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Published Version:
Towards a marine strategy for the deep Mediterranean Sea: Analysis of current ecological status / Danovaro R.; Fanelli E.; Canals M.; Ciuffardi T.; Fabri M.-C.; Taviani M.; Argyrou M.; Azzurro E.; Bianchelli S.; Cantafaro A.; Carugati L.; Corinaldesi C.; de Haan W.P.; Dell'Anno A.; Evans J.; Foglini F.; Galil B.; Gianni M.; Goren M.; Greco S.; Grimalt J.; Guell-Bujons Q.; Jadaud A.; Knittweis L.; Lopez J.L.; Sanchez-Vidal A.; Schembri P.J.; Snelgrove P.; Vaz S.; Angeletti L.; Barsanti M.; Borg J.A.; Bosso M.; Brind'Amour A.; Castellan G.; Conte F.; Delbono I.; Galgani F.; Morgana G.; Prato S.; Schirone A.; Soldevila E.. - In: MARINE POLICY. - ISSN 0308-597X. - STAMPA. - 112:(2020), pp. 103781.1-103781.18. [10.1016/j.marpol.2019.103781]

This version is available at: https://hdl.handle.net/11585/744200 since: 2020-03-02

Published:

DOI: http://doi.org/10.1016/j.marpol.2019.103781

R. Danovaro, E. Fanelli, M. Canals, T. Ciuffardi, M.-C. Fabri, M. Taviani, M. Argyrou, E. Azzurro, S. Bianchelli, A. Cantafaro, L. Carugati, C. Corinaldesi, W.P. de Haan, A. Dell'Anno, J. Evans, F. Foglini, B. Galil, M. Gianni, M. Goren, S. Greco, J. Grimalt, Q. Güell-Bujons, A. Jadaud, L. Knittweis, J.L. Lopez, A. Sanchez-Vidal, P.J. Schembri, P. Snelgrove, S. Vaz, L. Angeletti, M. Barsanti, J.A. Borg, M. Bosso, A. Brind'Amour, G. Castellan, F. Conte, I. Delbono, F. Galgani, G. Morgana, S. Prato, A. Schirone, E. Soldevila, Towards a marine strategy for the deep Mediterranean Sea: Analysis of current ecological status, Marine Policy, Volume 112, 2020, 103781, ISSN 0308-597X

The final published version is available online at: https://doi.org/10.1016/j.marpol.2019.103781

Marine Policy
February 2020, Volume 112 Pages 103781 (18p.)
https://doi.org/10.1016/j.marpol.2019.103781
https://archimer.ifremer.fr/doc/00609/72110/

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Abstract :

The Marine Strategy Framework Directive (MSFD), introduced in June 2008, was adopted to achieve a Good Environmental Status (GES) in the EU's marine waters and to protect resources of socio-economic interest. The MSFD exerts 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), including the deep-sea waters, seafloor and sub-seafloor of the Exclusive Economic Zones (EEZ). However, currently the MSFD focuses on coastal habitats and the shallow-water seafloor to the detriment of the deeper habitats. Despite the huge dimension of the deep sea (below 200 m of depth) covering more than 65% of the Earth's surface and including >95% of the global biosphere, the relevance of the dark portion of the seas and oceans is still almost completely neglected. Given the important bi-directional links between shallow and deep ecosystems, there is a clear need for extending the implementation of the MSFD into the deep sea, to define a sound ecosystem-based approach for the management and protection of deep-sea ecosystems and attain GES. We assembled data on drivers, anthropogenic pressures and impacts concerning the MSFD descriptors pertaining to the Mediterranean deep sea. We list deep-sea monitoring activities and the main sources providing benchmark conditions, and discuss knowledge and geographic coverage gaps. MSFD descriptors apply to the deep sea as to coastal waters, and ought to be monitored contemporaneously. We provide recommendations for guidelines for future deep-sea monitoring in the Mediterranean Sea.

Highlights

► MSFD fails to cover the huge dimension of deep-sea environments and important bi-directional link with shallow ones. ► Extending MSFD to the deep sea and defining an ecosystem-based approach for its management and protection is urgently needed. ► Data on drivers, anthropogenic pressures and impacts regarding the MSFD descriptors for deep-sea Mediterranean were reviewed. ► Deep-sea monitoring activities were discussed and knowledge and geographic coverage gaps evidenced. ► Recommendations for guidelines for future deep-sea monitoring were provided.

Keywords : Marine strategy framework directive, Deep-sea ecosystems, Mediterranean basin

List of acronyms and abbreviations

ABNJ: Areas Beyond National Jurisdiction
ACCOBAMS: Agreement on the Conservation of Cetaceans in the Black Sea Mediterranean Sea and Contiguous Atlantic Area
AUV: Autonomous Underwater Vehicle
CBD: Convention on Biological Diversity
CFP: Common Fisheries Policy
CWC: Cold-Water Corals
DCF: Data Collection Framework
DSF: Deep-Sea Fisheries
DSWC: Dense Shelf Water Cascading
EEZ: Exclusive Economic Zone
EFH: Essential Fish Habitats
EIA: Environmental Impact Assessment
EMT: Eastern Mediterranean Transient
EwE: Ecopath with Ecosim
FAO: Food and Agricultural Organization
FRA: Fishery Restricted Areas
GFCM: General Fisheries Commission for the Mediterranean
GSA: Geographical Sub Area
ICCAT: International Commission for the Conservation of Atlantic Tunas
LIW: Levantine Intermediate Water
MBES: Multibeam Echosounder
MEDIAS: Mediterranean International Acoustic Survey
MEDITS: Mediterranean International Trawl Survey
MS: Member States
MSFD: Marine Strategy Framework Directive
MSY: Maximum Sustainable Yield
NIS: Non-Indigenous Species
OMZ: Oxygen Minimum Zones
PAH: Polycyclic Aromatic Hydrocarbons
POM: Particulate Organic Matter
POP: Persistent Organic Pollutants
ROV: Remotely Operated Vehicle
SAC: Scientific Advisory Committee
SCA: Stomach Content Analysis
SSB: Spawning Stock Biomass
SIA: Stable Isotope Analysis
SSS: Side-Scan Sonar
STECF: Scientific, Technical and Economic Committee for Fisheries
VME: Vulnerable Marine Ecosystem
UNCLOS: United Nations Convention on the Law of the Sea
UNGA: United Nations General Assembly

1. Introduction

1.1 The Mediterranean Sea

The Mediterranean is a semi-enclosed basin between the European and African coasts with the narrow and shallow Strait of Gibraltar connecting its waters and life to the Atlantic Ocean. The Suez Canal creates a man-made connection to the Red Sea, recently doubled, which allows the penetration of tropical Indo-pacific species (Longhurst, 2017). Concomitantly, the Mediterranean Basin is experiencing major climatic-related changes that are strongly influencing its oceanography in terms of water mass characteristics (e.g., temperature, salinity, dissolved oxygen), currents, nutrients and relative sea levels. The interplay of these factors has, since historical times, strongly influenced the diversity and colonization of the Mediterranean Sea (Taviani, 2002; Danovaro et al., 2010).

Unique hydrology characterizes the present-day Mediterranean, including microtidal regime, oligotrophy, high salinity (37.5-39.5 psu), homoeothermic temperatures from 300–500 m to the bottom, with values at the seafloor ranging from ca 13-13.5 °C in the western basin to 13.5-15.5 °C in the eastern basin, and the almost complete lack of thermal boundaries (Emig and Geistdoerfer, 2005). These features uniquely make the Mediterranean one of the "warmest" deep-sea basins of the world. Strong energy gradients also characterize the Mediterranean, with primary production and food supply to the deep decreasing from the western to the eastern region of the basin and from shallower to deeper waters (Danovaro et al. 1999).

These historical, topographic and environmental characteristics complicate deep-sea biodiversity patterns of the Mediterranean Sea but raise intriguing questions. Numerous studies document that the Mediterranean Sea, although modest in size (0.82% and 0.32% of the global ocean surface and volume, respectively; Bianchi and Morri, 2000), is a biodiversity hot spot with overall ca 17000 species, which represent 7.5% of the species richness of the oceans (Danovaro and Pusceddu, 2007; Coll et al., 2010; Lejeusne et al., 2010). However, although data on the species richness of its deeper habitats are incomplete (two thirds of the deep species – excluding prokaryotes – have not been censused yet; Ramirez-Llodra et al., 2009; Coll et al., 2010, Danovaro et al., 2010), it appears that the biodiversity of the deep Mediterranean basin is lower than that of other oceans (Danovaro et al. 2010).

The biodiversity of the deep Mediterranean Sea depends largely from the heterogeneity of habitats, which include submarine canyons and seamounts, continental rise deposits, mud volcanoes and extreme environments such as hydrothermal vents, cold seeps and deephypersaline anoxic basins (Olu-Le Roy et al., 2004; Danovaro et al., 2010; Taviani, 2011, 2014; Fernandez-Arcaya et al., 2017). Even seemingly “featureless” soft bottom habitats host unique and vulnerable species and habitats (e.g. sponge fields, gorgonian and pennatulacean meadows) (Danovaro et al., 2010).

The Mediterranean basin is threatened by multiple stressors associated with the rapid expansion of coastal populations, urbanization, changes in agricultural, industrial and shipping patterns, overfishing and exploration and extraction of offshore minerals and hydrocarbons, which exert increasing pressures through habitat destruction, chemical pollution, and dumping of waste and litter (EEA, 1999; Danovaro et al., 1993). In concert with climate change, these stressors may act synergistically to affect the dynamics, and potentially the resilience, of fragile deep ecosystems (WWF/IUCN, 2004; UNEP/MAP, 2012). Direct stressors and processes also occur on the adjacent shelf and in the epi-mesopelagic zones, including Dense Shelf Water Cascading (DSWC) events down the continental slope, open-sea convection and severe coastal storms, which may transport sediments and organic matter to the continental slope and beyond, influencing deep-sea biodiversity and ecosystem functioning (Canals et al., 2006; Ulses et al., 2008; Sanchez-Vidal et al., 2012; Durrieu de Madron et al., 2013; Taviani et al., 2016). In particular, bottom-contacting fisheries, specifically bottom-trawling and longlines, represent the most significant anthropogenic threats to deep-water biota, severely impacting sensitive habitats and species such as cold-water corals (CWCs) and/or sponge gardens (Rogers, 1999; Koslow et al., 2000). Additional evidence attributes a significant proportion of deep-sea litter to the fishing industry (Bo et al., 2014; Tubau et al., 2015; D'Onghia et al., 2017; Mecho et al. 2017), along with land- and ship-based sources (Ryan et al. 2009).

