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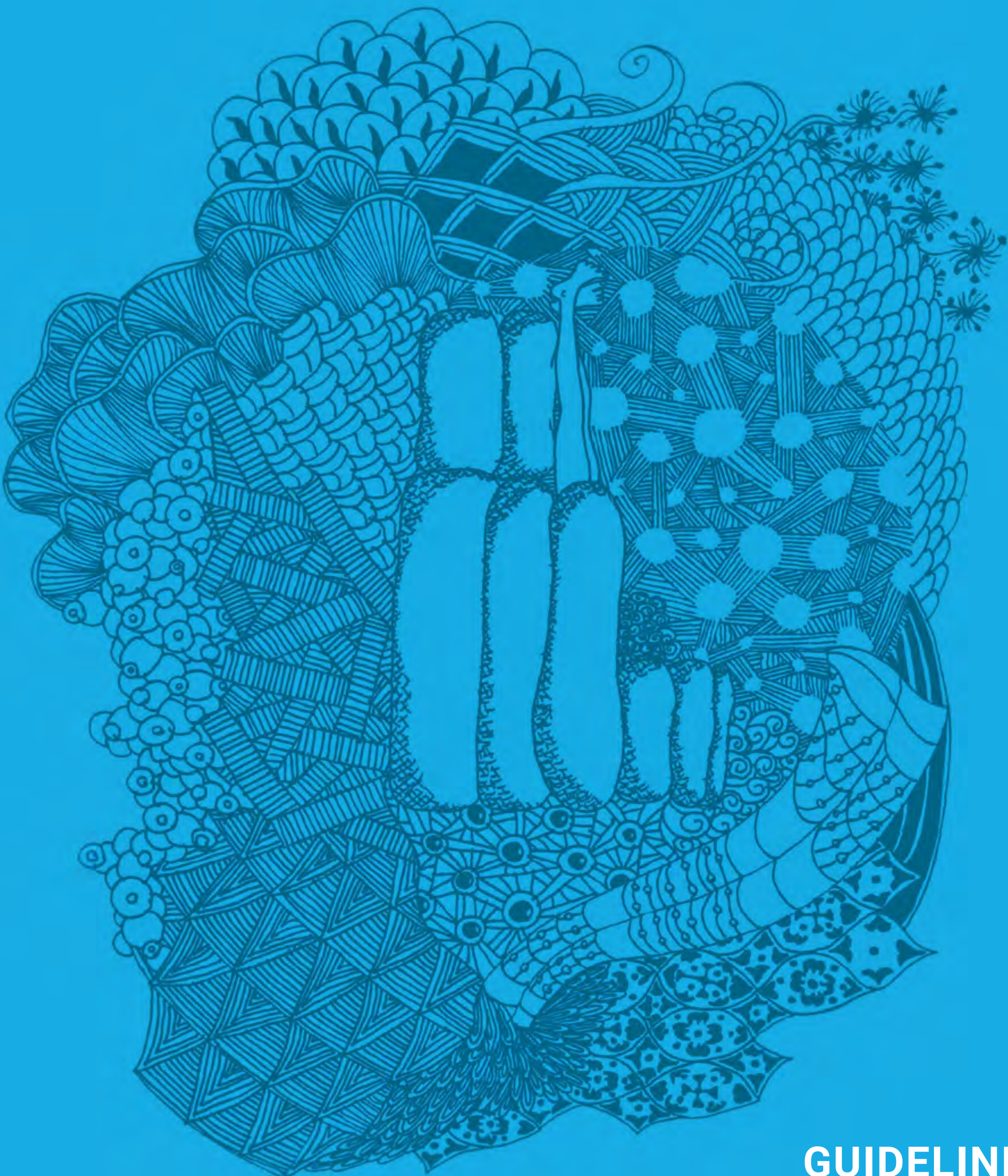

GUIDELINES FOR INVENTORYING AND MONITORING OF DARK HABITATS IN THE MEDITERRANEAN SEA

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Financial support

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For bibliographic purposes, this document may be cited as:

SPA/RAC–UN Environment/MAP, OCEANA, 2017. Guidelines for inventorying and monitoring of dark habitats in the Mediterranean Sea. By Vasilis GEROVASILEIOU, Ricardo AGUILAR, Pilar MARÍN. Ed. SPA/RAC -Deep Sea Lebanon Project, Tunis: 40 pp + Annexes

The original version of this document was prepared for the Specially Protected Areas Regional Activity Centre (SPA/RAC) by Ricardo AGUILAR & Pilar MARÍN, OCEANA and Vasilis GEROVASILEIOU, SPA/RAC Consultant with contribution from Tatjana BAKRAN PETRICIOLI, Enric BALLESTEROS, Hocein BAZAIRI, Carlo NIKE BIANCHI, Simona BUSSOTTI, Simonepietro CANESE, Pierre CHEVALDONNÉ, Douglas EVANS, Maïa FOURT, Jordi GRINYÓ, Jean Georges HARMELIN, Alain JEUDY DE GRISSAC, Vesna MAČIĆ, Covadonga OREJAS, Maria DEL MAR OTERO, Gérard PERGENT, Donat PETRICIOLI, Alfonso A. RAMOS ESPLÁ, Antonietta ROSSO, Rossana SANFILIPPO, Marco TAVIANI, Leonardo TUNESI, Maurizio WÜRTZ.

Layout:

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Cover photo credit:

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This document has been edited within the framework of the Deep-Sea Lebanon Project with the financial support of MAVA Foundation.

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CONTENT

A. CONTEXT AND AIMS

Dark habitats[1] are distributed throughout the Mediterranean basin from the sea surface (i.e. caves) to the deep-sea realm. Various habitats of unique scientific and conservation interest are included in this broad habitat category, such as dark caves, submarine canyons, seamounts and chemo-synthetic features supporting sensitive assemblages which require special protection. Therefore, dark habitats were considered under the Action Plan adopted in the Eighteenth Ordinary Meeting of the Contracting Parties to the Barcelona Convention (Turkey, December 2013). In the context of implementation schedule of the Dark Habitats Action Plan (UNEP-MAP-RAC/SPA, 2015a) a set of guidelines should be identified aiming to reduce the imminent pressures and threats affecting these vulnerable assemblages. This document aims to establish guidelines for inventorying and monitoring Mediterranean deep-sea habitats and marine caves in order to settle the basis for a regional-based assessment. Furthermore, it aims at reviewing the known distribution and main characteristics of these ecosystems. Although the Dark Habitats Action Plan covers entirely dark caves[2], inventorying and monitoring initiatives focusing on marine caves should consider the cave habitat as a whole. Therefore, this document presents methodologies which cover both semi-dark and dark caves.

Although scientific knowledge on dark habitats has increased during the last decades, there is still a significant gap today. The number of human activities and pressures impacting marine habitats has considerably increased throughout the Mediterranean, including deep-sea habitats (e.g. destructive fishing practices such as bottom trawling, oil and gas exploration, deep-sea mining); thus, there is an urgent need for establishing a regional monitoring system. Nevertheless, the development of comprehensive inventorying initiatives and monitoring tools becomes extremely challenging due to: (1) the scarcity of information on the current state of these habitats (distribution, density of key species, etc.) due to the high cost and difficulties for accessing, and (2) the lack of historic data and time series.
In this context, Marine Protected Areas (MPAs) may be considered as an essential tool for the conservation and monitoring of dark habitats. However, to date there is an obvious gap in the protection and monitoring of deep-sea habitats as they are mainly located in off-shore areas where information also remains limited. This issue should be addressed by Contracting Parties at the earliest convenience in order to put in place control systems aiming at the implementation of Ecosystem Approach (EcAp) procedures, and particularly an Integrated and Monitoring Assessment (IMAP) at regional level.

I. HABITATS AND SPECIES ASSOCIATED WITH MARINE CAVES

Marine caves harbour a variety of sciaphilic communities, usually distributed according to the following scheme: (a) a (pre-)coralligenous[3] algae-dominated community at the entrance zone, (b) a semi-dark zone dominated by sessile filter-feeding invertebrates (mainly sponges and anthozoans), and (c) a dark zone which is sparsely colonized by sponges, serpulid polychaetes, bryozoans and brachiopods (Pérès, 1967). Nevertheless, there is a lamentable dearth of information on the gradients of physical-chemical parameters acting on the marine cave biota (Gili *et al*., 1986; Morri *et al*., 1994a; Bianchi *et al*., 1998).
A general description of the semi-dark and dark cave communities which are considered in the present document can be found bellow.

Semi-dark cave communities

Hard substrates in semi-dark caves are typically dominated by sessile invertebrates (sponges, anthozoans and bryozoans). The most frequently recorded sponge species are *Agelas oroides, Petrosia ficiformis* (often discoloured), *Spirastrella cunctatrix, Chondrosia reniformis* (often discoloured), *Phorbas tenacior* and *Axinella damicornis* (see Appendix I). The sponge *Aplysina cavernicola* has been also described as a characteristic species of the semi-dark community in the north-western Mediterranean basin (Vacelet, 1959). Sponges of the class Homoscleromorpha (e.g. *Oscarella spp*. and *Plakina spp.*) may also have significant contribution to the local sponge assemblages. Three anthozoan facies have been recorded in semi-dark caves (mostly on ceilings) (Pérès, 1967; Zibrowius, 1978): (i) facies of the scleractinian species *Leptopsammia pruvoti*, *Madracis pharensis* (particularly abundant in the eastern basin), *Hoplangia durotrix, Polycyathus muellerae, Caryophyllia inornata* and *Astroides calycularis* (southern areas of the Central and Western Mediterranean); (ii) facies of *Coral-*

[1] Dark habitats are those where either no sunlight arrives or where the light that does arrive is insufficient for the development of plant communities. They include both shallow marine caves and deep habitats (usually at depths below 150/200 m).
[2] <0.01% of the light at the sea surface level according to Harmelin et al. (1985).
[3] Coralligenous and semi-dark cave communities have been integrated into the Action Plan for the conservation of the coralligenous and other calcareous bio-concretions in the Mediterranean Sea (UNEP-MAP-RAC/SPA, 2008).

lium rubrum which is more common in the north-western Mediterranean but can be found only in deeper waters (below 50 m) in the north-eastern basin; and (iii) facies of *Parazoanthus axinellae*, which is more common close to the cave entrance or in semi-dark tunnels with high hydrodynamic regime (more common in the Adriatic Sea). Facies of erect bryozoans (e.g. *Adeonella spp*. and *Reteporella spp*.) often develop in semi-dark caves (Pérès, 1967; Ros *et al.*, 1985).

