Ionian Sea, ANTARES in the Ligurian Sea and Pylos in the South Ionian Sea) are equipped with hydrophones for passive acoustic monitoring.

There is general lack of comparability of the approaches which has made the results of the assessment of the D11 descriptor quite diverse. The coherence across EU member states can be ensured by the inclusion of three subprograms related to in situ noise measurement, traffic monitoring and impulse sound register.

11.3.2   Anthropogenic impulsive sound in water. (D11C1) - Primary

The spatial distribution, temporal extent, and levels of anthropogenic impulsive sound sources do not exceed levels that adversely affect populations of marine animals. Member States shall establish threshold values for these levels through cooperation at Union level, taking into account regional or subregional specificities

Anthropogenic continuous low-frequency sound in water in number of days per quarter (or per month if appropriate) with impulsive sound sources; proportion (percentage) of unit areas or extent in square kilometres (km2) of assessment area with impulsive sound sources per year.

Impulsive noise can be monitored by setting up a register of anthropogenic activities which use loud noise sources. By knowing the date and location of such activities, the proportion of days within a given period, and over a given geographical scale, in which activities generating impulsive sounds take place can be computed, monitored and managed.

In order to establish the register, for each of the above activities, the basic information required to derive the number of days in which activities using impulsive sources occur in an area, are:
  - Position data (geographic position: lat/long)
  - Period of operation (start – end)
  - Source Level
  - Number of hours of activity per day
  - Duty cycle (ON/OFF ratio) or % of time ON
  - Frequency range
  - Source level (for mid-frequency sources)

11.3.3   Anthropogenic continuous low-frequency sound in water. (D11C2) - Primary

The spatial distribution, temporal extent, and levels of anthropogenic impulsive sound sources do not exceed levels that adversely affect populations of marine animals. Member States shall establish threshold values for these levels through cooperation at Union level, taking into account regional or subregional specificities

Anthropogenic continuous low-frequency sound in water in number of days per quarter (or per month if appropriate) with impulsive sound sources; proportion (percentage) of unit areas or extent in square kilometres (km2) of assessment area with impulsive sound sources per year.

D11C2 can be measured by in-situ acoustic measurements for gathering fundamental field data to establish information on the ambient noise in a given location. Besides this, especially for deep-sea monitoring, models can be applied because they reduce the time required to establish a trend. Indeed, the expected trend in shipping noise, based on observations in deep water, is of the order of 0.1 dB/year, thus taking many years, possibly decades, to reveal such small trends without the help of spatial averaging. Models can also help reducing the number of stations required to establish a trend over a fixed amount of time, therefore reducing the cost of monitoring. The use of models can help with the choice of monitoring positions and equipment, i.e., selecting locations where explosions or seismic surveys are dominant. It is also highly recommended to use models to produce noise maps, which are a valuable tool to quickly understand the ensonification levels over large areas, and a fundamental tool to calculate the extent of potentially impacted (non-GES) areas.

11.4   Knowledge On Threshold

For GES assessment related to D11, three thresholds need to be established: a spatial and a temporal threshold concerning D11C1 and a noise threshold concerning D11C2.

Because of the lack of data on the response of deep-sea communities to anthropogenic stress, none of the member states has defined thresholds nor even baseline levels.

Regulators have often sought to establish a particular noise level that would trigger management action, such as temporary shut-down of the noise source until the cetacean moves away (Weilgart, 2007). Such a noise level has been very difficult to determine, particularly as there is such a wide variety of responses between species, situations, and noise sources, especially in the deep sea.

Dedicated research projects should be carried out to provide new baseline information on both the temporal and spatial coverage, i.e. number of days over a year and number of cells over a grid respectively, at which activities using impulsive noise sources occur. On the other hand, baseline knowledge about ambient noise levels throughout the Mediterranean is limited, and the effects of noise are not sufficiently known to robustly determine whether existing levels are too high, or if GES is being achieved. A thorough review of available literature on ambient noise in the Mediterranean Sea is needed to identify the threshold for ambient noise.

11.5   CONCLUSIONS

11.5.1   Gaps

Management implications of noise impacts include difficulties in establishing ''safe'' exposure levels. Knowledge about threats and their impacts on deep-sea organisms is also limited. Spatial and temporal thresholds should be reviewed for implementing both criteria. There is an urgent need to review the necessary baseline information on space-time distribution of impulsive/continuous noise sources and ambient noise data available for the Mediterranean Sea in order to identify the thresholds.

A Mediterranean impulsive noise register (ACCOBAMS, 2017) should be established. The idea of register was initiated by the MSFD, expanded to the Mediterranean Sea area through the Ecosystem Approach Initiative led by the UNEP/MAP Barcelona Convention. Finally, the responsibility for register's establishment was given to ACCOBAMS (ACCOBAMS, 2016).

It is also important to improve our knowledge about the actual status of species, which can be identified through the IUCN Red List Assessment or the conservation status under the Habitats Directive. For cetaceans specifically, the assessment in the ACCOBAMS area together with the IUCN was made in 2006 and the following will be implemented after the ACCOBAMS survey. Availability of data is an issue. A significant amount of data about noise sources is available via the web, but there is no central place for these data. Also, there is an issue of confidentiality regarding implemented seismic surveys.

National budgets are also very limited and the majority of the countries is not able to adequately finance implementation of conservation mechanisms and measures. There are many requirements coming from the EU legislation and strategies, hence the EU provides a good share of the funding in the Region. Some of the EU funding possibilities include transboundary programmes like INTERREG, LIFE or Structural and investment funds, for which allocation is negotiated bilaterally between countries and the EC 8 (ACCOBAMS, 2016).