Given the increasing pressures on deep-sea habitats, scientists and managers are becoming conscious of the need to develop standardised tools and harmonized observation systems for long-term biological monitoring, in order to enable the collection of scientifically-validated data and a better understanding of the consequences of the present and future anthropogenic impacts (Danovaro et al., 2017, Aguzzi et al., 2019; Danovaro et al., 2019).

1.2 Implementing the Marine Strategy Framework Directive in the deep Mediterranean Sea

The Marine Strategy Framework Directive (MSFD 2008/56/EC) represents the EU’s Integrated Maritime Policy tool to achieve Good Environmental Status (GES) of marine waters, with an initial target for 2020. The MSFD applies to the area of marine waters over which a

Member State exercises jurisdictional rights in accordance with the UNCLOS (see Figure 1, for the definition of territorial waters and EEZs in the Mediterranean). These include also deep-sea waters, seabed and sub-seafloor. At present, MSFD implementation focuses mostly on coastal habitats or those impacted by commercial fisheries (Raicevich et al., 2017). However, over long-time scales, global nutrient and carbon cycles depend on a functioning deep sea (e.g. Snelgrove et al., 2018). Moreover, the life-cycle stages of some coastal species use offshore environments, thus achieving GES for marine ecosystems associated with continental shelves, must link to the achievement of GES for deep Mediterranean environments and Areas Beyond National Jurisdiction (ABNJ or "High Seas"). Otherwise, the MSFD will largely disregard the precautionary principle and undermine an ecosystem-based approach to marine management.

An effective governance and management of the Mediterranean Sea requires consideration of the complexity of these environmental issues, and meaningful international cooperation (de Vivero and Rodriguez Mateos, 2015). Given the transboundary nature of most of the deep waters, their inclusion in MSFD complicates the requirement for each Member State to apply the Directive to areas within its national jurisdiction. This emphasizes the need for Member States (MS) to cooperate in order to ensure coordinated and harmonized development of marine strategies at the scale of region/sub-region in the Mediterranean Basin, where EU MS and developing countries co-exist.

MSFD implementation currently suffers from a lack of standardized and consistent methodology for deep waters. To address this gap, we identify approaches, variables, and methodologies to enable MSFD implementation in the deep Mediterranean Sea. This synthesis summarises available information on MSFD descriptors for the deep Mediterranean Sea, with respect to the criteria listed in the European Commission Decision (COMM/DEC/2017/848), and anthropogenic pressures, uses and human activities affecting the marine environment (Table 2 of Annex III of COMM/DEC/2017/848).

2. State of the knowledge of MSFD descriptors in the deep Mediterranean

2.1 Descriptor 1: Biological diversity

Descriptor 1 (D1) states that *"The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions"* (MSFD, 2008/56/EC, Annex I, summarised in Table 1). The species groups specified in Part II of the Annex to COMM/DEC/2017/848, these include birds, mammals, reptiles, fish and cephalopods, some of which are present, diverse and abundant in the deep sea, such as fishes and cephalopods, in addition to deep diving and feeding cetaceans. Deep-sea organisms play an important role in marine food webs, either as predators or as important prey of a large set of high trophic level predators, including other fishes and cephalopods and marine mammals (Fanelli et al., 2012, 2013; Quetglas et al., 2013).

Data on these components from the deep Mediterranean Sea are largely included in the MEDITS database, which also represent the only extensive time series available for the deep Mediterranean (Bertrand et al., 2002), although with the limitation that it is a destructive sampling method with discrete sampling time, which exclude the possibility to detect any displacements of demersal species (Aguzzi et al., 2009; 2013). MEDITS is funded as part of the EU Data Collection multi-annual sampling program (DC-MAP), which limits the sampling frequency to yearly surveys confined to the northern part of the Mediterranean Basin. MEDITS mostly targets demersal fish (including deep-sea sharks), but includes also commercial invertebrates and other macro and mega-invertebrates (as by-catch species). MEDITS provides detailed information on their abundance and biomass, including the population structure of several species (including length frequency distributions by sex and maturity stages for different target species), which allow us to obtain information on the size spectra, maturity ogives, sex ratios and mortality rates. This information contributes to both the census of shallow and deep marine biodiversity and stock assessments carried out by the GFCM and the STECF of the European Commission (see Vasilakopoulos et al., 2014; Cardinale et al., 2017) [see Descriptor 3 below]. In the case of meso- and bathypelagic species (MEDIAS Handbook, 2015; i.e. Galil, 2004; Papiol et al., 2013; Fanelli et al., 2013, 2015; Valls et al., 2014), species of non-commercial interest, hard bottom habitats between 200 and 800 m depth, and in general all habitats below 800 m depth, only scattered information without temporal datasets exist.

Another important gap concerns the smaller size biota, such as meiofauna, which are a key component in the deep-sea ecosystems and are driven by water depth, regional setting and geomorphological characteristics of the deep Mediterranean habitats (Bianchelli and Danovaro, 2019). Meiofauna are highly diversified (possibly hyper-diverse), and play a fundamental ecological role in the biogeochemical cycles and in food webs and are sensitive to environmental and anthropogenic pressures (Pusceddu et al., 2014). Since this component, which increases its ecological relevance, in terms of abundance and functional role, with increasing water depths (Danovaro et al., 2015), has been recently suggested for inclusion in the D1 for the implementation of the MSFD (Semprucci et al., 2014; Bianchelli et al., 2016a; 2018). It is even more evident that it should be taken into consideration in the implementation of the MSFD in the deep sea.

The MSFD deep-sea habitats included in the COMM/DEC/2017/848 are: a) upper bathyal rocks and biogenic reefs, b) upper bathyal sediments, c) lower bathyal rock and biogenic reef, d) lower bathyal sediments, and e) abyssal seafloor. These include other benthic habitats such as: canyons (which may include rocky and sedimentary substrates), rocky bottoms with coral banks (including Cold-water corals) or large bivalves, different types of sedimentary bottoms in bathyal or abyssal plains (muds, sands or coarser sediment), chemosynthetic ecosystems (hydrothermal vents and mud volcanoes), and seamounts.

Previous studies on the deep Mediterranean Sea reported a west–east decreasing gradient of food availability (Danovaro et al., 1999; Danovaro et al., 2008), which explains the presence of a significant decreasing gradient in the abundance and biomass of most deep-sea benthic components along that gradient, from meiofauna to megafauna (Sardá et al., 2004; Bianchelli and Danovaro, 2019; Fanelli et al., 2018). The CWCs apparently follow the same gradient (Taviani et al., 2017; Chimienti et al., 2019), and the presence of Levantine Intermediate Water (LIW) likely strongly influences their distribution (Freiwald et al., 2009; Taviani et al., 2016, 2017).

Trawl surveys provide most of the available information on deep-sea habitats and their characteristics (see Table 1, Annex III, MSFD), but only for soft bottom habitats including those dominated by *Isidella elongata* and *Funiculina quandrangularis* (Lauria et al., 2017; Vasilis et al., 2019). Oceanographic cruises using ROVs offer the possibility to conduct non-destructive image and sample collections able to contribute significantly to the study of deep-sea habitats. Most ROV surveys to date have focused on CWC habitats and coral gardens, and provide important information on the composition, abundance, and biomass of the communities within these habitats (Taviani et al., 2005, 2011, 2015, 2019; Schembri et al., 2007; Fabri et al., 2014, 2017; Bo et al., 2015; Evans et al., 2016; Fanelli et al., 2017; Chimienti et al., 2019; Moccia et al., 2019). Most available information focuses on deep-sea canyons (Migeon et al., 2012), seamounts (Wurtz and Rovere, 2015) and mud volcanoes (Mascle et al., 2014), with major data gaps for deep-sea pelagic habitats, notwithstanding there is an increasing information on deep-water zooplankton and micronekton (Koppelmann et al., 2009; Fanelli et al. 2011, 2014; Cartes et al., 2013; Denda and Christiansen, 2014; Danovaro et al., 2017; Conese et al., 2019). Descriptor 1 is directly linked to D2, D3, D4 and D6 (habitats), and monitoring efforts in the deep sea can therefore gather contextual information on all these descriptors. The ecosystem criteria listed in COMM/DEC/2017/848, which link Descriptors 1 and 4, consider trophic guilds. These are highly relevant to the deep sea and can therefore be immediately described, as the already available data would provide the required background information.

2.2 Descriptor 2: Non-indigenous species introduced by human activities

The number of recorded Non-Indigenous Species (NIS) in the Mediterranean Sea greatly exceeds that in other European seas (Galil et al., 2014; Zenetos, 2019). Their establishment alters biotic assemblages and ecosystem functions (Galil, 2007; Katsanevakis et al., 2007; Fanelli et al., 2015; Galil et al., 2016, 2017; Goren et al., 2016; Azzurro et al., 2019). The Suez Canal is an important pathway for Red Sea species, which indeed represent 2/3 of the NIS in the Mediterranean Sea (Galil et al., 2017). In the past, it was assumed that NIS could establish only in shallow waters, however, the deep sea is not immune to species invasions. NIS have been rarely documented in the deep sea, a notable exception is the red king crab *Paralithodes camtschaticus* in the Barents Sea (Jørgensen and Nilssen, 2011). Yet, a growing number of Erythraean species were reported from the deeper part of the continental shelf, beyond the shelf break and in the upper slope (Özcan et al., 2008; Corsini-Foka et al., 2010; Innocenti et al., 2017; Özgür Özbek et al., 2017). For example, the lethally poisonous silver-cheeked toadfish, *Lagocephalus sceleratus*, has been collected from 350-400 m depth off Spain (Izguerdo-Munoz and Izguerdo-Gomez, 2014). The invasive lionfish, *Pterois miles*, that was initially present only in the upper shelf has been recently recorded at depths down to 110-150 m (Yağlioğlu and Ayas, 2016; Jimenez et al., 2019). In the southern Levantine Sea, three carnivorous species of Erythraean origin have been observed at 200 m depth: the crocodile toothfish *Champsodon nudivittis*, the burrowing goby *Trypauchen vagina* and the red-eye round herring *Etrumeus golanii* (Galil et al. 2018). The presence of deeper dwelling populations suggests that thermal niche assessments based only on a species' native range may underestimate their ability to tolerate lower temperatures (Parravicini et al. 2015). Wider thermal tolerance of some Erythraean species may facilitate their bathymetric and geographic expansion to depths where unique, diverse, and fragile mesophotic 'animal forests' occur. The lately observed "descent" of NIS from the upper to lower continental shelf may be an indication of temperature-dependent range expansion at increasing water depths, and appears to be accelerating. Therefore, even if abundances of NIS at levels of true invasions have not been reported yet in the deep Mediterranean, these vulnerable environments should be monitored also for D2, as they could be future targets of NIS invasions.