Dark cave communities

The shift from semi-dark to dark cave communities is evidenced through a sharp decrease in biotic coverage, biomass, three-dimensional complexity, species richness, and the appearance of a black mineral coating of Mn-Fe oxides on the substrate (Pérès, 1967; Harmelin *et al.*, 1985). This community is usually sparsely colonized by sponges, serpulid polychaetes, bryozoans and brachiopods (Pérès, 1967). Common sponge species are *Petrosia ficiformis* (usually discoloured), *Petrobiona massiliana* (mainly in Western Mediterranean caves), *Chondrosia reniformis* (usually discoloured), *Diplastrella bistellata, Penares euastrum, P. helleri,* and *Haliclona mucosa* (see Appendix I). Serpulid polychaetes are among the dominant taxa in caves, with the typical species being *Serpula cavernicola* and *Spiraserpula massiliensis* (Zibrowius, 1971; Bianchi & Sanfilippo, 2003; Sanfilippo and Mòllica, 2000). In some caves, the species *Protula tubularia* has been found to form aggregates which constitute the basis for the creation of bioconstructions; these "biostalactites" are constructed by invertebrates (serpulids, sponges, and bryozoans), foraminiferans and carbonate-forming microorganisms (Sanfilippo *et al*., 2015). Encrusting bryozoans (e.g. *Celleporina caminata* and *Onychochella marioni*) can also produce nodular and crest-like formations in the transitional zone between semi-dark and dark cave communities

Figure 1. Facies of *Corallium rubrum* in a semi-dark cave

(Harmelin, 1985). Brachiopods (e.g. *Joania cordata*, *Argyrotheca cuneata*, and *Novocrania anomala*) are common in dark cave habitats (Logan *et al.*, 2004). The species *N. anomala* is frequently found in high numbers, cemented on cave walls and roofs (Logan *et al.*, 2004). A number of deep-sea species belonging to various taxonomic groups (e.g. sponges and bryozoans) have been recorded in sublittoral dark caves, regardless of depth (Zibrowius, 1978; Harmelin *et al.*, 1985; Vacelet *et al.*, 1994). Several motile species often find shelter in dark caves such as the mysids *Hemimysis margalefi* and *H. speluncola*, the decapods *Stenopus spinosus, Palinurus elephas*, and *Plesionika narval* (more common in southern and eastern Mediterranean areas) and the fish species *Apogon imberbis* and *Grammonus ater* (Pérès, 1967; Ros *et al.*, 1985, Bussotti *et al.*, 2015).

I.1. METHODS FOR THE STUDY OF MARINE CAVE COMMUNITIES

Bearing in mind the aims pursued and the investigative tools to be implemented, this summary will be subdivided into two parts, inventorying methods and monitoring methods.

I.2. INVENTORYING MARINE CAVE COMMUNITIES

Inventorying of marine cave communities requires two levels:

- Locating the marine caves (geo-referencing, topography, mapping, etc.)
- Characterization of the communities (diversity, structure, species cover, etc.)

I.2.a. Locating marine cave communities

Diving is necessary for the inventorying, exploration and mapping of marine caves, except for shallow caves of the semi-submerged type which can be often spotted and accessed at the sea surface level[4]. To a certain level, basic information on the location, depth and morphology of marine caves could be derived from local diving and fishing communities, prior to any cave mapping initiative.

Topography plays a crucial role in the structuring of marine cave communities and, thus, recording of basic topographic features is important for cave inventories as well as for the design of appropriate sampling schemes and monitoring protocols. Good knowledge of the cave's topography prior

Figure 2. Shrimp *Stenopus spinosus* in an obscure cave

[4] Semi-submerged caves are not always dark enough to allow the development of typical cave communities.

to underwater fieldwork is important for safety reasons (Rastorgueff *et al*., 2015). The most striking topographic features to be considered during marine cave inventorying are: depth, orientation and dimensions of the cave entrance(s); cave morphology (e.g. blind cave or tunnel); submersion level (e.g. semi-submerged or submerged cave); maximum and minimum water depth inside the cave; and total length of the cave. Definitions for these topographic attributes are available in the World Register of marine Cave Species (WoRCS) thematic species database of the World Register of Marine Species (Gerovasileiou *et al*., 2016a). Unique abiotic and biotic features, such as micro-habitats that could support distinct communities and rare species (e.g. sulphur springs, freshwater springs, bioconstructions etc.) should be also recorded.

Diving in marine caves, even in the shallower ones, is logistically challenging and requires the adoption of appropriate safety measures under the precautionary approach, even for experienced divers. The cave bottom is often covered by silty sediment which could easily be stirred up by divers, thus reducing visibility and making it difficult – or impossible – to locate the cave entrance. Therefore, a dive reel with calibrated line (e.g. distance markers every 1 m) is necessary along with standard SCUBA equipment (e.g. dive computer, lights, magnetic compass, dive slate). Additional equipment is needed for taking distance measurements (e.g. tape measure, portable echosounder and waterproof range finder for semi-submerged caves).

To date, a wide array of methodologies and equipment have been developed mostly for the mapping of terrestrial and groundwater caves and channels, including remotely operated options (e.g. Fairfield *et al*., 2007; Stipanov *et al*., 2008; Poole *et al*. 2011) and software platforms for three-dimensional (3D) cave modelling (e.g. Sellers & Chamberlain, 1998, Boggus & Crawfis, 2009; Gallay *et al*., 2015; Oludare Idrees

Figure 3. Exploration dive in an underwater cave

& Pradhan, 2016). Regarding marine cave biological research in the Mediterranean Sea, a rapid and cost-efficient protocol for the 3D mapping and visualization of entirely and semi-submerged marine caves with a simple, non-dendritic morphology, has been developed and described by Gerovasileiou *et al*. (2013). The method can be applied by two divers in 1-2 dives and enables the automatic production of 3D depictions of cave morphology using the accompanying "cavetopo" software. A GPS device is necessary for geo-referencing the location of the access point to the surveyed marine cave at the sea surface level. Recently, in the framework Grotte-3D Project three submerged caves in Parc National des Calanques (France) were depicted in high-resolution 3D models using photogrammetry (Chemisky *et al*., 2015). A useful protocol for inventorying semi-submerged caves, has been provided by Dendrinos *et al*. (2007); however, in areas which support Mediterranean monk seal (*Monachus monachus*) populations, such initiatives should be undertaken during periods with low in-cave seal activity (e.g. late spring / early summer) to minimize potential disturbance.

Most of the Mediterranean marine caves studied are semi-submerged or shallow and very few exceed the maximum depth of 30 m, probably due to the logistic constraints in underwater work. The inventorying of deeper and complex cave formations requires highly specialized skills and diving equipment (e.g. Close Circuit Underwater Breathing Apparatus – CCUBA) inducing a greater extent of risks than conventional SCUBA diving. The exploration of deep-sea caves and overhangs requires the use of Remote Operating Vehicles (ROV), though involving several limitations.

I.2.b. Characterization of marine cave communities

Marine caves support well diversified and unique biological communities (Pérès & Picard, 1949; Pérès 1967; Riedl 1966; Harmelin *et al*., 1985). The general principles and methods for the characterization of hard substrate cave communities are similar to those described for the coralligenous ones (UNEP-MAP-RAC/SPA, 2015b). The use of non-destructive quantitative methods (e.g. photoquadrats) for studying the structure of sessile communities is highly recommended (e.g. Martí *et al*., 2004; Bussotti *et al*., 2006; Gerovasileiou & Voultsiadou, 2016). These methods minimize both human impact on these fragile communities and duration of underwater work, still providing reference conditions for monitoring at given sites (Bianchi *et al*., 2004). Given the limitations of the visual identification of several benthic taxa, the collection of supplementary qualitative samples is often necessary. The adoption of biological surrogates (e.g. morphological diversity) for the study of cave sessile benthos could further facilitate their non-destructive study and monitoring (Parravicini *et al*., 2010; Nepote *et al*., 2017; Gerovasileiou *et al*., 2017).

Advanced image processing software dedicated to marine biological research integrate methods and tools for the accurate extraction of species coverage / abundance from photoquadrats (e.g. Teixidó *et al*., 2011; Trygonis & Sini, 2012). Semi-quantitative evaluations through underwater visual census could also provide valuable information in certain cases.

Visual census methods can be also applied for studying the structure of mobile cave fauna; specifically, a modified transect visual census method (Harmelin-Vivien *et al*., 1985) adapted to cave habitats has been developed and applied in several Mediterranean caves for the study of fish assemblages (Bussotti *et al*., 2002; 2006; Bussotti & Guidetti, 2009; Bussotti *et al*., 2015) as well as for decapod crustaceans (Denitto *et al*., 2009).

Sampling with hand-held corers is necessary for studying soft sediment communities of the cave bottom (Todaro *et al*., 2006; Janssen *et al*., 2013; Navarro-Barranco *et al*., 2012 ; 2014).

I.3. Monitoring marine cave communities according to the recommendations of the Integrated Monitoring and Assessment Programme (IMAP)

The lack of quantitative data and long time-series from marine caves in most Mediterranean areas is a major impediment to evaluating changes in their ecological status. However, there is evidence of alterations through time in caves of the north-western Mediterranean, suggesting that there might be an unregarded decrease in quality at a broader scale (Parravicini *et al*., 2010; Rastorgueff *et al*., 2015; Gubbay *et al*., 2016). The most important pressures affecting marine cave communities are: mechanical damage of fragile species caused by unregulated diving activities, physical damage and siltation due to coastal and marine infrastructure activities, marine pollution (e.g. sewage plant outflow, marine litter), extractive human activities (e.g. red coral harvesting), water temperature rise, and potentially non-indigenous species (Chevaldonné & Lejeusne, 2003; Guarnieri *et al*., 2012; Giakoumi *et al*., 2013; Gerovasileiou *et al*., 2016b).