11.5.2 Recommendations

It is highly recommended, whenever possible, to use deep monitoring stations, either autonomous or cabled, to limit the influence of surface and sub-surface noise. Recommendations for the placement of measurement devices are listed as follows: i) monitoring in both high traffic and low traffic areas, also searching and including spots where the noise is supposed to be the lowest; ii) monitoring may be more cost effective if existing oceanographic stations (e.g. EMSO/INFN networks) included noise monitoring along with the other oceanographic variables already being monitored; iii) consider local topography and bathymetry effects e.g. where there are pronounced coastal landscapes or islands/archipelagos it may be appropriate to place hydrophones on both sides of the feature; iv) as far as possible avoid locations close to other sound producing sources that might interfere with measurements, e.g. oil and gas exploration or offshore construction activities. Areas of particularly high tidal currents may also affect the quality of the measurement. v) Monitoring station should be primarily located in important cetacean habitat, such as all the off-shore IMMAs (Important Marine Mammal Areas) of the Mediterranean, as the Alboran sea, the Pelagos Sanctuary in the Ligurian Sea and the Strait of Sicily-Western Ionian Sea (see Fig. 11.3).
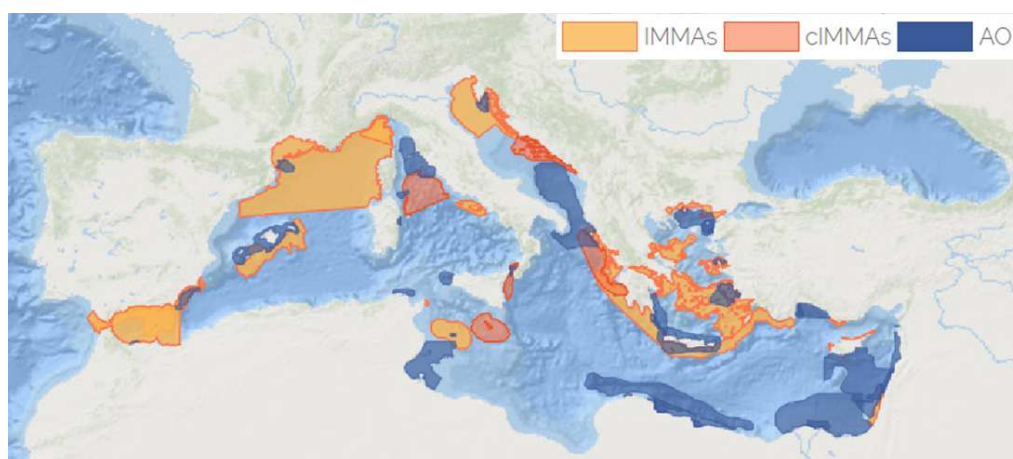

Figure 11.3. Map of the Important Marine Mammal Areas (IMMAS), candidate IMAMS (CIMMAs) and Areas of Interest (AoI) which are presently pending further assessment by marine mammal experts working with the IUCN-MMPATF (Marine Mammal Protected Area Task Force) (source IMMA e-Atlas).

For research purposes, and with a view to robustly support the implementation of mitigation policies, it is recommended to increase time resolution to describe fine scale temporal variations of noise levels to be possibly correlated with human activities (e.g. ship traffic). Ship noise and seismic airgun surveys are increasingly recognized as significant disturbing factors that affect fin whale acoustic habitat and threat populations' survival. The impact of these factors should be evaluated especially in critical areas, such as feeding and breeding grounds (Castellote et al., 2010), where vital functions of a population may be heavily at risk. In this context, the development of new monitoring techniques actively contributes to promote good conservation and management practices for the species and for areas of strategic ecological importance. Hence, the long-term analysis of noise levels, together with the study of the possible acoustic, behavioral and physical responses to noise by the animals, are fundamental tools in understanding the impact of the underwater noise on the conservation of species.

The general lines to improve coherency at the regional level are explicitly given in the revised decision (Commission Decision 2017/848/EC), which brings new elements about collaboration at the European level and about the establishment of thresholds. In particular, the expectation that the

threshold values should reflect the potential risks to the marine environment tend to promote the idea that the GES should be defined consistently with thresholds.

The thresholds have to be defined through a collaborative process under the Common Implementation Strategy auspices. However, the decision acknowledges the fact that the process should lead to specific thresholds relevant to a region, subregion or subdivision. In this context, a practical solution could be that MS agree on guidance for GES definition (e.g. indicator and/or criteria thresholds and how to apply them). The thresholds should be adapted to lead to a GES definition which account for subregional particularities (scales, species or other specific ecosystems) in setting the threshold values, as pointed out in Walmsley et al. (2017).

Possible improvements lie in an optimization of the monitoring strategies (spatial resolution, long term monitoring positions, data sharing, ambient noise models benchmark...). In terms of environmental and anthropogenic activity data, which are critical in sound mapping, a possible improvement lies in a better link between others EU policies and projects for instance to feed models with EU referenced data set (as for instance for maritime surveillance data). In addition, a peculiar attention has to be paid for neighboring subregions to ensure coherency and relevance in cross-border assessments.

Finally, the use of statistical -correlation between D11 and D1 indicators and criteria has been proposed and is a possible way to build relevant impact indicators. For the next cycles, the possibility to cross-correlate D1 and D11 indicators at least to a minimum extend (some sub region or subdivision, some sensitive species whenever data set are available and relevant) is to be studied as a complement of impact indicators based upon the behavioral response of species (QuietMED, 2017).

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