2.3 Descriptor 3: Populations of commercially exploited fish and shellfish

Descriptor 3 determines that Member States should maintain commercially exploited stocks of fish and shellfish in a healthy state. This descriptor implies sustainable exploitation that does not exceed the Maximum Sustainable Yield (MSY), i.e. the maximum yield catch that can be taken annually without reducing the fish stock productivity. Heavy fishing pressures, such as overexploitation or overfishing, produce negative environmental and socio-economic impacts, ranging from loss of significant potential yield of targeted stocks to severe stock depletion and fisheries collapse (Gascuel et al., 2016). Overfishing can also reduce fish stocks dramatically to the point where they lose internal genetic diversity and, with it, their capacity to adapt to environmental change (Pinsky and Palumbi, 2014; Allendorf et al., 2014). Fish communities may also change, such as altered size structures, when fisheries target or discard particular-sized individuals of a species, may potentially affect predator and prey dynamics (Fanelli et al., 2010), i.e. Descriptor 4 addresses the question of trophic relationships and marine food webs. The MSFD builds on existing EU legislation such as the Common Fishery Policy (CFP), and the criteria describing stock status follow internationally acknowledged best practices. The SAC of the GFCM, the STECF of the European Commission and the ICCAT (for highly migratory species, such as tunas or swordfishes, which account for more than 10 % of the value of the total catches in the Mediterranean) collectively monitor exploitation of fisheries resources in the Mediterranean marine sub-regions. The FAO and GFCM oversee collection of fisheries monitoring data in the Mediterranean Sea within GSAs (Geographical Sub Areas management divisions, according to resolution GFCM/33/2009/2, www.gfcm.org, for the correspondence between GSA numbers and their names Fig. 2), often assessing stocks over one or several GSAs. However, the MSFD sub-regions do not match with the GSAs. Furthermore, when we focus our attention on depths >200 m, the distinction between shallow and deep-water species is often irrelevant because distribution, exploitation and assessment of many stocks often cover wide depth ranges.

Descriptor 3 stipulates the need for fishery-induced mortality, yielding (but not exceeding) MSY (D3C1), and that populations of all commercially exploited species should remain within safe biological limits (D3C2), with a population age and size distribution (D3C3) indicative of a healthy stock. Fulfilling D3 criteria for deep-sea stocks requires: (1) sustainable exploitation consistent with high long-term yields, (2) maintaining full reproductive capacity in order to maintain stock biomass, and (3) maintaining or increasing the proportion of older and larger fish/shellfish, an indicator of a healthy stock. Achieving GES also for a deep-sea stock requires fulfilling all three of these attributes and, for the reasons highlighted above, D3 indicators require trans-national cooperation at the level of each MFSD sub-region.

In the Mediterranean Sea, the enforcement of the CFP and, more recently, of the MSFD, continues to fall far short of achieving its objectives for exploited living marine resources (e.g., Colloca et al., 2013; Vasilakopoulos et al., 2014). Notwithstanding the enforcement of the EU-Data Collection Regulation (EU, 2000) in the early 2000s by all EU Member States, and the rapid increase in the number of assessed stocks by the GFCM and the STECF, industries continue to exploit Mediterranean Sea marine resources above MSY levels, with few signs of population recovery (Vasilakopoulos et al., 2014; Cardinale et al., 2017; Colloca et al., 2017).

Management practices of DSF and VMEs in the Mediterranean were reviewed in 2016 and 2017 (FAO, 2016 and GFCM, 2017). UNGA Resolutions 51/2006 and the FAO International Guidelines for the Management of Deep-sea Fisheries in the High Seas (FAO, 2009), identify DSF in the Mediterranean Sea, as those: a) using bottom-contact gears or b) using deep-pelagic trawls and c) targeting species associated with the sea floor between 300/400 m and 1000 m depth (FAO, 2016 and GFCM, 2017). This grouping considers shallower fisheries that extend below 400 m. These reports identify deep-water red shrimps (*Aristaeomorpha foliacea* and *Aristeus antennatus)* as the primary DSF targets in the Mediterranean deep-sea habitats, which are harvested mostly at 300/400–800 m depths, along with European hake *(Merluccius merluccius)*, Norway lobster *(Nephrops norvegicus)* and deep-water rose shrimp (*Parapenaeus longirostris)*. In addition, gillnet fisheries and demersal long-liners target both *M. merluccius* and the blackspot seabream (*Pagellus bogaraveo)*. Also in Spain, deep-sea fisheries target *Plesionika* shrimps below 300 m depth (see also IDEM 2018a). GFCM banned the use of towed dredges and trawl nets at depths beyond 1,000 m in 2005 (Recommendation GFCM/29/2005/1), protecting over ca. 1,700,000 km^2 of Mediterranean Sea seafloor habitats (about 59% of the GFCM area of application) (FAO, 2018).

To date, the GFCM has established a number of Fishery Restricted Areas (FRAs) to protect Essential Fish Habitats (EFHs) or/and VMEs from excessive fishing mortality or the significant adverse impact of fishing activities through bottom-contact fishing gears, respectively. The FRAs encompass a total marine area of ca. 22,500 km^2 (FAO, 2018). Four of the FRAs were declared within a multiannual management plan for deep-sea fisheries, in order to protect EFHs for spawners of several species that are heavily exploited, to maintain habitat of the continental slope (canyons and submarine canyons), and to preserve all the species of the area (commercially exploited or not), i.e., one in the Gulf of Lion in France (Le Corre and Farrugio, 2011), one in the Jabuka-Pomo Pit (Elahi et al., 2018) and two FRAs south of Sicily. The existence of management plans, however, does not necessarily imply regular completion of accurate stock evaluation. Moreover, the existence of the FRA does not necessarily imply banning bottom trawling and may simply represent an effort management tool (to prevent further effort increase as seen in freezing fishing effort in the Gulf of Lion FRA).

Member States shall establish a list of commercially exploited species to which the criteria apply in each assessment area through regional or sub-regional cooperation, and update that list for each six-year evaluation period, taking into account Council Regulations (EU) 1251/2016, 1380/2013, 1343/2011, and 1967/2006, in accordance with article 43 (3) of the Treaty on the Functioning of the European Union, article 9 of Regulation (EU) No 1380/2013 and article 19 of Regulation (EC) No 1967/2006.

The MSFD criteria available for coastal environments cannot often be directly utilised for deep-sea species. This is because stock assessments are limited and not sufficiently monitored, hampering the assessment of stock exploitation at MSY (Criterion D3C1). The available information decreases eastwards and southwards. In addition, a gap of knowledge is also present in terms of time-series coverage of Spawning Stock Biomass (SSB) (Criterion D3C2) trend data hampering the possibility to define appropriate reference points.

The third criterion, i.e. “Healthy age and size structure” (criterion D3C3), assumes that a stock with sufficient large, and therefore old, fish corresponds to a healthy stock, thus reflecting good status. The larger and older fish stocks indicate healthier conditions, but this criterion has not been developed because GES lacks accepted thresholds (European Environment Agency, 2018).

This gap suggests a need to identify and test suitable indicators, metrics, and thresholds for populations and age size distributions for each deep-sea stock (ICES, 2016, 2017). In conclusion, our analysis points out that there is a potential to inform D3 criteria, but more data need to be collected in the future to propose sound stocks analyses and reference conditions, likely though the extension of the EU Data Collection Multiannual Programme (DC-MAP, EU Regulation 2016/1701) to include more deep-sea species.

2.4 Descriptor 4: Marine food webs

Descriptor 4 addresses the functional aspects of marine food webs, especially the rates of energy transfer within the system and levels of productivity in key components. In the context of the MSFD, this descriptor reaches GES when "*All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and population levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity*" (MSFD, 2008/56/EC, Annex I).

Deep-sea food webs critically depend on input of organic carbon from the photic zone (Thomsen et al., 2017). The microbial loop and viral infections can play an important role in the functioning of deep-sea food webs both in the water column and in sediments, and in the control of the biogeochemical cycles (Danovaro et al., 2008). At the same time, deep-sea meiofauna represents a potential basic linkage in energy transfer from the benthic detritus and microbes to the macro- megafauna and demersal fishes (Van Oevelen et al., 2011; Gambi et al., 2014).

D4 is one of the most controversial MSFD Descriptors in terms of protocols, criteria, and thresholds (ICES, Report 2015). D4 is generally investigated along with Descriptors D1 and D6 or D3. Studies of food web properties typically utilize two different approaches: a) Stomach Contents' Analyses (SCA) and b) Stable Isotope Analyses (SIA), and fatty acid trophic markers. Modelling techniques, in contrast, can provide insights regarding the potential structure of food webs (Rombouts et al., 2013). SIA identified three trophic levels among deep-sea supra-benthos (Fanelli et al., 2009) and four levels within macrozoobenthos (Iken et al., 2001; Fanelli et al., 2011a) and macrozooplankton/micronekton (Fanelli et al., 2011b). Fishes, decapods, and cephalopods dominate higher trophic levels in deep-sea demersal communities. Despite the availability of a large dataset for the deep Mediterranean Sea (MEDITS, 2002), this dataset includes few commercial species from bathyal depths: the European hake *Merluccius merluccius* and the greater forkbeard *Phycis blennoides*, some sharks (mostly *Etmopterus spinax* and *Galeus melastomus*), decapods (the red shrimps *Aristaeus antennatus* and *Aristaeomorpha foliacea*, the rose shrimp *Parapenaeus longirostris* and the Norway lobster *Nephrops norvegicus*). We lack sufficient data on other dominant deep-sea species, such as macrourids, or key predators such as deep-sea sharks (e.g., *Centroscymus coelolepis*; Stefanescu et al., 1994; Massutí et al., 2004; Anastosopoulou et al., 2016).