Following the IMAP recommendations, it is suggested that future monitoring schemes for marine caves should mainly consider common indicators related to biodiversity (EO1):

EO1 – Common Indicator 1 "Habitat distributional range":

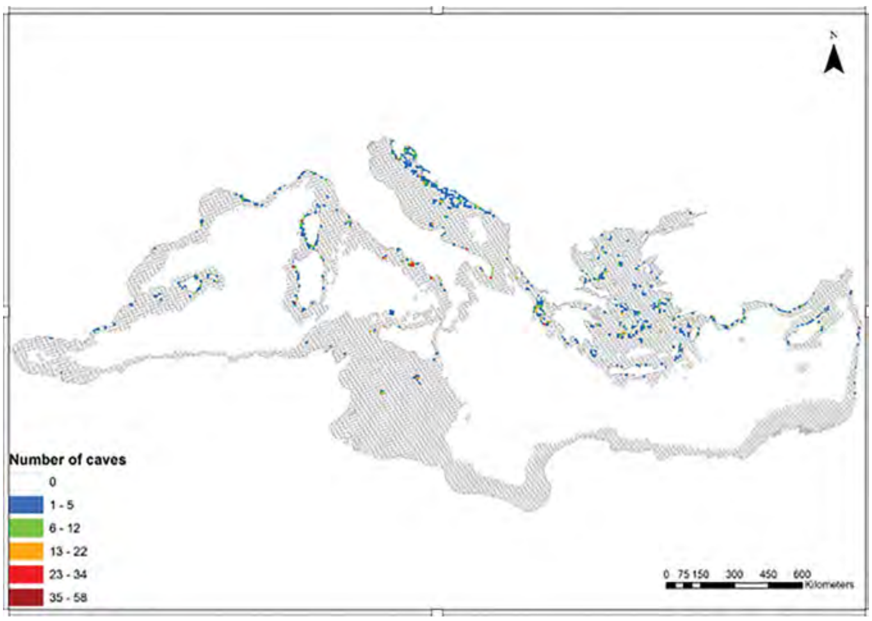
To date approximately 3,000 marine caves (semi- and entirely submerged) have been recorded in the Mediterranean basin (see Map 1) according to the latest census (Giakoumi *et al*., 2013); most of these caves (97%) are located in the North Mediterranean which encompasses a higher percentage of carbonate coasts and has been more extensively studied. More data on the number of known caves recorded in different Mediterranean regions are presented in the case studies below. Nevertheless, the number of underwater caves penetrating the rocky coasts of the Mediterranean basin remains unknown and comprehensive mapping efforts are necessary to fill distribution gaps, especially in the eastern and southern marine regions.

EO1 – Common Indicator 2 "Condition of the habitat's typical species and communities":

A list of species that are frequently reported in Mediterranean marine caves is presented in Appendix I[5]. However, most of the present knowledge concerns the biota associated with the rocky walls and vaults of caves, while less information is available about the infauna in cave floor sediments (Bianchi & Morri, 2003). Marine caves are characterized by a high degree of natural heterogeneity and their communities present qualitative and quantitative differences in species composition across different Mediterranean ecoregions (Gerovasileiou & Voultsiadou, 2012; Bussotti *et al*., 2015). For instance, species which have been traditionally considered cave characteristic in the western basin (e.g. *Corallium rubrum*) may be rare or even absent in the eastern basin and vice versa. Thus, the list is annotated with comments on the distribution of certain taxa.

The elimination of fragile sessile invertebrates (e.g. the bryozoans *Adeonella spp*. and *Reteporella spp*.) or particular growth forms (e.g. massive and erect invertebrates) and the replacement of endemic cave mysids by thermotolerant congeners are among the most striking examples of negative alterations on cave communities (Chevaldonné & Lejeusne, 2003; Guarnieri *et al*., 2012; Nepote *et al*., 2017). The damage to fragile organisms can be more easily avoided in caves inside MPAs, which can be protected, managed and monitored more effectively. The use of taxonomic surrogates as well as morphological and functional descriptors for sessile benthos could greatly assist monitoring initiatives (Parravicini *et al*., 2010; Gerovasileiou & Voultsiadou, 2016; Gerovasileiou et al., 2017; Nepote *et al*., 2017).

An ecosystem-based index for the evaluation of the ecological quality of marine cave ecosystems was recently developed and tested in the western Mediterranean basin (Rastorgueff *et al*., 2015). According to this approach, the following features could be indicative of high quality status: high spatial coverage of suspension feeders with a three-



Carte 1. Distribution of marine caves in the Mediterranean Sea;
different colours represent the number of caves in 10 x 10 km cells (from Giakoumi *et al*., 2013).

[5] This species list is not exhaustive but includes species reported from a considerable number of semi-dark and dark caves at the Mediterranean scale according to data from the Mediterranean marine cave biodiversity database by Gerovasileiou & Voultsiadou (2012, 2014).

dimensional form (e.g. *C. rubrum*) and large filter feeders (e.g. the sponges *Petrosia ficiformis* and *Agelas oroides*) along with the presence of mysid swarms and several species of omnivorous and carnivorous fish and decapods.

Monitoring of marine cave communities and sessile invertebrates with slow growth rates could be also benefited from methods quantifying 3D features, using photogrammetry (e.g. Chemisky *et al*., 2015).

Other indicators of the IMAP

Other indicators of the IMAP which could be considered on a supplementary basis, especially in areas of higher risk, are: EO2 – trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species, particularly the invasive ones, in relation to their main vectors and pathways of spreading (mainly in the Levantine Sea); EO8 – length of coastline subject to physical disturbance due to the influence of man-made structures, that should be also used for the assessment of EO1 on habitat extent (Common Indicator 1); EO9 – occurrence, origin (where possible), and extent of acute pollution events (e.g. slicks from oil, oil products and hazardous substances) and their impact on biota affected by this pollution; and EO10 – trends in the amount of litter in the water column and on the seafloor, including microplastics.

A fill-in form that could be used as a basis for recording (a) basic topographic features, (b) characteristic species from different functional components of the ecosystem-based approach by Rastorgueff *et al.* (2015), (c) protected species, and (d) pressures and threats is available in Appendix II.

I.4. REGIONAL OVERVIEW

Western Mediterranean

To date 1,046 marine caves have been recorded in the Western Mediterranean basin (Giakoumi *et al*., 2013). The rocky coasts of the Tyrrhenian Sea and the Algero-Provençal Basin have been extensively studied for their cave biodiversity, with 822 and 650 taxa recorded from these two areas respectively (Gerovasileiou & Voultsiadou, 2014). The first and some of the most influential studies on the diversity and structure of marine cave communities were carried out in the French, Italian and Catalan coasts (e.g. Pérès & Picard, 1949; Riedl, 1966; Harmelin *et al*., 1985; Ros *et al*., 1985; Bianchi & Morri, 1994; Bianchi *et al*., 1996). A synthesis of the existing knowledge on Italian marine caves, accumulated in fifty years of research, was compiled by Cicogna *et al*. (2003). The fully submerged caves of Figuier, Jarre, Riou, Trémies and Triperie in the karstic coasts of Marseille-Cassis area are among the species-richest Mediterranean caves while the famous Trois Pépés cave has been characterized as a unique "deep-sea mesocosm" in the sublittoral zone, supporting deep-sea faunal elements in its inner dark sectors (Vacelet *et al*., 1994; Harmelin, 1997). Submarine caves in the region of Palinuro (Tyrrhenian Sea) have been found to host sulphur springs which support trophic webs based on chemosynthesis (Bianchi *et al*., 1994; Morri *et al*., 1994b; Southward *et al*., 1996), presenting analogies with deep-water chemosynthetic ecosystems.
The number of species reported from marine caves decreases towards the insular and southern sectors of the Western Mediterranean basin, according to differences in temperature and trophic conditions (Uriz *et al*., 1993) and to a notable decrease in research effort (Gerovasileiou & Voultsiadou, 2014). For instance, the Alboran Sea is one of the least studied areas regarding its marine cave fauna (but see Navarro-Barranco *et al*., 2014; 2016). Nevertheless, recent research expeditions in the framework of the MedKeyHabitats project have provided baseline information for the previously understudied Alboran coasts of Morocco (PNUE/PAM-CAR/ASP, 2016).

In the framework of the recent evaluation of ecological quality status in 21 Western Mediterranean caves using the CavEBQI index, 14 caves were found in favourable status (good/high ecological quality) and no cave was found to be of bad ecological quality (Rastorgueff *et al*., 2015). However, a comparison of data obtained in 1986 and 2004 from the Bergeggi cave (Ligurian Sea, Italy) revealed a decrease in ecological quality attributed to summer heat waves (Parravicini *et al.*, 2010; Rastorgueff *et al.*, 2015).

Ionian Sea and Central Mediterranean

The western coasts of the Ionian Sea are among the best-studied Mediterranean areas regarding their marine cave biodiversity, with almost 700 taxa reported in this area (Gerovasileiou & Voultsiadou, 2014). To date 375 marine caves are known from the Ionian Sea and the Tunisian Plateau/Gulf of Sidra (Giakoumi *et al*., 2013). Most of the regional inventories, mapping initiatives and biodiversity studies have taken place in the Salento Peninsula (e.g. Onorato *et al*., 1999; Bussotti *et al*., 2002; 2006; Denitto *et al*., 2007; Belmonte *et al*., 2009; Bussotti & Guidetti, 2009) and in Sicily (e.g. Rosso *et al*., 2013; 2014; Sanfilippo *et al*. 2015). Piccola del Ciolo cave, which is one of the most studied Mediterranean marine caves, was evaluated to be of high ecological quality using CavEBQI index (Rastorgueff *et al*., 2015). A preliminary survey of marine cave habitats has recently taken place in Malta (Knittweis *et al*., 2015).

On the eastern Ionian Sea, a considerable number of caves are located in the National Marine Park of

Zakynthos (NMPZ), Greece. Marine caves in this area were recently studied and evaluated for their ecological status (V. Gerovasileiou – HCMR / NMPZ, unpublished data).

Adriatic Sea

Up to date 708 marine caves have been recorded in the Adriatic Sea (Giakoumi *et al*., 2013), supporting approximately 400 taxa (Gerovasileiou & Voultsiadou, 2014). The coasts of Croatia are among the most well-studied Mediterranean areas concerning their marine and anchialine caves, in terms of geology (e.g. detailed mapping initiatives by Surić *et al.*, 2010) and biodiversity (e.g. Riedl, 1966, Bakran-Petricioli *et al.*, 2007; 2012; Radolovic *et al.* 2015). Specifically, Y-Cave on Dugi Otok Island is one of the species-richest caves in the Mediterranean basin while deep-sea sponges have been found in caves of the islands Hvar, Lastovo, Veli Garmenjak, Iški Mrtovnjak and Fraškerić (Bakran-Petricioli *et al.*, 2007). Recently, inventories for marine cave habitats and their communities have taken place in Montenegro and Albania in the framework of the MedKeyHabitats project.

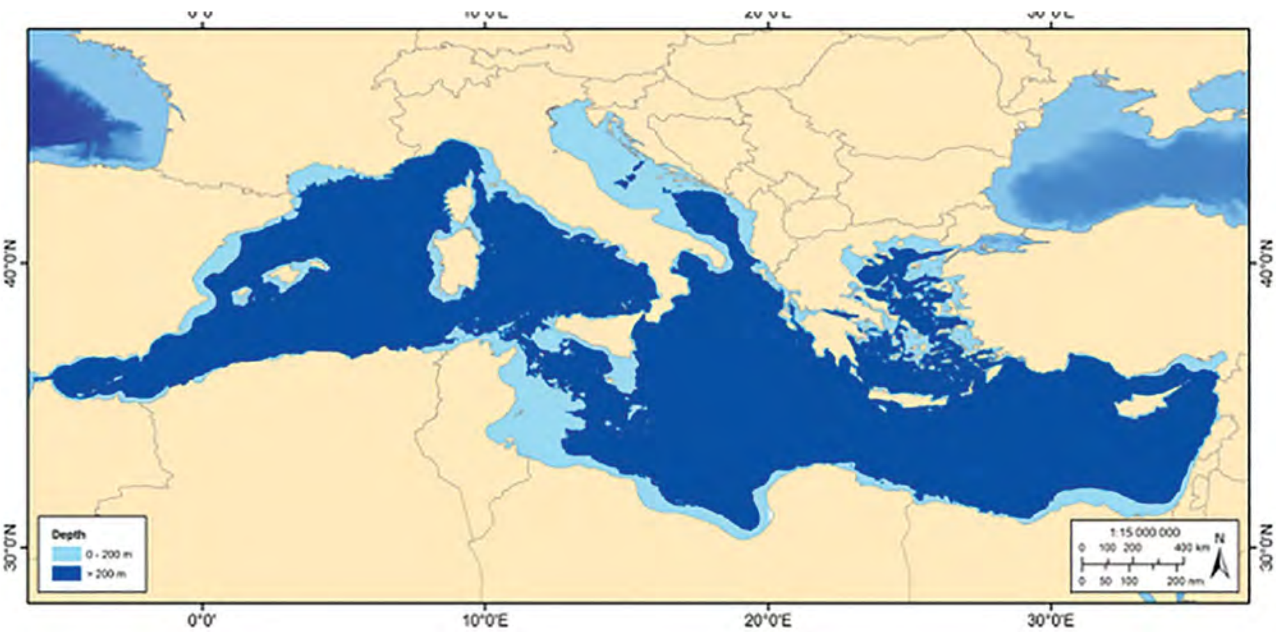
Aegean Sea and Levantine Sea

The coasts of the eastern Mediterranean basin host approximately one third (738) of the marine caves recorded in the Mediterranean Sea, mostly across the complex coastline of the Greek Islands in the Aegean Sea (Giakoumi *et al.*, 2013). A total of 520 taxa have been found in caves of the Aegean and the Levantine seas (324 and 157, respectively) (Gerovasileiou *et al.*, 2015). Lesvos Island in the North Aegean Sea hosts two of the best-studied marine caves with regard to their diversity (approximately 200 taxa recorded in each cave), community structure and function (Gerovasileiou & Voultsiadou, 2016; Sanfilippo *et al.*, 2017). Several caves scattered across the Aegean ecoregion were recently studied for their biodiversity (e.g. Rastorgueff *et al.*, 2014; Gerovasileiou *et al.*, 2015), community structure and ecological quality (V. Gerovasileiou, unpublished data). One of the most well-known insular areas concerning their marine cave formations is encompassed within the National Marine Park of Alonissos and Northern Sporades, hosting numerous cave habitats, critical for the survival of the endangered Mediterranean monk seal *M. monachus* (Dendrinos *et al.*, 2007).

The coasts of Lebanon host most of the studied Levantine caves (e.g. Bitar & Zibrowius, 1997; Logan *et al.*, 2002; Pérez *et al.*, 2004; Vacelet *et al.*, 2007; Morri *et al.*, 2009). Fourty six non-indigenous species have been recorded in 80% of the marine caves and tunnels known to exist in the Levantine Sea, mostly at their entrance and semi-dark zones (Gerovasileiou *et al.*, 2016b), indicating a potential new threat for cave communities that should be further monitored.

II. HABITATS AND SPECIES ASSOCIATED WITH SEAMOUNTS, CANYONS, APHOTIC HARD (AND SOFT) BEDS AND CHEMOSYNTHETIC PHENOMENA

Dark habitats are those where either no sunlight arrives or where the light that does arrive is insufficient for the development of plant communities. They include both caves and deep habitats, usually at depths below 150/200 m (see Map 2).



Map 2. Deep-sea areas in the Mediterranean Sea below 200 m depth

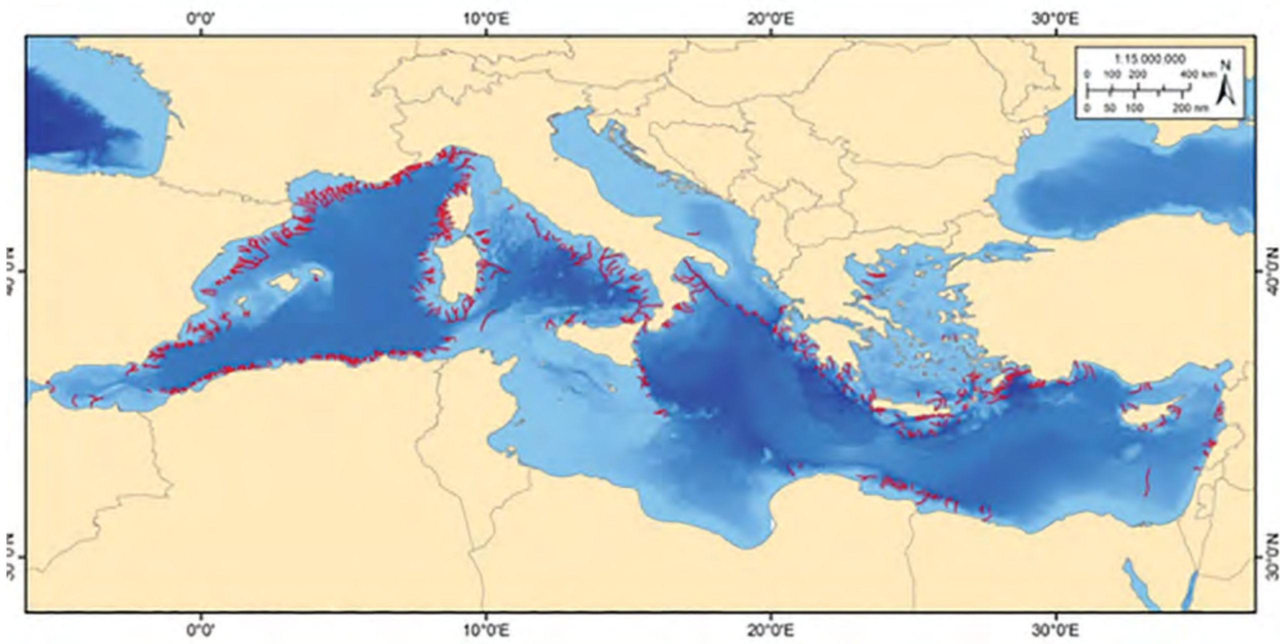
In some geoformations that typically contain dark habitats it can also occur that, given their wide bathymetric range, parts of these are in the photic zone. This is the case of the summits of seamounts and in the heads of canyons. The importance of maintaining their integrity justifies considering all of these habitats to be included within the classification of dark habitats.

Dark habitats can be found in very diverse and extensive areas of the Mediterranean, given that this sea has an average depth of about 1,500 m, with many of its seabeds in aphotic zones.
As agreed and set out in the Dark Habitats Action Plan, the existing biological communities will be analysed as follows:

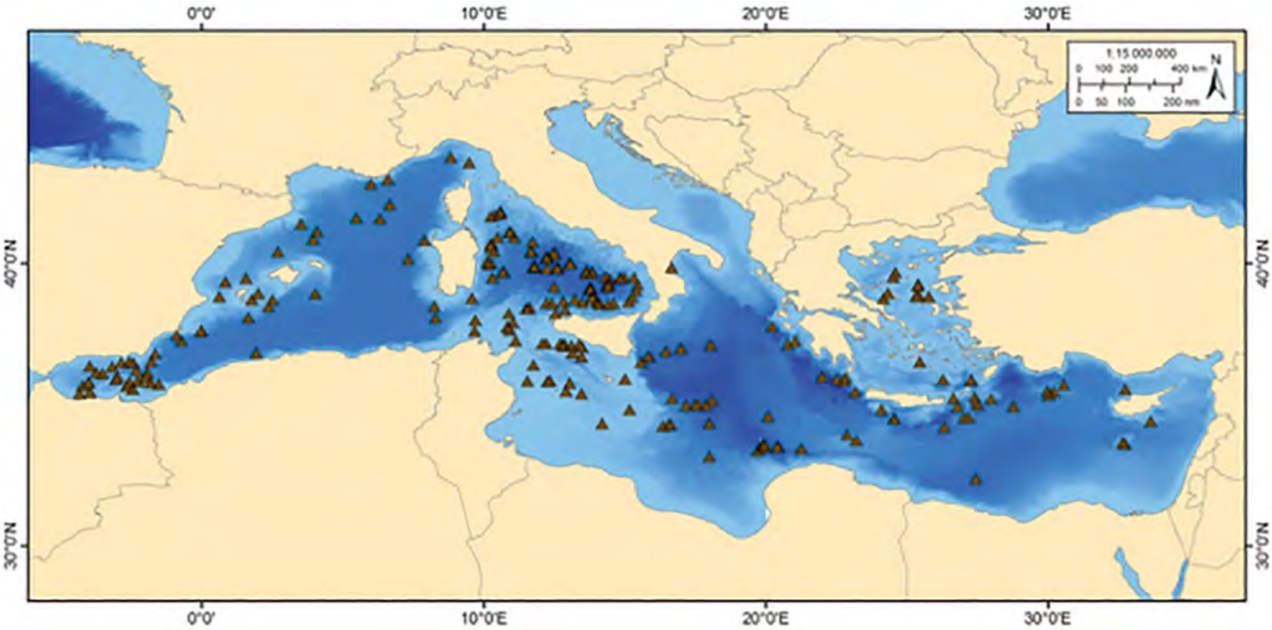
- Assemblages of underwater canyons
- Engineering benthic invertebrate assemblages
  - º Black coral and gorgonian forests on hard substrata
  - º Beds with *Isidella elongata* and beds with *Pennatula* on crumbly substrata
  - º Associations of big sponges and "Deep water corals" present on both types of substrata
- Deep-sea chemosynthetic assemblages
- Assemblages associated with seamounts

However, mention will also be made to other recently discovered types of dark habitats which are more difficult to include within this categorisation, but which, thanks to advances in scientific knowledge, are being added to the lists of deep-sea habitats.