Most studies on trophic functional groups have focused on macro- and megafauna (i.e. fish, decapods, cephalopods and echinoderms), whereas few studies are available on other biotic compartments such as meiofauna or mesozooplankton (Danovaro et al., 2010). The northwestern

Mediterranean portion is better studied (Fanelli et al., 2009, 2011a, b, 2013, 2015, 2016; Papiol et al., 2013; Cresson et al., 2014), whereas the Ionian and Aegean seas have been much less investigated (Carlier et al., 2009; Koppelmann et al., 2009; Tecchio et al., 2013; Cartes et al., 2014; Naumann et al., 2015). On the other hand, these types of studies rarely consider some key species/taxa, such as mesopelagic fishes or megazooplankton (Fanelli et al., 2014; Valls et al., 2014).

COMM/DEC/848/2017 sets criteria and methodological standards for monitoring and assessment of GES within the theme “Ecosystems”. For example, selection criteria require that at least one of the three trophic guilds monitored should focus on primary producers. This criterion is the major drawback for this descriptor given that, aside from the few, very localized ecosystems that depend on chemosynthesis (hydrothermal vents, cold seeps, or wood and whale falls; Luna et al., 2012; Molari et al., 2013), the vast majority of the deep sea lacks primary production. The data gap between experimental and functional data adds further complication.

Stable isotope analysis may comply with the primary criterion D4C1 (diversity of trophic guilds) and the secondary criterion D4C3 (distribution of individuals across the trophic guild). Moreover, in combination with abundance, biomass, and other biological data available from MEDITS data, it may offer inputs into ecosystem models that could generate useful outputs, such as identification of unrecognized keystone species, a gap not presently considered. Italy addresses D4 under its fishery monitoring program (i.e. D3) and specifically with three subprograms aimed at: i) defining, testing, and applying ecosystem indicators through models (essentially EwE, http://www.ecopath.org); ii) identifying functional groups through the application of stable isotope analysis of monitored species within the DCF; and iii) integrating analysis of commercial species with those for benthos, zooplankton and Particulate Organic Matter (POM) samples every three years. Spain and France recently introduced SIA and SCA of species collected during MEDITS or MEDIAS surveys for use in D4.

In conclusion, the analysis of D4 can be realistically initiated for the deep Mediterranean, using the available technologies, protocols and monitoring programs and adapting the criteria, by neglecting the relevance of primary production, which could be replaced by the analysis of the inputs of primary organic matter and/or by starting from primary consumers and/or including the chemosynthetic primary production.

2.5 Descriptor 5: Human-induced eutrophication

Eutrophication refers to "*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 is achieved with respect to eutrophication 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).

In coastal environments, eutrophication means the increase of primary production generally monitored through the chlorophyll-*a* (Chl *a*) content and/or macroalgal biomass (Ferreira et al., 2007; Xiao et al., 2007; Borja et al., 2008; Bricker et al., 2008; Nixon, 2009). The effects of eutrophication are the lowering of oxygen concentrations, losses of submerged aquatic vegetation, and mass mortalities especially at the sediment water interface (Claussen et al., 2009; Ferreira et al., 2011). The global occurrence and expansion of hypoxic/anoxic events at bathyal depths worldwide point to the need for better understanding and monitoring of the effects of such phenomena on deep-sea benthic communities (Doya et al., 2015; Breitburg et al., 2018). Recent models foresee an oxygen decline from 1 to 7% in the next 100 years (Keeling et al., 2010) with an increase in the extension of Oxygen Minimum Zones (OMZs) (Stramma et al., 2008). Farther, OMZs, with their naturally occurring low pH and oxygen, offer some hints as to the structure of deep-sea ecosystems affected by eutrophication (Levin, 2003; Moffit et al., 2015).

Deep-sea ecosystems have been historically considered as a food-poor environment, and this is typically true for the deep Mediterranean Sea, especially in its eastern basin, but some areas may experience symptoms of eutrophication and oxygen depletion (Danovaro et al., 2014). For instance, it has been reported that massive phytodetritus exports from highly productive coastal waters to the deep-sea floor (Billet et al., 1983). Excessive C inputs in combination with the high bottom temperatures can cause episodic oxygen depletion in the deep sea (Ferreira et al., 2011; Danovaro et al., 2014). Recent studies highlighted that deep-sea trophic status can be also affected by climate change, as the Western basin is expected to become more oligotrophic and the Eastern basin more eutrophic (Piroddi et al., 2017). In addition, predicted increasing surface temperatures may affect water mass stratification and the formation of cold oxygenated deep water, modifying global ocean circulation and the dissolved oxygen availability in deep-water masses (Ramirez-Llodra et al., 2011). Local scale eutrophication could affect deep-sea sediments facing highly productive areas of the Mediterranean Sea, such as the Gulf of Lions, the northern Aegean Sea and the Ionian Sea receiving inputs from the Adriatic Sea.

However, the MSFD, in relation to the qualitative Descriptor 5, calls for an assessment of nutrients and organic matter inputs (Annex III of Directive 2017/845) and the use of the following criteria (Directive 2017/848): i) nutrient concentrations in the water column, ii) chlorophyll a in the water column, iii) harmful algal blooms, iv) photic limit, v) dissolved oxygen at the bottom of the water column, vi) opportunistic macroalgae, vii) macrophyte communities and viii) macrofaunal communities. These criteria can only be partially applied to the deep sea. Firstly, primary producers (e.g., macrophyte, macroalgae, harmful algal bloom) must be excluded, and the assessment of trophic status using variables measured in the water column can lead to misleading classifications (Dell'Anno et al., 2002, see also Fabri et al., 2018). Considering that oxygen depletion is one of the main causes of benthic faunal mortality, it is important to measure physical-chemical parameters and indicators also in the sediments (Mercado et al., 2015). In addition, the concentration of organic matter accumulated in surface sediments can provide a good indication of the eutrophication process occurring on the seafloor (Dell’Anno et al., 2002; Pusceddu et al., 2009). The current conceptual framework suggests the need to introduce new criteria and indicators, related to benthic ecosystems and, particularly, to the deep sea.

A group of core indicators is already utilised to monitor eutrophication in open waters, including: i) nutrients (nitrate, ammonium, phosphate), ii) dissolved oxygen and iii) phytoplankton (chlorophyll a, dominance). Zooplankton biomass is considered a potential, though not fully mature indicator (UNEP(DEPI)/MED, 2007 and references therein) because of incomplete knowledge of its relationship to eutrophication. The monitoring of benthic ecosystems should include i) quantity and quality of sedimentary organic matter, and ii) biodiversity and taxonomic composition of benthic invertebrates (Dell'Anno et al., 2002; Pusceddu et al., 2009, 2011, 2014; Bianchelli et al., 2016a). Among indicators recently proposed to assess benthic trophic status of marine ecosystems, the quantity and biochemical composition of sedimentary organic matter has received the widest application, both in coastal and deep-sea ecosystems (Pusceddu et al., 2009; see also Fabri et al., 2018). The concentrations of biopolymeric C (defined as the sum of C deriving from proteins, carbohydrates and lipids) and its algal fraction have been used to assess impacts of humans on benthic trophic status in different oceanic and coastal regions and varying water depths, within the Mediterranean basin (Dell'Anno et al., 2002; Pusceddu et al., 2009, 2011, 2014; Bianchelli et al., 2016b). Changes in the quantity and quality of organic matter in the deep sea lead to responses from all benthic components, from prokaryotes to foraminifera, and from meiofauna to macrofauna (Danovaro et al., 1999). Also the functional traits of macrofauna have been widely used as indicators of alteration and for measure the health status of marine benthic ecosystems (Borja et al., 2008). Further, meiofauna could be considered a good indicator as it is highly sensitive to environmental changes, and particularly to organic enrichment due to eutrophication (Pusceddu et al., 2011). For these reasons, meiofauna have been recently proposed for the monitoring of eutrophication effects and for assessing the environmental quality of both coastal and deep-sea ecosystems (Bianchelli et al., 2016a; Pusceddu et al., 2016).

2.6 Descriptor 6: Sea floor integrity

Descriptor 6 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*" (Commission Decision 2010/477/EU). This involves addressing "*physical damage, having regard to substrate characteristics*", and the "*condition of benthic community*", the latter being directly related to Descriptor 1. The relevant pressures identified in the Commission Decision (EU) 2017/848 generically refer to "physical loss (due to permanent change of seabed substrate or morphology and to extraction of seabed substrate)" and to "physical disturbance to seabed (temporary or reversible)". Four primary criteria address these points, three of which (D6C1 to DGC3) are specific for Descriptor 6, while two are also relevant for Descriptor 1 (D6C4 and D6C5).

The deep Mediterranean seafloor experiences two dominant physical disturbances associated with human activities: i) bottom-contact fisheries and ii) oil and gas activities (Boschen et al., 2013; D'Onghia et al., 2017; Lauria et al., 2016; Holler et al., 2017). Fisheries using bottom-contacting gear lead to direct alteration of seafloor morphology at large, medium and small scales (Puig et al., 2012; Martin et al., 2014). Bottom trawling is a key driver for large-scale seascape change as it smoothens the natural topography (Puig et al., 2012). Direct and indirect biological effects of bottom trawling have been demonstrated in terms of biogeochemical changes (e.g. less total amino acid concentration in sediments) and faunal desertification (Pusceddu et al., 2014). The Mediterranean Sea shows the highest fisheries footprint per unit landings in Europe (Eigaard et al., 2017), with peak intensities in the Tyrrhenian and the Adriatic Sea. In the Catalan margin, trawling impact is major down to 800 m depth (Puig et al., 2012). Sediment resuspension from fishing grounds can propagate to wider and deeper areas eventually leading to suffocation and burial of VME (Martin et al., 2008). Downslope moving gravity-driven resuspension flows enhance sedimentation rates far beyond fishing grounds, such as in canyon axes. Other types of fishing gear such as bottom touching longlines and gillnets could also have a significant adverse impact on vulnerable benthic communities and organisms such as black corals, gorgonians, scleractinians and many other habitat-forming species (GFCM, 2017), because of breaking while pulling, ghost fishing or entanglement.

Activities undertaken by the offshore oil and gas industry may cause physical loss of the natural deep seabed. Physical (and chemical) impacts on the seafloor and subseafloor range from the installation of drilling rigs, wellheads and other structures on the seabed to the accumulation of litter including lost or abandoned equipment, consumables and other materials. Today's deep-water (>200 m) oil and gas production in the Mediterranean Sea, or advanced prospects for it, takes place essentially offshore Egypt, Israel, Lebanon, Syria and Cyprus (The Petroleum Economist Ltd, 2013; Galil and Herut, 2011). The environmental approach for the hydrocarbon industry in the Mediterranean Sea is developed in the Offshore Protocol of the Barcelona Convention, adopted in October 1994, which obliges countries to perform comprehensive EIAs after entering into force in December 2012. The EU adopted the Directive on Safety of Offshore Oil and Gas Prospection, Exploration and Production Activities in July 2013, which provides a blueprint of the best international practice also for non-EU countries in the Eastern Mediterranean that are new to the energy industry (Livnat, 2014). Further disturbance occurs in case of cable deployment, for not the cables and pipelines per se, rather for the impact of the anchoring of the supply vessel during the deployment of the cable.

Dumping of industrial waste in the deep Mediterranean Sea is a matter of concern for habitat integrity. Submarine canyons with heads close to the coast are favoured sites for direct deep-sea disposal (Ramirez-Llodra et al., 2015). Two aluminium-processing plants have discharged red mud waste in the deep Mediterranean Sea: one in France (Cassidaigne Canyon, Gulf of Lion) (Dauvin, 2010; Fontanier et al., 2012, 2014; see also Fabri et al., 2018) and one in Greece (Gulf of Corinth, Antikyra Bay) (Varnavas et al., 1986; Varnavas and Archilleopoulos, 1995; Poulos et al., 1996). Since 1988, Coal Fly-Ash (CFA) from the Hadera power plant, in Israel, has been dumped into a 16 km^2 disposal site some 70 km offshore, at a water depth of 1400 m, where a 0.5-1.0 cm thick ash layer has been noticed (Kress et al., 1996, 1998) together with severe impoverishment of benthic fauna. Israel allowed also long-term disposal of dredged sediments and industrial waste

(1,900,000 m^3) polluted with Hg, Cd, Pb, tributyltin and organotins, and PCBs at a site 1300 m deep (Herut et al., 2010).

Different proved tools is currently available to assess seafloor integrity. High-resolution maps of benthic substrata and habitats are increasingly required both to underpin environmental and socioeconomic impact assessments and to help in developing effective management measures (Kenny et al., 2003; Brown et al., 2011; Stephens and Diesing, 2014; Holler et al., 2017; Fabri et al., 2018). Multibeam Echo-Sounders (MBES) and side scan sonars (SSS), map seabed areas with 100% spatial coverage at a resolution finer than 1 m^2, depending on the depth of data collection and on distance-to-bottom of the sensors (Kenny et al., 2003). Ground-truthing methods, such as the use of remotely operated vehicles (ROVs) and autonomous underwater vehicles (AUVs) (Fabri et al., 2014; Lastras et al., 2016), are widely available and could be applied according to the size and the nature of the area of interest (Kenny et al., 2003; Brown and Blondel, 2009; Brown et al., 2011; Holler et al., 2017). Habitat suitability models try to predict the distribution of some habitats such as CWCs (Lo Iacono et al., 2012; Bargain et al., 2017, 2018; Fabri et al., 2017; Angeletti et al., 2019; Lo Iacono et al., 2018; Giusti et al., 2014, 2017; Lauria et al., 2017). However, because such models often include a large degree of uncertainty, decisions based entirely on modelling approaches may involve significant risk.

The revision of the MSFD (through the COMM. DEC. 2017/848/EU) emphasised 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”, but for this to be implemented, a very long time perspective is needed. All impacts described in this section have immediate effects (and sometimes also delayed effects) on seafloor communities, which in most cases could represent either a tipping point (e.g. large-scale seascape change) or require long time before any significant recovery could take place. A time-span of 12 years is possibly too short and it is urgent to proceed with a sound extensive evaluation of the current status of the deep benthic habitats in the Mediterranean Sea before human impact severely modifies or erases them from the face of our planet.

Descriptor 7: Permanent alteration of hydrographical conditions

Descriptor 7 is geared towards addressing the problem of the permanent alteration of hydrographical conditions. These conditions are often affected by the presence of coastal infrastructure and other man-made activities (ports, artificial reefs, etc.). However, in most cases these structures impact coastal areas and only rarely can reach higher depths. Conversely, global climate change, combined with episodic climate-driven events, can alter the “hydrographical conditions” also at depths. In recent decades, deep Mediterranean waters have experienced drastic changes resulting in an alteration of the stratification associated to temperature increases and salinity shifts (Schroeder et al., 2009, 2016). At the end of the 1980s, climate change, changing hydrographic properties, surface circulation, and deep-water convection caused a ‘regime shift’ on a global scale (Reid et al., 2016) and throughout the Mediterranean basin (Conversi et al., 2010). During that period, the main site of deep-water formation shifted from the southern Adriatic to the Aegean sub-basins. This “Eastern Mediterranean Transient” (EMT; 1987-1994) event resulted in increased oxygen consumption (Roether and Well, 2001; Klein et al., 2003) and, in the eastern Ionian, in a nutricline shoaling by about 150 m (Klein et al., 1999). Other rapid hydrological changes have also occurred in the Western Mediterranean Sea. The “Western Mediterranean Transient” (WMT; 2004/05 and 2005/06) was characterised by the formation of warmer and denser new deep waters over the continental shelf as a result of cooling and evaporation of the surface layer and downslope cascading (Canals et al., 2006; Palanques et al., 2006; Schroeder et al., 2009). The high volumes of newly formed deep waters generated during intense cascading and convection events dramatically altered the hydrological structure of the basin, completely de-stratifying the water column and transferring massive heat and salt to the deep layers (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 in the last decade that greatly altered the structure of the intermediate and, especially, the deep layers of the Western Basin (Durrieu de Madron et al., 2013). Cascading events transport huge amounts of nutrients and organic matter, to bathyal depths (Canals et al., 2006; Sanchez-Vidal et al., 2008; Danovaro et al., 1999; Company et al., 2008). 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 deep-water formation (Fabri et al., 2018). Moreover, progressive increase in heat and salt content in the intermediate layer, advected from east to west, favoured new dense water formation in the North-Western Mediterranean basin. Multiple heat and saline anomalies characterised the Mediterranean Sea from 1950 to 2000 (Rixen et al., 2005; Kress et al., 2014) and although these alterations cannot be considered permanent, all of these changes have long-term effects.

The multidisciplinary Mediterranean Targeted Projects MTP-I and MTP-II/MATER (1993-2000; Monaco and Peruzzi, 2002), the MEDAR/MEDATLAS database (Fichaut et al., 2003), the

SeaDataNet (i.e., https://www.seadatanet.org/) and EMODnet (http://www.emodnet-physics.eu) infrastructures created datasets on temperature, salinity oxygen, silicate, nitrates and phosphates, but in some cases with insufficient coverage in the eastern region (including Tunisia, Libya, Croatia and Turkey; Simoncelli et al., 2015).

Since the 2000's, national and international programmes (e.g. EU-PERSEUS and MedSeA, IT-VECTOR, FR-MERMEX, E-RADMED) produced hydrological data on the whole Mediterranean Basin. Regular cruises, mooring lines, and deployment of new instruments and infrastructure (Argo floats, gliders) now support intensive collection of *in situ* observations in the North-Western Mediterranean Sea. Argo floats, autonomous profiling floats, drift at a given depth for a given time period. After drifting for a set time, they sink to 2000 m and profile temperature and salinity during the upcast. These data proven useful in describing deep-water formation (Smith et al., 2008). Moreover, in the last decade, glider technology mainly in the North-Western Mediterranean Sea has enabled repeated cross-basin transects at depths from the surface to 1000 m. In addition, numerical models implemented at regional or local scales use these data to elucidate water mass formation and spreading, and basin-scale hydrological dynamics (Bonaldo et al., 2015; Estournel et al., 2005).

Long-term monitoring of basic hydrological parameters (temperature and salinity), collected as time series with appropriate temporal resolution (i.e. sampling intervals that resolve all relevant timescales) represent a science priority in the context of climate change study for key locations in the Mediterranean Sea (e.g. straits and channels, zones of dense water formation and spreading, deep basins) (Schroeder et al., 2013; Aguzzi et al., 2019). The HYDROCHANGES network aims to address this need by deploying moorings fitted with Conductivity, Temperature, Depth sensors (CTDs) at key locations for monitoring temperature and salinity (Schroeder et al., 2013). The FixO3 (Fixed point Open Ocean Observatory network, http://earthvo.fixo3.eu/) programmes and the EMSO (European Multidisciplinary Seafloor and water column Observatories, http://emso.eu/) EU Infrastructure provide additional near-bottom data from fixed-point observations. For example, DYFAMED and LION deep-water stations, and ANTARES neutrino telescope in the North-Western Mediterranean or the KM3NET in the Ionian Sea (Tamburini et al., 2013; Aguzzi et al., 2018), continuously monitor sets of specific parameters. Long time series provided by these mooring stations have contributed pivotal findings on the deep dynamics of the Mediterranean Sea in last years. In order to partially interpolate data within homogeneous habitats, scientists have generated gridded products through objective analysis of available observations (such as numerical models with data assimilation delivered by Copernicus downstream services (http://marine.copernicus.eu/services-portfolio/access-to-products/).

2.8 Descriptor 8: Concentrations of contaminants giving rise to pollution effects

The input of xenobiotic substances represent one of the major threats for ocean health (Halpern et al., 2008). Hydrophobic pollutants, such as organo-halogenates and polycyclic aromatic hydrocarbons (PAH), enter the marine environment through effluent discharges, atmospheric deposition, runoff and other means (Iwata et al., 1994). Once in the water column, the adsorption onto particulate matter transfers these compounds from the surface to the deep waters and sediments (Buesseler, 1998). Particle settling is also favoured by biological processes, and by lateral transport from continental shelves (Heussner et al., 2006; Martin et al., 2006; Zuñiga et al., 2009). DSWC is a massive mechanism of pollutant transfer to the open deep ocean (Canals et al., 2006). Higher fluxes of organohalogen pollutants and Polycyclic Aromatic Hydrocarbons (PAH) occur during these cascading events (Salvadó et al., 2017). PAH settling fluxes in the north-western Mediterranean Sea vary widely (Lipiatou et al., 1993; Raoux et al., 1999), with highest concentrations of contaminants in the Alboran Sea (Dachs et al., 1996), and much lower values in Sardinia and in the Southern Ionian Sea (Bouloubassi et al., 2006; Tsapakis et al., 2006). However, these values greatly exceed atmospheric deposition of PAH in central sites of the Western Mediterranean Sea, thus highlighting the role of river discharge (Heussner et al., 2006; Bonnin et al., 2008; Palanques et al., 2008). Qualitative differences are also observed in relation to these transfer processes. Sediments of coastal areas, continental shelves and slopes have higher proportions of petrogenic PAHs whereas the deep basin of the north-western Mediterranean Sea is characterized by high amounts of pyrogenic PAHs.