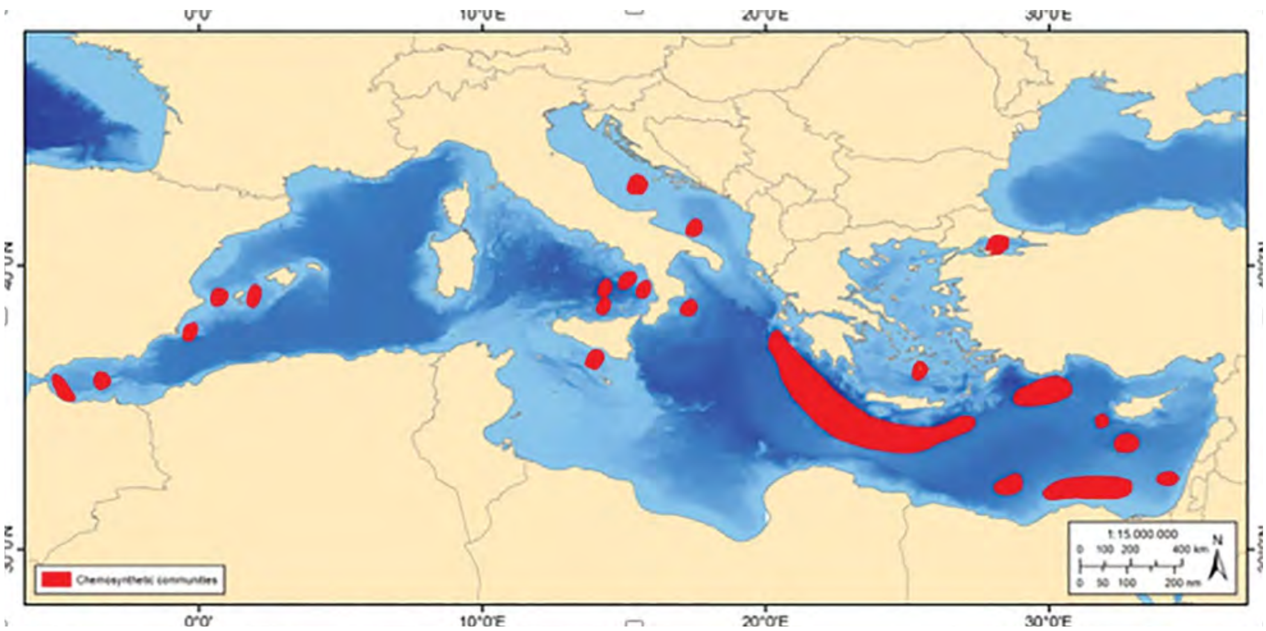
In the Mediterranean, 518 large canyons have been identified (Harris & Whiteway, 2011), along with around 242 underwater mountains or seamount-like structures (Würtz & Rovere, 2015) and there are some twenty sites where deep-water chemosynthetic assemblages have been confirmed (Taviani, 2014). However, there are still many other canyons, underwater structures and sites involving the release of gas that have not yet been studied, which is certain to change these figures. Also, 80% of the Mediterranean seabeds are at a depth of more than 200 m, and could therefore potentially be home to dark habitats (see Maps 3, 4 and 5).



Map 3. Distribution of Mediterranean submarine canyons
(compiled by the authors based on data available from different sources)



Map 4. Distribution of Mediterranean seamounts
(compiled by the authors based on data available from different sources)



Map 5. Identified areas with chemosynthetic assemblages
(compiled by the authors based on data available from different sources)

II. 1. INVENTORY: LOCATION AND CHARACTERIZATION

The habitat-forming species most characteristic of aphotic zones are sponges and anthozoans, although other phyla and classes, such as mollusks, polychaete tube-worms, bryozoans, cirriped crustaceans, etc., may also have a predominant role in some cases or be a fundamental part of mixed habitats, through the formation of complex bioconstructions or large communities that provide three-dimensional structures.

Habitats dominated or formed by stony corals (Scleractinia)

The best known are cold water coral reefs (CWC) mainly formed by *Lophelia pertusa* and *Madrepora oculata*. They usually occur in rocky substrates (e.g. seamounts, canyons or escarpments) although they could also be found in highly silted areas.

Their bathymetric range is usually between about -200 m and down to more than -1,000 m, and they have been found both in the Western and Eastern Central Mediterranean in places such as the Cabliers, Chella and Avempace seamounts in the Alboran Sea (de la Torriente *et al.*, 2014; Pardo *et al.*, 2011; Lo Iacono *et al.* 2014), in canyons in the Gulf of Lion and the surrounding area such as Cassidaigne and Creus (Fourt & Goujard, 2012; Orejas *et al.*, 2009; Gori *et al.* 2013a; Bourcier & Zibrowius, 1973), in the southern Catalan canyons (e.g. La Fonera canyon, Lastras *et al.* 2016), south of Sardinia in the Nora Canyon (Taviani *et al.*, 2016b), in the Gulf of Naples (Taviani *et al.*, 2016c), offshore Santa Maria di Leuca in the Northern Ionian Sea (D'Onghia *et al.*, 2012; Mastrototaro *et al.*, 2010; Savini *et al.*, 2010; Taviani *et al.*, 2005a,b; Vertino *et al.*, 2010), south of Malta and other sites in the Strait of Sicily (Evans *et al.*, 2016; Freiwald *et al.*, 2009; Schembri *et al.*, 2007; Taviani *et al.*, 2009, 2011a), next to the Jabuka-Pomo depression (Županović, 1969), in the Bari canyon and off Apulia in the Southwestern Adriatic (Angeletti *et al.*, 2014; D'Onghia *et al.*, 2015; Freiwald *et al.*, 2009), in the Montenegrin canyons (Angeletti *et al.*, 2014, 2015a), in the Adriatic Sea, trough off Thassos in northern Aegean Sea (Vafidis *et al.*, 1997), in the Marmara Sea (Taviani *et al.*, 2011b), in the deep waters of the Hellenic Arc in the south of the Aegean/ Levantine Basin (Fink *et al.*, 2015), among others.

Other stony corals that form important marine habitats are the tree corals (*Dendrophyllia spp*.). *D. cornigera* can form dense aggregations in deep seabeds, although in the Mediterranean it is rare to find places with dense populations. Its bathymetric range can vary from shallow water to depths of more than 600 m. It has been found mainly in the western basin, on seamounts in the Alboran Sea (de la Torriente *et al.*, 2014; Pardo *et al.*, 2011), in submarine canyons in the Gulf of Lion and Corsica (Orejas *et al.*, 2009; Gori *et al.* 2013a; Fourt & Goujard, 2014), in the Balearic Archipelago continental shelf and slope (Orejas *et al.*, 2014), on seamounts in the Tyrrhenian Sea (Bo *et al.*, 2011) in the Ligurian Sea (Bo *et al.*, 2014), in some areas of the Central Mediterranean (Würtz & Rovere, 2015), including the banks of the Ionian Sea (Tursi *et al.*, 2004), and in the southern Adriatic Sea (Angeletti *et al.*, 2015; Freiwald *et al.*, 2009).

Dendrophyllia ramea is more common in shallower waters. Recently, however, *D. ramea* communities have been found in deep waters in the Eastern Mediterranean, such as the deep seabeds of Cyprus (Orejas *et al.*, in press) and the submarine canyons off Lebanon (R. Aguilar Pers. Obs.). Both species can occur on rocky and soft seabeds. Furthermore, in the northern part of the Sicilian coast, between 80 and 120 m depth, a huge population of *D. ramea* with several colonies was recently discovered. Many colonies showed severe injure caused by lost fishing gear (S. Canese Pers. Obs.). Probably this species presented a more diffuse abundance and distribution in the past.

Other colonial stony corals that have been found forming dense aggregations in certain places are *Madracis pharensis*, - a typical component of cave assemblages that is particularly abundant in the coralligenous outcrops of the Eastern Mediterranean - abundant in the heads of canyons and coastal waters of Lebanon, in depths of down to nearly 300 m, sometimes in mixed aggregations with brachiopods, mollusks and polychaetes (R. Aguilar Pers. Obs.). Colonies of *Anomocora fecunda* have been found on the seamounts of the Alboran Sea (de la Torriente *et al.*, 2014) on seabeds at depths of between 200 and 400 m.

There are also solitary corals that sometimes create important aggregations. This is the case of the pan-Mediterranean *Desmophyllum dianthus*, a solitary coral with a pseudocolonial habit found in both canyons and deep seabeds, alone or even participating in the formation of reefs with *Lophelia pertusa* and *Madrepora oculata* (de la Torriente *et al.*, 2014; Freiwald *et al.*, 2009; Fourt *et al.*, 2014; Galil & Zibrowius, 1998; Montagna *et al.*, 2006; Taviani *et al.*, 2011b, 2016a, b).

Members in the genus *Caryophyllia* settle on rocky and detritic bottoms and may become important at places. For example, *Caryophyllia calveri* is one of the most common solitary coral species in deep rocky bottoms, being capable of forming dense communities, sometimes along with other

Figure 4. *Dendrophyllia cornigera*, Catifas Bank

scleractinians such as *Javania cailleti, Stenocyathus vermiformis* and other *Caryophyllia spp*. As it has been found in seamounts, escarpments or rocky bottoms (Aguilar *et al.*, 2014; Aguilar *et al.*, 2013; Mastrototaro *et al.*, 2010; Galil & Zibrowius, 1998).

In the case of soft bottoms, mainly in detritic sands, beginning in the deep circalittoral sand and extending to depths of down to 400/500 m, *Caryophyllia smithii* f. *clavus* can cover significant areas (de la Torriente *et al.*, 2014), similar to *Flabellum spp*. in the Atlantic (for example: Serrano *et al.*, 2014; Baker *et al.*, 2012).

Habitats formed by black corals

Antipatharians or black corals are represented in the Mediterranean by just a few species, although this number may increase with the recent finding of at least one previously unknown species.
The species that reach the highest densities are *Antipathella subpinnata, Leiopathes glaberrima*, and (in some occasions) *Parantipathes larix* that can form monospecific assemblages (e.g. Bo *et al.*, 2009, 2015; Ingrassia *et al.*, 2016). *Antipathes dichotoma* can also occur on high densities, but many times are part of other black coral communities alongside gorgonians. They have a wide bathymetric distribution with some species occurring at relatively shallow depth (≈60 m) (Bo *et al.*, 2009), and others extending to the superficial bathyal zone (and even sometimes in the deep circalittoral zone) and reaching depths of over 1,000 m. It is known that some *Leiopathes sp*. inhabit depths down to 4,000 m (Molodtsova, 2011).

Dense aggregations have been found on seamounts in the Alboran (de la Torriente *et al.*, 2014), the Balearic Archipelago (Grinyó, 2016), and Tyrrhenian Seas (Ingrassia *et al.*, 2016; Fourt *et al.*, 2014), in South Western Sardinia (Cau *et al.*, 2016; Bo *et al.*, 2015), on the escarpments to the south of Malta (Evans *et al.*, 2016; Deidun *et al.*, 2014), in the Ionian Sea (Mytilineou *et al.*, 2014) and in the eastern Adriatic Sea (Angeletti *et al.* 2014; Taviani *et al.*, 2016a). Sporadic occurrences have been also reported from various sites in the Mediterranean, like the Malta Escarpment and offshore Rhodes (Angeletti *et al.*, 2015a; Taviani *et al.*, 2011b).

Antipathella subpinnata normally occupies shallower areas on seamount summits (de la Torriente *et al.*, 2014; Bo *et al.*, 2009;), but can reach greater depths and have a wide distribution in the Mediterranean, mainly in the Western and Central basins but also in the Aegean Sea (Bo *et al.*, 2008; Vafidis & Koukouras, 1998). *Antipathella wollastoni* has also been recorded in the Mediterranean near the Strait of Gibraltar (Ocaña *et al.*, 2007).
Recently other black coral species have also been observed forming dense aggregations. An example of this is *Parantipathes larix* in some areas of the Alboran Sea (Pardo *et al.*, 2011) and in deep waters off the Tuscan and Pontin archipelago in the Tyrrhenian Sea (Bo *et al.*, 2014b, Ingrassia *et al.*, 2016), also in Corsica and Provence region (Fourt *et al.*, 2014), and a new species of Antipatharia (not yet described) on seamounts in the south of the Alboran Sea, such as Cabliers Bank.

Figure 5. *Antipathes dichotoma* and *Leiopathes glaberrima*, Malta

Parantipathes larix has a wide bathymetric distribution, from 120 m and down to over 2,000 m (Opresko and Försterra, 2004; Fabri *et al.*, 2011; Bo *et al.*, 2012b), while the new black coral has only been found in depths between 180/400 m although its distribution could be more extensive.

All black coral species are found on hard bottoms, although they can withstand some sedimentation and may occur on rocky bottoms slightly covered by sediments. They can also occur on seamounts, in canyons or on deep sea environments where hard substrates are present.

Habitats dominated by gorgonians

Deep Mediterranean gorgonian assemblages (Alcyonacea excluding Alcyoniina) can be highly diverse and present a wide geographic and bathymetric distribution.

Most are species that attach to a hard substrate, although some can withstand high levels of sedimentation and a few species can occur in soft bottoms, both detritic and muddy.

Some of the assemblages that reach higher densities are those formed by the Atlanto-Mediterranean gorgonian *Callogorgia verticillata*. Dense "forests" have been found that can begin in the deep circalittoral zone and extend to a depth of more than 1,000 m (Angeletti *et al.*, 2015a; Evans *et al.*, 2016: de la Torriente *et al.*, 2014). These forests may be monospecific or may be formed by several gorgonian species (e.g. *Bebryce mollis, Swiftia pallida*), antipatharians (e.g. *L. glaberrima* and A. *dichotoma*) or scleractinian white corals (e.g. *L. pertusa, Dendrophyllia spp*). A frequent association of this species is with the whip coral (*Viminella flagellum*), especially in the deep circalittoral and upper bathyal zones (Lo Iacono *et al.*, 2012: Giusti *et al.*, 2012), where it is more common.

Other species that commonly occur on hard substrates of the continental slope is *Acanthogorgia hirsuta* that can occur as isolated colonies (Grinyó *et al.*, 2016) or forming dense assemblages (Fourt *et al.*, 2014b; Aguilar *et al.*, 2013), sometimes with other gorgonians such as *Placogorgia spp.*, on the slopes of seamounts or on the gently inclining edges of escarpments (de la Torriente *et al.*, 2014). It is also a species observed as part of the Alcyonacea that grow among coral rubbles or with other communities of deep-seabed corals and gorgonians, usually below 250/300 m.

Eunicella cavolini and *E. verrucosa* are the only species of the genus *Eunicella* that can be found in rocky bottoms at great depths. *Eunicella. cavolini* was observed down to 280 m in the Nice canyon (Fourt & Chevaldonné Pers. Obs.), however, they are more common on the tops of seamounts, forming monospecific assemblages or mixed with *Paramuricea clavata* (De la Torriente *et al.*, 2014; Aguilar *et al.*, 2013).

The latter is not usually found beyond 140/150 m, but becomes very abundant on the summits of seamounts, like the Palos, the Chella Banks (Aguilar *et al.*, 2013), or in heads of some canyons (Pérez-Portela *et al.*, 2016), such as Cassidaigne canyon where it occurs at a depth around 200 m (Fourt *et al.*, 2014). It shares this characteristic with *E. cavolini*, which has been found on rocky bottoms in the heads of canyons in the Balearic Sea (Grinyó *et al.*, 2016) and the Gulf of Lion (Fourt & Goujard, 2012).

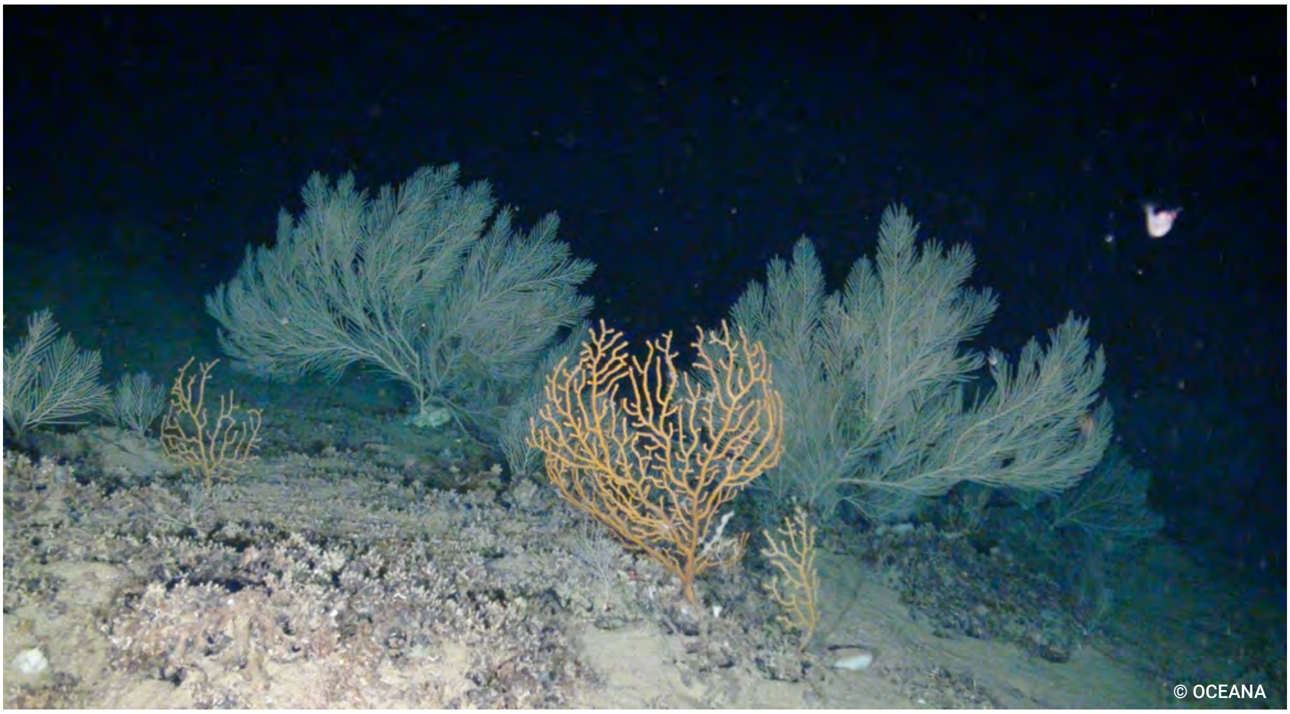


Figure 6. *Callogorgia verticillata* and *Placogorgia* sp., Ses Olives Seamount

There is a wide range of small gorgonians that can form dense thickets (Grinyó *et al.*, 2016; Angiolillo *et al.*, 2014) or co-occur alongside larger species such as *C. verticillata*, antipatharians or alongside cold water coral reef building species (Evans *et al.*, 2016). Among these species, we find *Bebryce mollis, Swiftia pallida, Paramuricea macrospina* and *Villogorgia bebrycoides*, which can occur on unstable substrata and coarse detritic bottoms, from the shelf edge (or even the deep circalittoral zone) to depths of 600/700 m (Aguilar *et al.*, 2013; Angeletti *et al.*, 2014; Evans *et al.*, 2016; Grinyó *et al.*, 2015; Taviani *et al.*, 2016b).
Swiftia pallida / S. dubia forms important single species thickets in the upper bathyal zone, usually between 200 and 700 m, although it may have a greater bathymetric range. It is widely distributed throughout the Mediterranean, having been found in seamounts of the Alboran Sea (de la Torriente *et al.*, 2014) to places as far away as the canyons off Lebanon (Aguilar Pers. Obs.) and Israel (Zvi Ben Avraham). It can occur on rocky and deep detritic bottoms, tolerating a certain level of sedimentation.
Muriceides lepida and *Placogorgia massiliensis*, on the other hand, occur as accompanying species in one of the assemblages described above, although they can also be the dominant species in some escarpments or in combination with sponge aggregations or other benthic communities (Evans *et al.*, 2016; Maldonado *et al.*, 2015). Both can be found in the Western and Central Mediterranean in zones ranging from a depth of 300 m to over 1,000 m.
The case of *Dendrobrachia bonsai* is similar, although it is a species associated with greater depths (usually below 400/500 m). It has been found forming thickets in deep rocky bottoms or as the predominant species in areas of escarpments and canyons with a steep inclination (Evans *et al.*, 2016; de la Torriente *et al.*, 2014; Sartoretto, 2012).