Organochlorine compounds such as Polychlorobiphenils (PCBs) and chlorinated pesticides, characterised a group of Persistent Organic Pollutants (POPs) of worldwide concern due to their toxic effects (Harmon, 2015). Notwithstanding the discontinued use of these compounds in most world areas, thanks to relevant national regulations and international agreements such as the Stockholm Convention, their extensive occurrence is still observed, 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.

Radioactive compounds in the Mediterranean Sea are derived from the fallout of nuclear weapon testing and the Chernobyl accident. In sediments, concentrations of ^{137}Cs and $^{239+340}$Pu have been measured in various parts of the Mediterranean Sea, including deep basins. The concentrations are generally higher in coastal ecosystems because land-based sources can exceed atmospheric inputs (Durrieu de Madron et al., 2011; Garcia-Orellana et al., 2009). Concentrations in biota are presently undistinguishable from those in areas without point sources. Hence, the relevance of these contaminants lies in their usefulness as process tracers, more than on their impact on the environment. Studies on radionuclides in marine organisms 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 (Harmelin-Vivien et al., 2012). Finally, neglected impacts that can be very important in several areas of world are military activities. Information on their impacts on the environment are relatively scarce and are often studied after several years from their production and without any baseline available (Lawrence et al., 2015; Danovaro et al., 2019).

Atmospheric inputs constitute one of the major sources of Trace Elements (TE) to the deep Mediterranean Sea (Migeon et al., 2012; Guerzoni et al., 1999), where TE concentrations in waters are typically higher than in other areas of the world ocean. In addition, 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 hydrologic regime of the Mediterranean Sea tends to transfer the pollutants and nutrients to the Atlantic by bottom water flow transport. Our knowledge on the concentrations, fluxes, and behaviour of trace elements, radionuclides and organic substances in the deep waters and sediment and their toxicological impacts on habitats and organisms is scarce (Durrieu de Madron et al., 2011). Pollutants with hydrophobic properties, e.g. PCBs and mercury, accumulate in biota and thus in the food web. In case of chronic pollution events, the concentration of contaminants should be analysed in sediments collected by sediment cores, which enable the reconstruction of temporal trends. Sample collection of water at different depths and analysis of the dissolved and particulate matter would also be important. Abundance of populations and estimates of the extent of habitats adversely affected by chronic pollution should be assessed concurrently.

Nonetheless, information on pollutants in the deep sea is almost completely lacking, and this represent the main gap in the application of the criteria needed to determine the D8.

2.9 Descriptor 9: Contaminants in fish and other seafood for human consumption

Descriptor 9 focuses on the accumulation of toxic, persistent and liable substances in wild deep-sea organisms used for human consumption (i.e., mostly teleost and decapod crustaceans) and the contaminants considered by D9 are only part of those of interest for the D8 (cfr. Regulation EC 1881/2006 and its amendments EC 2006, 2008). Each Member State may ignore specific contaminants and/or include additional ones (EC 2017) (Fliedner et al., 2018). In any case the monitoring of the contaminants accumulated in the deep-sea biota should at least consider the following compounds for which regulatory levels have been set: i) heavy metals (lead, cadmium and mercury); ii) PAHs; iii) dioxins (including dioxin-like PCBs). In addition, the following contaminants of relevance should be monitored: i) non-dioxins like PCBs; ii) phthalates; iii) organochlorine pesticides; iv) organotin compounds; v) brominated flame retardants; vi) polyfluorinated compounds. Also, artificial radionuclides should be monitored in case of nuclear accidents or any other radioactive emergencies that could lead to or has led to significant radioactive contamination of food.

Contaminants in fish and other seafood might derive from numerous anthropogenic sources described for the D8. Chemical contamination in fish and seafood results from a complex process that balances 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 requires understanding which contaminants exceed regulatory limits, how much they alter food webs, and what metabolic processes are involved in detoxification. The presence of xenobiotics in the deep Mediterranean organisms has been repeatedly documented (Galil et al., 1995; Storelli et al., 2009) with deep-sea Mediterranean fishes tending to exhibit higher levels of metal accumulation than those of populations inhabiting other areas such as the Atlantic Ocean (Damiano et al., 2011). Red-shrimps, *Aristeus antennatus and Aristaeomorpha foliacea* may be useful indicator species of levels of deep-sea contamination (e.g., see data on *A. antennatus*, Koenig et al., 2012). Contaminants in fish muscle and liver have been investigated in the most abundant deep-sea megafaunal species, e.g. *Alepocephalus rostratus*, *Coelorinchus mediterraneus*, *Coelorhinchus caelorhincus*, *Trachyrincus trachyrincus* and *Nezumia sclerorhynchus*, *Chimaera monstrosa*, *Lophius budegassa*, *Lepidion lepidion* (Koenig et al., 2013c), revealing mercury concentration exceeding 0.5 µg g^{-1} muscle wet weight in all species but one. This represents the threshold value indicated by the European Commission as acceptable for human consumption. High mercury concentration is a distinct feature of the Mediterranean Sea (the so-called "Mediterranean mercury anomaly"; Cossa and

Coquery, 2005; Cossa et al., 2012). The Hg concentration is even higher in some deep-sea fish species (Koenig et al., 2013c; Cresson et al., 2014; Chouvelon et al., 2018). Most deep-sea species are long-lived and slow-growing, which favours the bioaccumulation of pollutants (Drazen and Haedrich, 2012; Koenig et al., 2013a, b, c). Since some deep-sea species are of commercial interest, the high contamination level poses serious risks for human health (Rotllant et al., 2006; Carbery et al., 2018). Despite this, few studies have investigated the concentrations of contaminants in the deep Mediterranean fauna fished for human consumption (Storelli et al., 2004, 2007; Koenig et al., 2013c; Cresson et al., 2014). In this regard, the Gulf of Lion is the best-investigated area, whereas the Levantine Basin (with the exception of Israel waters; Galil et al., 1995) are the least studied. A comparison of the concentrations of xenobiotics in fish collected in the NW Mediterranean Sea, in 1996 and 2009 (Koenig et al., 2013a; Porte et al., 2000; Solé et al., 2001) indicate that their contamination did not change over time.

The application of the criteria of Descriptor 9 should consider the concentration, the thresholds, and the contamination sources. Regulators should also consider the species of interest for human diets and their ability to bioaccumulated pollutants. GES would be achieved if all contaminants occur at levels below those established for safe human consumption.

2.10 Descriptor 10: Marine litter

Two primary and two secondary criteria are associated to Descriptor 10: i) the composition, amount and spatial distribution of litter (D10C1) and of micro-litter (D10C2) “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” (primary) and ii) the amount of litter ingested by marine animals, which should not reach a level that adversely affect the health of the species (D10C3) and the number of individuals which are adversely affected due to litter, such as by entanglement, other types of injury or mortality, or health effects (D10C4) (secondary). Each sub-region should assess the outcomes for all criteria and as well as threshold values.

Marine litter represents a threat for the health of the deep Mediterranean Sea due to its limited exchange with other basins, dense population, touristic and industrialized coastlines, and heavy maritime traffic (UNEP, 2015). The sources of marine litter to the deep-sea floor of the Mediterranean Sea are either from land (river discharge, storm drains, sewage treatment plants and industrialized areas) or marine (fishing activities, commercial and recreational shipping, aquaculture, direct dumping), and include plastics (accounting for >70% of the total), glass, metal, clinker, cardboard and fabrics (Galgani et al., 2000; Ramirez-Llodra et al., 2013; Fabri et al., 2014; Pham et al., 2014; Tubau et al., 2015; UNEP, 2015; Mecho et al., 2017). The quantity and composition of marine litter differs among regions and changes with depth, probably as a result of a complex set of interactions between hydrodynamics, geomorphology, and anthropogenic sources (Pham et al., 2014; Tubau et al., 2015; UNEP, 2015). The abundance of marine litter items in the deep Mediterranean Sea varies from 500 items km^{-2} on the continental slopes off Malta and Cyprus (Mifsud et al., 2013; Ioakeimidis et al., 2014), the Tyrrhenian Sea (Angiolillo et al., 2015), or the Adriatic Sea (Galgani et al., 2000), to more than 2,000 items km^{-2} in the Antalya Bay in the Eastern Mediterranean or in the submarine canyons of the Gulf of Lion and of the Catalan Sea (Galgani et al., 2000; Tubau et al., 2015). Astonishingly high litter abundance of up to 1.3 million of items km^{-2} were reported at 300-600 m depth in the Messina Strait canyons (Central Mediterranean Sea) (Pierdomenico et al., 2019). Litter abundance found in submarine canyons and depths greater than 500 m typically exceeds that at shallower depths, suggesting that submarine canyons can act as primary conduits of litter from the coast to the deep sea (Galgani et al., 2000; Tubau et al., 2015). Superposition of highly efficient source-to-sink sedimentary transport (with flash-flood generated hyperpycnal flows) and strong urbanization of the coastal area promote the occurrence of large litter hotspots in the deep sea (Pierdomenico et al., 2019). In addition to large marine debris, concern has grown about microplastics (i.e., <1-5 mm in diameter; Desforges et al., 2014), which can directly enter the ocean also through cosmetic abrasives (i.e. microbeads), preproduction plastic pellets, or textile fibres known as primary plastics. Additionally, combined mechanical, biological, photic and thermal actions can break down larger plastic objects into numerous small fragments, which are defined as secondary microplastics. Depending on the density of the polymer, microplastics may sink and behave as very fine-grained sediments (for example polyester; Woodall et al., 2014), or they may float and subsequently sink following colonization by organisms, adsorption to phytoplankton, and/or aggregation with organic debris. Fibres appear to dominate the microplastics reported in Mediterranean deep-sea sediments (van Cauwenberghe et al., 2013; Woodall et al., 2014; Sanchez-Vidal et al., 2018). Recent studies indicate that the Mediterranean deep-sea floor might act as a long-term sink for microplastics where the abundances of such particles can exceed those in surface waters (Sanchez-Vidal et al., 2018). Marine litter in the Mediterranean deep sea may significantly affect different ecological compartments and, consequently, human health, with potentially severe economic impacts. Biotic effects of large and small items include entanglement, ingestion, colonization and rafting (Gregory, 2009; Murray and Cowie, 2011; Anastasopoulou et al., 2012; Ramirez-Llodra et al., 2013; Bo et al., 2014; Pham et al., 2014; Angiolillo et al., 2015; Tubau et al., 2015). However, information on the actual effects of (micro)plastics on deep-sea organisms and trophic webs is still limited (Taylor et al., 2016).