In the case of *Nicella granifera*, so far this has only been found in the Western Mediterranean, in seamounts between the Alboran and the Balearic Seas (Aguilar *et al.*, 2013). It has a deep bathymetric distribution, usually below 400 m.
Finally, the red coral (*Corallium rubrum*) shows a wide bathymetric range that stretches from shallow-water caves in the infralittoral zone to depths greater than 1,000 m in the bathyal zone (Rossi *et al.*, 2008; Taviani *et al.*, 2010; Knittweis *et al.*, 2016). Although it may form single-species forests on rocky bottoms or be the predominant species on escarpments and in caves (Cau *et al.*, 2016b), it has also been found as part of mixed forests associated with white corals, antipatharians or large gorgonians (Constatini *et al.*, 2010; Freiwald *et al.*, 2009; Evans *et al.*, 2016).

On soft bottoms, the most characteristic community is that of the bamboo corals (*Isidella elongata*). It is a species which is almost exclusive to the Mediterranean and which usually appears in muddy bottoms below depths of 400 m. It has been found on seamounts in the Alboran and Balearic Seas (de la Torriente *et al.*, 2014; Aguilar *et al.*, 2013; Mastrototaro *et al.*, 2017), deep seabeds in the Spanish slope (Cartes *et al.*, 2013), in front of the canyons in the Gulf of Lion (Fabri *et al.*, 2014), over the Carloforte Shoal

(Bo *et al.*, 2015), in the bathyal plain of Malta (Aguilar Pers. Obs.), and in the Ionian Sea (Mytilineou *et al.*, 2014), among other places.

Other soft-bottom species include *Spinimuricea spp.* (Topçu & Öztürk, 2016; Bo *et al.*, 2012b; Aguilar *et al.*, 2008), at depths ranging from the circalittoral zone to the upper bathyal, on detritic bottoms either in coastal areas and in deep-sea areas, sometimes alongside pennatulaceans and Alcyoniidae. The species *Eunicella filiformis*, develops freely on detritic seabeds (Templado *et al.*, 1993) with a distribution similar to that of *Spinimuricea spp*.

Habitats dominated by pennatulaceans

Since these are species that bury part of the colony in the substrate, they require soft bottoms, either sandy or muddy, between the infralittoral zone and the depths of the bathyal zone. They can therefore appear in all kinds of soft bottoms on seamounts and in canyons, and on bathyal plains and shelf edges, etc.
Species of the genus *Pennatula* and *Pteroeides* can form mixed communities that become numerous on the shelf edges and the beginning of the slope (e.g. Chella Bank) (de la Torriente *et al.*, 2014; Aguilar *et al.*, 2013; Gili & Pagès, 1987). The species may vary according to the depth, with *Pennatula rubra* being more frequent in shallower areas, while *P. phosphorea* occupies deeper seabeds, at depths reaching the muddy areas of the bathyal zone. Their distribution is pan-Mediterranean.
Virgularia mirabilis and *Veretillum cynomorium* are also species with a wide bathymetric and geographical distribution. Found all over the Mediterranean on seamount slopes, the shelf edges, plains, and in canyons, etc. (Aguilar *et al.*, 2013; Gili & Pagès, 1987), they occupy muddy-sandy bottoms, from the infralittoral to the bathyal zones, sometimes also mixing with other pennatulaceans or forming monospecific communities.

Funiculina quadrangularis also shares characteristics with other pennatulaceans, but it is a species typical of deep soft bottoms, found throughout the Mediterranean, at depths ranging from the circalittoral to the depths of the bathyal zone. It forms dense forests in shelf areas, gently sloping areas in canyons, and muddy-sandy interstices on seamounts, etc. (Fabri *et al.*, 2014; de la Torriente *et al.*, 2014; Morri *et al.*, 1991). It may appear in mixed communities with other pennatulaceans, bamboo corals, or other soft-bottom species, such as various bryozoans and sponges.
Recently, another pennatulacean whose distribution was believed to be exclusively Atlantic, has been discovered in several areas of the Mediterranean (Balearic Sea, Central Mediterranean and Ionian Sea). This is *Protoptilum carpenteri* (Mastrototaro *et al.*, 2015, 2017; Aguilar Pers. Obs.), which has a preference for the same substrate and looks very similar to *Funiculina quadrangularis*, which has sometimes led to it going unnoticed.

Finally, *Kophobelemnon stelliferum* is a typical species of deep muddy bottoms (usually below 400/500 m) - although sometimes shallower (Fourt *et al.*, 2012) - which, like other pennatulaceans, can appear mixed with other biological communities characteristic of these seabeds (*Isidella elongata, Funiculina quadrangualris, Kinetoskias sp*). It has been found on deep seamount summits such as Avempace in the Alboran Sea (Pardo *et al.*, 2011), or in bathyal zones of the Ionian Sea such as Santa Maria di Leuca (Mastrototaro *et al.*, 2013).

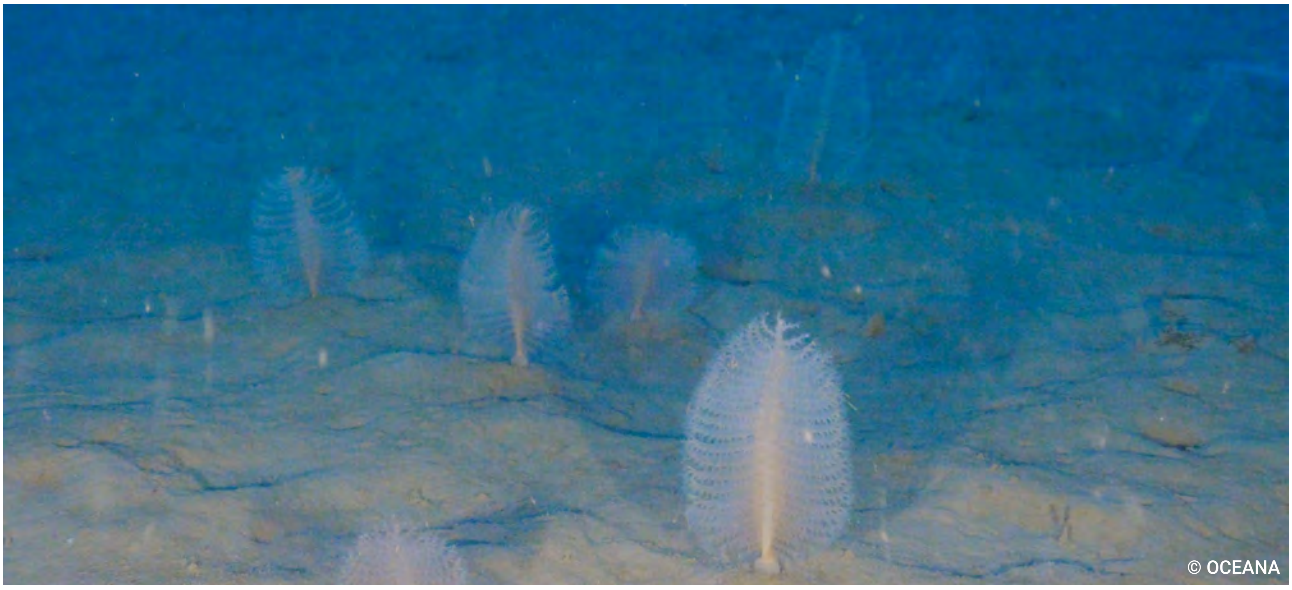


Figure 7. Pennatulaceans - *Pennatula rubra*, Lebanon

Habitats with other anthozoans

Other groups of anthozoans, such as Alcyoniidae, sea anemones (Actinaria) and cerianthids also give rise to communities characteristic of dark habitats.
These include newly discovered or rediscovered species, such as *Chironephthya mediterranea* (López-González *et al.*, 2014) and *Nidalia studeri* (López-González *et al.*, 2012), which create dense aggregations in the lower circalittoral and bathyal zones, at depths of between approximately 150 m and 400 m. They can be found on hard bottoms, and on the substrates with gravel and coarse sediments of seamounts, slope edges and submarine canyons. Their known geographical distribution stretches from the Western to the Central Mediterranean, although a wider distribution has not been ruled out.
Equally important are species such as *Alcyonium palmatum* and *Paralcyonium spinulosum* (Marin *et al.*, 2014; UNEP-MAP-RAC/SPA, 2013; Bo *et al.*, 2011; Marin *et al.*, 2011b; Templado *et al.*, 1993), since their plasticity in the occupation of both soft and hard bottoms allows them to colonise large areas of the Mediterranean, in both shallow and dark habitats, usually found on seamounts' summits. It is not uncommon for them to associate with other anthozoans.
With regard to anemones, at present only *Actinauge richardii* can be considered as a dark habitat species which forms communities of importance. Habitual in sedimentary bottoms, preferably sandy, between the circalittoral and the bathyal zones, it is found in large numbers on the gentle slopes of seamounts in the Western Mediterranean or in bathyal plains in the Central Mediterranean (Aguilar Pers. Obs.).

Finally, tube anemones or cerianthids are another order of anthozoans with colonies that can reach high densities in detritic and muddy bathyal seabeds. Thus, for example, *Cerianthus membranaceus* can occur in compact groups of individuals scattered over a wide area, like in the slopes or around canyons (Lastras *et al.*, 2016; Aguilar *et al.*, 2008) whereas *Arachnanthus spp.* usually appears in groups of hundreds or thousands of individuals slightly separated from each other (Aguilar *et al.*, 2014; Marín *et al.*, 2011).