Marine litter in deep sea produces economic impacts primarily on the fishery sector, damaging vessels and fishing equipment due to entanglement of catch, loss of target species through ghost fishing, or reduced reproductive capacity of benthic organisms consuming microplastics (Newman et al., 2015). Furthermore, marine litter may contain pollutants (hazardous plastic additives, POPs) that exert toxic and endocrine disruptive effects on marine organisms that ingest plastics (Oehlmann et al., 2009).

2.11 Descriptor 11: Introduction of energy including underwater noise

Descriptor 11 deals with introduction of energy into the marine environment. Underwater noise can be pulsed or continuous. MSFD currently focuses on two criteria: anthropogenic pulsed (D11C1) and continuous low-frequency (D11C2) sounds in water. D11C1 addresses the space-time distribution of pulsed noise sources, whereas D11C2 addresses levels of continuous noise, using in situ measurements and models. Pulsed noise may cause direct acute effects such as hearing loss, tissue damage, and death of individuals of sensitive species such as cetaceans. Whereas continuous or chronic noise exposure mainly causes stress and behavioural alterations, with negative effects on deep-sea organisms (Nowacek et al., 2015). The proposed strategy on noise monitoring recommends several adaptations in the case of the deep Mediterranean. Particularly, both indicators are closely related to the acoustic biology of deep-diving marine mammal species, such as sperm whale and Cuvier's beaked whale. Pulsed noise can be monitored by setting up a register of anthropogenic activities, reporting on date, location, proportion of days within a given period and over a given geographical scale in which activities generating pulsed sounds occur. This could be done through the deployment of hydrophones (e.g., permanent or semi-permanent PAM) on new infrastructures (or implementation of the existing infrastructures) and their subsequent future development into larger geographic network: Rountree et al., 2019).

A variety of phenomena generates noise in the ocean, either from physical processes, such as wind-generated waves, earthquakes, precipitation, or from biological phenomena such as whale songs, dolphin clicks, and fish vocalizations (Montgomery and Radford, 2017); not all reach the deep sea. Fish produce sounds for their navigation, habitat selection and mating, as well as to communicate (Simpson et al., 2005). Marine mammals use sound as a primary tool for underwater communication (Wartzok and Ketten, 1999), mating and social interaction (Edds-Walton, 1997), and for tracking the prey (Au, 1993).

Anthropogenic noise can reach the deep sea, through commercial shipping, oil and gas exploration, fishing, and scientific research; all of these sources currently contribute to the general noise budget of the ocean (Montgomery and Radford, 2017). The impact of noise on marine is being increasingly investigated (Wenz, 1962; Hildebrand, 2009). Noise sources are divided into three frequency bands: low (10 to 500 Hz), medium (500 Hz to 25 kHz) and high (>25 kHz). Anthropogenic sources dominate the low-frequency band, and include commercial shipping and seismic emissions for hydrocarbon exploration. Minimal attenuation of low-frequency sound allows long-distance propagation. Sea-surface agitation (breaking waves, spray, bubble formation and collapse, and rainfall) and various sonars (e.g. military and multibeam seabed mapping), as well as small vessels produce most medium frequency sound. Greater attenuation limits propagation of noise in the mid-frequency band over long distances, and only local or regional (10s of km distant) sound sources contribute to this ambient noise field. At high frequencies, extreme acoustic attenuation confines all noise sources to the area close to the receiver.

Oil industry operations have traditionally focused on shallow, continental shelf waters, but exploration is moving in deeper waters (>500-1000 m). Expansion of oil exploration into deeper water has increased the potential for long-range propagation of seismic reflection signals. Indeed, sound in deep waters can propagate greater distances than in shallow-water ecosystems, by moving through the deep sound channel (Hildebrand, 2009). Seismic surveys currently target all regional seas in the south-eastern Mediterranean, apart from the Aegean Sea (Maglio et al., 2016). This expansion stresses the transboundary aspect of seismic surveys and calls for international cooperation.

Anthropogenic noise can cause physical and biological damage, such as behavioural changes and stress, especially in marine mammals, sea turtles and fish (Popper et al., 2014; Peng et al., 2015). The occurrence of low frequency noise in the deeper part of the basins is particularly important for deep-diving marine mammals, such as toothed whales, because the ambient noise they use as a background for echolocation decreases rapidly with depth (Foote et al., 2004; André et al., 2011; Azzellino et al., 2011). Indeed, small odontocetes produce high frequency sounds (ranging from 70 kHz to more than 150 kHz), while sperm whales, *Physeter macrocephalus*, during diving, make sound with frequencies ranging to more than 30 kHz which are detectable within 10-

15 km. The fin whale *Balaenoptera physalus*, the only mysticete constantly present in the Mediterranean Sea, emits mostly infrasonic signals (20-40 Hz), which are emitted in long sequences and can be detected at large distances.

Research on sea turtles in the South-Eastern Mediterranean region revealed that they can detect low frequency sounds that overlap with seismic airgun frequencies, these are high-intensity, low-frequency impulsive noise at regular intervals, mostly between 10 and 300 Hz (Carroll et al., 2017; Nelms et al, 2016). Airguns can stress the sea turtles, *Caretta caretta* (DeRuiter et al., 2012). The impacts of anthropogenic noise on sharks and rays are poorly studied, with most research to date focusing outside the Mediterranean region (Weilgart, 2017). Fish sensitivity to certain frequencies varies among species (Carroll et al., 2017). Recent studies demonstrate negative effects of seismic survey airgun operations even in zooplankton (McCauley et al., 2017).

In situ acoustic measurements can document continuous low-frequency sound, gathering field data on ambient noise in a given location. Understanding the large-scale influence of artificial noise on marine organisms and ecosystems represents the main gap to the application of the D11 on the deep sea. Deep-sea observatories offer new opportunities to assess the presence and effects of noise in on deep-sea life (Aguzzi et al., 2019). Deep-sea cabled observatories (i.e. NEMO-SN1 in the Western Ionian Sea, Caruso et al. 2015, Favali et al., 2013; ANTARES in the Ligurian Sea, André et al., 2017; and PYLOS in the South Ionian Sea, http://www.fixo3.eu/observatory/pylos/) are equipped with hydrophones for passive acoustic monitoring. Besides these measurements, and especially for monitoring continuous low-frequency sound in deep sea, modelling approaches (both for single sources or distributed sources of noise, from the most advanced Dynamic Ambient Noise Prediction System elaborated by the U.S. for modelling multiple sources, to the Acoustic Integration Model used for modelling the effects of noise on cetaceans; NRC, 2003) may reduce the time required to establish trends and patterns.

3. Future implementation

In order to effectively implement the deep-sea MSFD, we need to identify the criteria to achieve or maintain GES in open waters and deep-sea bottoms, including "*spatial protection measures, contributing to coherent and representative networks of marine protected areas, adequately covering the diversity of the constituent ecosystems, such as special areas of conservation pursuant to the Habitats Directive, special protection areas pursuant to the Birds*

Directive, and marine protected areas as agreed by the Community or Member States concerned in the framework of international or regional agreements to which they are parties" (MSFD, 2008/56/EC, Article 13).

The MSFD takes an overarching and integrated approach by focusing on achieving GES and targets, and we therefore recommend exploring and assessing synergies between the different treaties, directives, and conventions (e.g. see Descriptor 6 section) so that, wherever possible, the programme of measures and proposed MSFD monitoring simultaneously address the requirements of other legislations.

Our analysis indicates that the 11 Descriptors promulgated by the MSFD (MSFD, 2008/56/EC) can be adapted and applied to the deep sea. Several Descriptors (D1, D2, D3, D6, D8, D10) can be readily implemented, others (D4, D9 and D11) require additional data in order to set up benchmark and threshold values, while two (D5, D7) require changes in the assumptions and/or modification in the concept of "permanent".

Priority ecological variables, spatial distribution, extent of pressures and impacts ought to be identified and standardized in order to establish targets and indicators addressing the distinct conditions in the deep sea. The expertise, tools and resources required for deep-sea monitoring are not universally available to all Mediterranean Member States (MS), nor to countries in the southern and easternmost Mediterranean. These limitations may be overcome by initiating deep sea MDFD-focused monitoring in already data-rich locations (presumably off MS), and pioneering joint-effort monitoring in collaboration with non-MS, to enhance awareness, capacity building, and gain much needed data on scantily studied regions. Given the costs entailed by scientific and technical expertise, tools and infrastructure required for deep-sea research and monitoring, we advocate for EU-level financial support for MS/non-MS collaboration spanning joint fieldwork, training (early career research fellowships, workshops) and *public awareness* communication.

Since the millennium, increased awareness of the vulnerability of deep-sea ecosystems has changed attitudes concerning their protection and conservation (Ramirez-Llodra et al, 2011). Yet, for effective regulatory measures, legislators and managers require scientific evidence, which follows from basic scientific research and monitoring. Ecosystem-based management of the Mediterranean deep sea pressingly requires comprehensive analysis of available data, new data from yet unexplored regions, and impact assessment studies. Mounting evidence points to the vulnerability of the deep biota to anthropogenic disturbance that may result in biodiversity loss, urgent implementation of the MSFD in the Mediterranean deep sea will go a long way towards conserving its unique biodiversity and habitats.

Acknowledgements

This study has been supported by the DG ENV project IDEM (Implementation of the MSFD to the Deep Mediterranean Sea; contract EU No 11.0661/2017/750680/SUB/EN V.C2). MC and AS-V from University of Barcelona acknowledge support from the Spanish government through Red BAMAR (ref.: CGL2016-81854-REDT), a network on marine litter, and RTD projects NUREIEV (ref. CTM2013-44598-R) and NUREIEVA (ref. CTM2016-75953-C2-1-R) on far-field and near-field impacts of the Portman Bay, SE Spain, coastal submarine mine tailings disposal site. Generalitat de Catalunya autonomous government funding to CRG Marine Geosciences (ref. 2017 SGR 315) within its support scheme to excellence research groups is equally acknowledged.

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Table 1. List of the descriptors, their definition if GES is achieved and if they are a state or pressure descriptors. Only D3 is both a state and pressure descriptor as it related to aspects such as the level of fishing activity (pressure) and population age, size distribution and biomass indices (state).

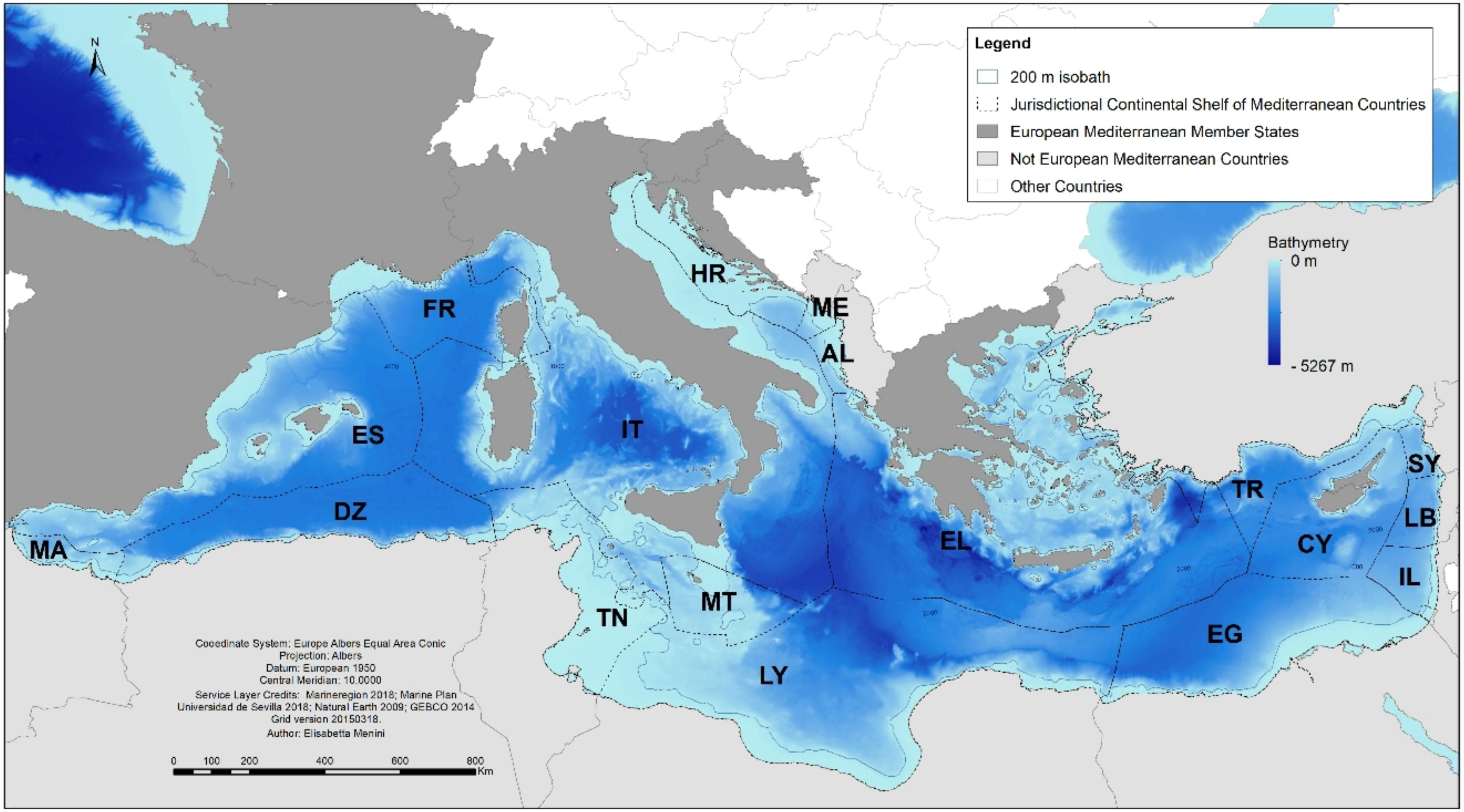
Descriptor	GES	State	Pressure
1	Biodiversity is maintained	X	
2	Non-indigenous species do not adversely alter the ecosystem		X
3	The population of commercial fish species is healthy	X	X
4	Elements of food webs ensure long-term abundance and reproduction	X	
5	Eutrophication is minimised		X
6	The sea floor integrity ensures functioning of the ecosystem	X	
7	Permanent alteration of hydrographical conditions does not adversely affect the ecosystem		X
8	Concentrations of contaminants give no effects		X
9	Contaminants in seafood are below safe levels		X
10	Marine litter does not cause harm		X
11	Introduction of energy (including underwater noise) does not adversely affect the ecosystem		X

Figure captions

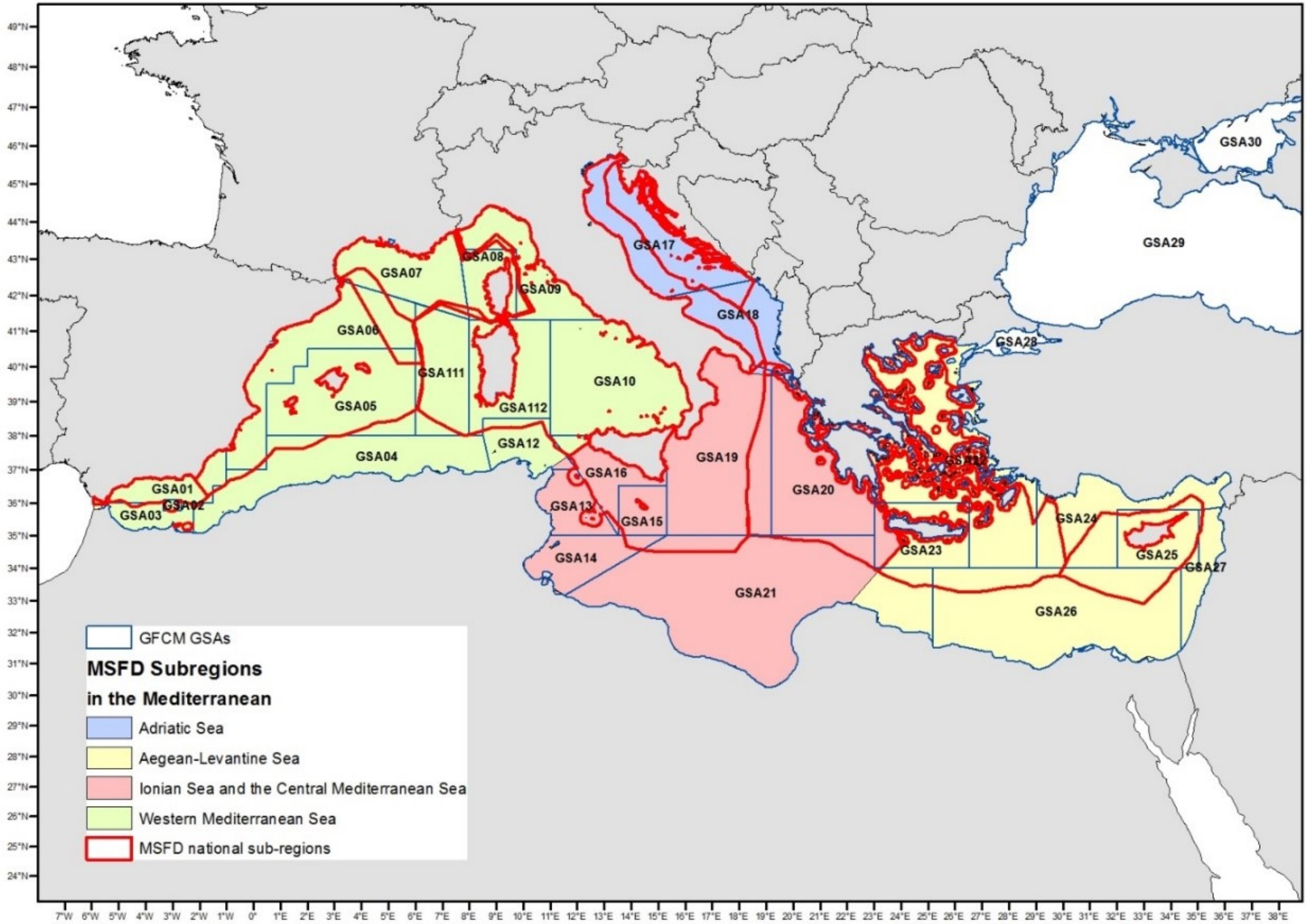
Figure 1. Jurisdictional Continental Shelf and deep Mediterranean Sea, with indication of jurisdictional continental shelf per each country (including non-EU countries).

Figure 2. Location of GFCM Geographical Sub-Areas (GSAs) and MSFD Mediterranean national sub-regions. FAO divisions are shown in thumbnail map.

Jurisdictional Continental Shelf and Deep Mediterranean Sea



Approximantive percentages of Jurisdicional Contintental Shelf per Country related to the total area of deep sea, calculated with the 200 meters isobath limit, in the Mediterranean Sea: Albania (AL): 0,3%; Algeria (DZ): 6,1%; Croatia (HR): 0,5%; Cyprus (CY): 4,8%; Egypt (EG): 7%; France (FR): 3,5%; Greece (EL): 20,6%; Israel (IL): 1,1%; Italy (IT): 21,2%; Lebanon (LB): 0,95%; Lybia (LY): 15,1%; Malta (MT): 2,3%; Montenegro (ME): 0,15%; Morocco (MA): 0,6%; Spain (ES): 11,1%; Syria (SY): 0,5%; Tunisia (TN): 1,5%; Turkey (TR): 2,5%.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GFCM GSAs
MSFD Subregions in the Mediterranean
Adriatic Sea
Aegean-Levantine Sea
Ionian Sea and the Central Mediterranean Sea
Western Mediterranean Sea
MSFD national sub-regions
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