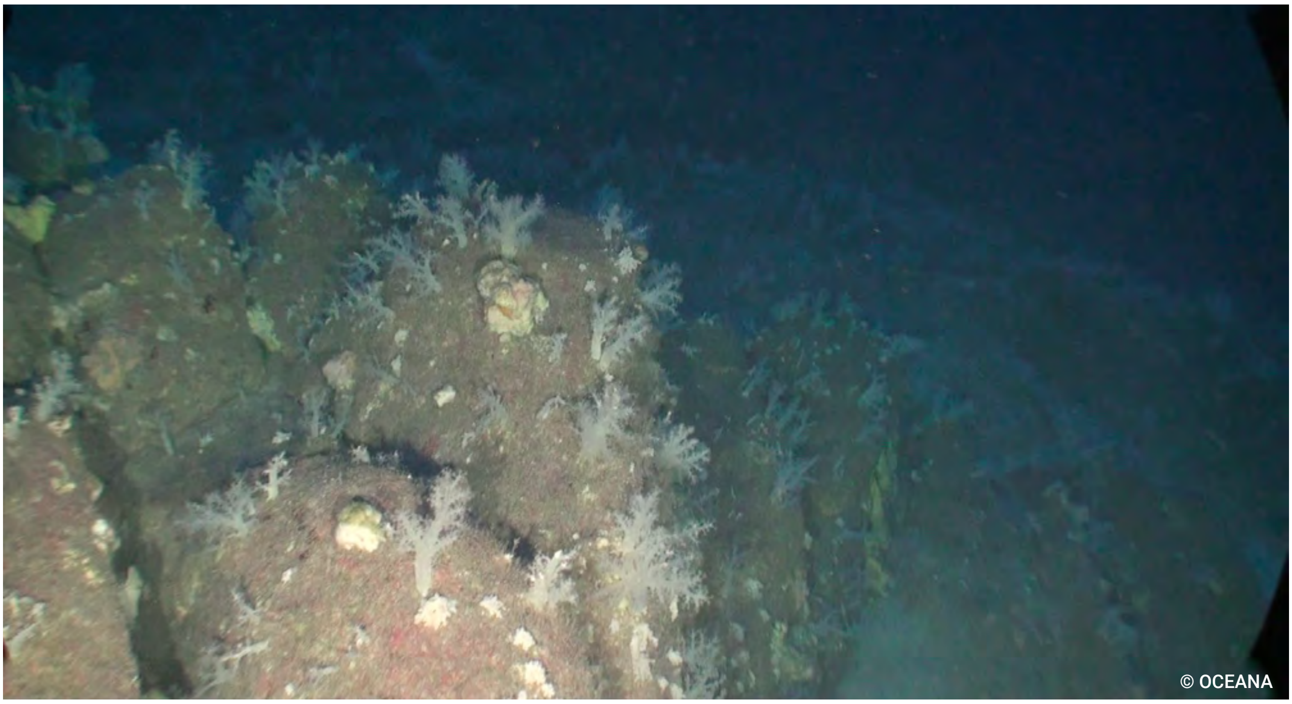


Figure 8. Anthozoans - *Chironephthya mediterranea*, Palos Seamount

Sponge grounds with demosponges

Various demosponges give rise to dense aggregations, on some occasions as the dominant species and on others in combination with corals and gorgonians. *Poecillastra compressa* and *Pachastrella monilifera* appear to have the most extensive geographical distribution within the Mediterranean and an important role in deep ecosystems (Angeletti *et al.*, 2014; Bo *et al.*, 2012; Calcinai *et al.*, 2013; Taviani *et al.*, 2016a), while those of the genus *Phakellia* are more common in the Western Mediterranean (de la Torriente *et al.*, 2014; Aguilar *et al.*, 2013). They may begin to appear in the lower circalittoral, but their presence is more common in the bathyal zone.
The Eastern Mediterranean is home to large Dictyoceratida of the genera *Spongia, Ircinia, Sarcotragus, Scalarispongia* as well as Agelasida (i.e. *Agelas oroides*), etc., which are common in shallow

areas reaching as deep as the heads of canyons, shelf edges and upper bathyal zones (Aguilar Pers. Obs.).
Both Axinellida and Haplosclerida can also show similar behaviour, becoming abundant in the deep circalittoral and upper bathyal zones, especially on seamounts and other rocky bottoms (Aguilar *et al.*, 2013; Bo *et al.*, 2012b; Bo *et al.*, 2011).
Desma-bearing demosponges or Tetractinellida (ex-"Lithistida"), can form large aggregations, even reef formations, in deep zones of the bathyal, like the one of *Leiodermatium pfeifferae* found in a seamount at a depth of more than 700 m near the Balearic Islands (Maldonado *et al.*, 2015) and on Mejean bank between 380 and 455 m (Fourt & Chevaldonné Pers. Obs.). It is not known whether other "rock sponges" present in the Mediterranean, such as *Leiodermatium lynceus* or *Neophrissospongia nolitangere*, and which give rise to similar formations in the Atlantic, could also do so in the Mediterranean.

In soft bottoms, the presence of sponge aggregations is limited to a few species, such as *Thenea muricata*, which is common in muddy bottoms of the bathyal zone throughout the Mediterranean (Evans *et al.*, 2016; Fourt *et al.*, 2014; de la Torriente *et al.*, 2014; Pansini & Musso, 1991), sometimes with the presence of the carnivorous sponge *Cladorhiza abyssicola*, while *Rhizaxinella pyrifera* is more common in sandy-detritic bottoms, but can also be found in cold seeps on mud volcanoes (Olu-Le Roy *et al.*, 2004).

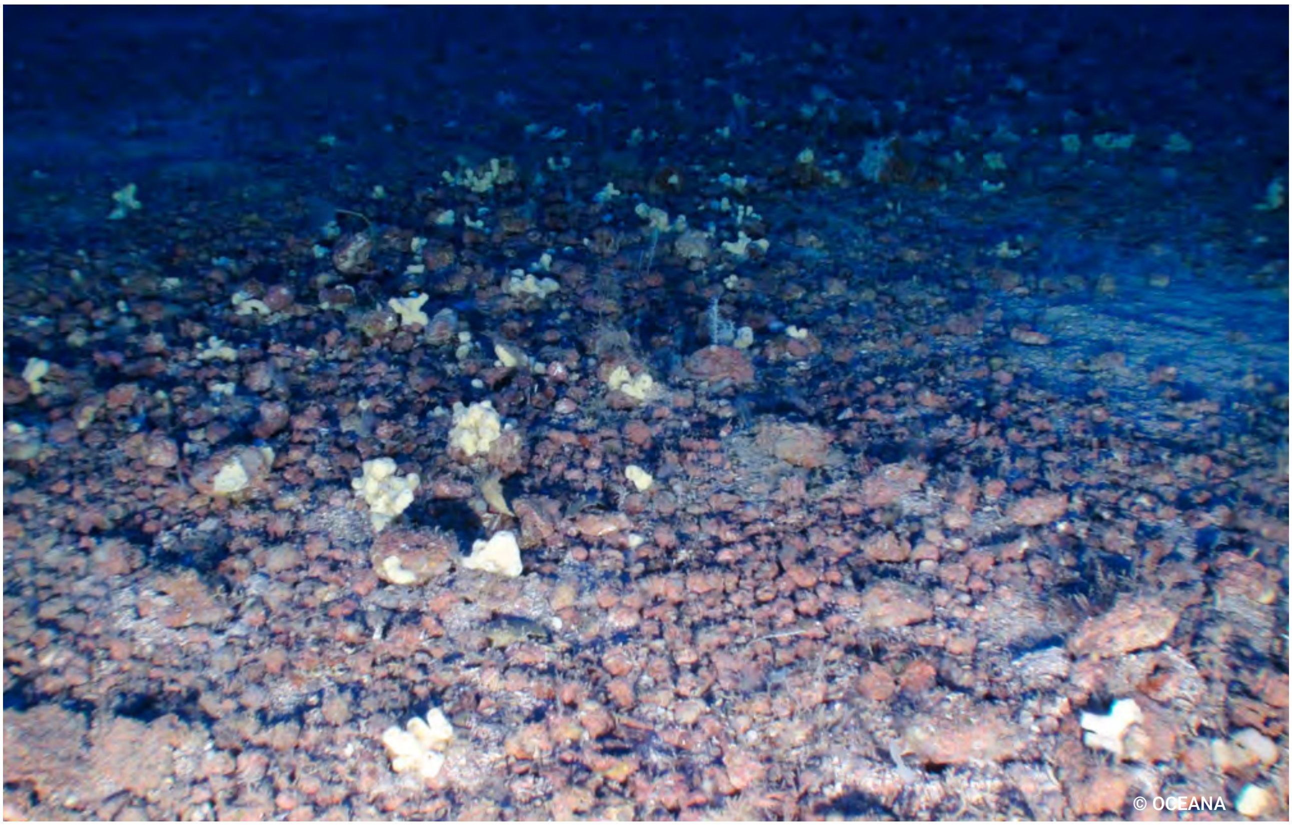


Figure 9. Demosponges - *Aplysina aerophoba* and rhodolith bed, Seamount Ausias March

Sponge grounds with hexactinellids

The large glass sponge *Asconema setubalense* is the most important in the formation of these aggregations of sponges in the Western Mediterranean (Boury-Esnault *et al.*, 2015; Aguilar *et al.*, 2013), mainly on rocky bottoms on seamounts at depths below 200 m, but has not been found beyond the Alboran Sea.
With a much wider distribution in the Mediterranean, reaching the eastern basin, *Tetrodictyum reiswegi* (Boury-Esnault *et al.*, 2017; 2015; Aguilar *et al.*, 2014) is smaller than the previously mentioned sponge and usually less numerous, although it can form aggregations on hard bottoms on seamounts, escarpments, and in canyons, etc., at depths of 200-2,500 m.

It is not known whether other species of hexactinellids that inhabit the Mediterranean can form aggregations similar to those that they create in the Atlantic, as in the cases of the genera *Aphrocallistes* or *Farrea* (Boury-Esnault *et al.*, 2017).
Another sponge, *Pheronema carpenteri*, can also give rise to important formations, but in this case on muddy bottoms. In the Mediterranean it has been found from the Alboran to the Tyrrhenian Sea at depths between 350 m and more than 2,000 m (Boury-Esnault *et al.*, 2015).

Figure 10. Hexactinellids - *Asconema setubalense,* Chella Bank

Mixed habitats of sponges and corals

All the species of anthozoans and sponges mentioned above which have a similar bathymetric distribution and substrate preference may form mixed habitats. Depending on other environmental conditions, one or another taxon may be dominant.

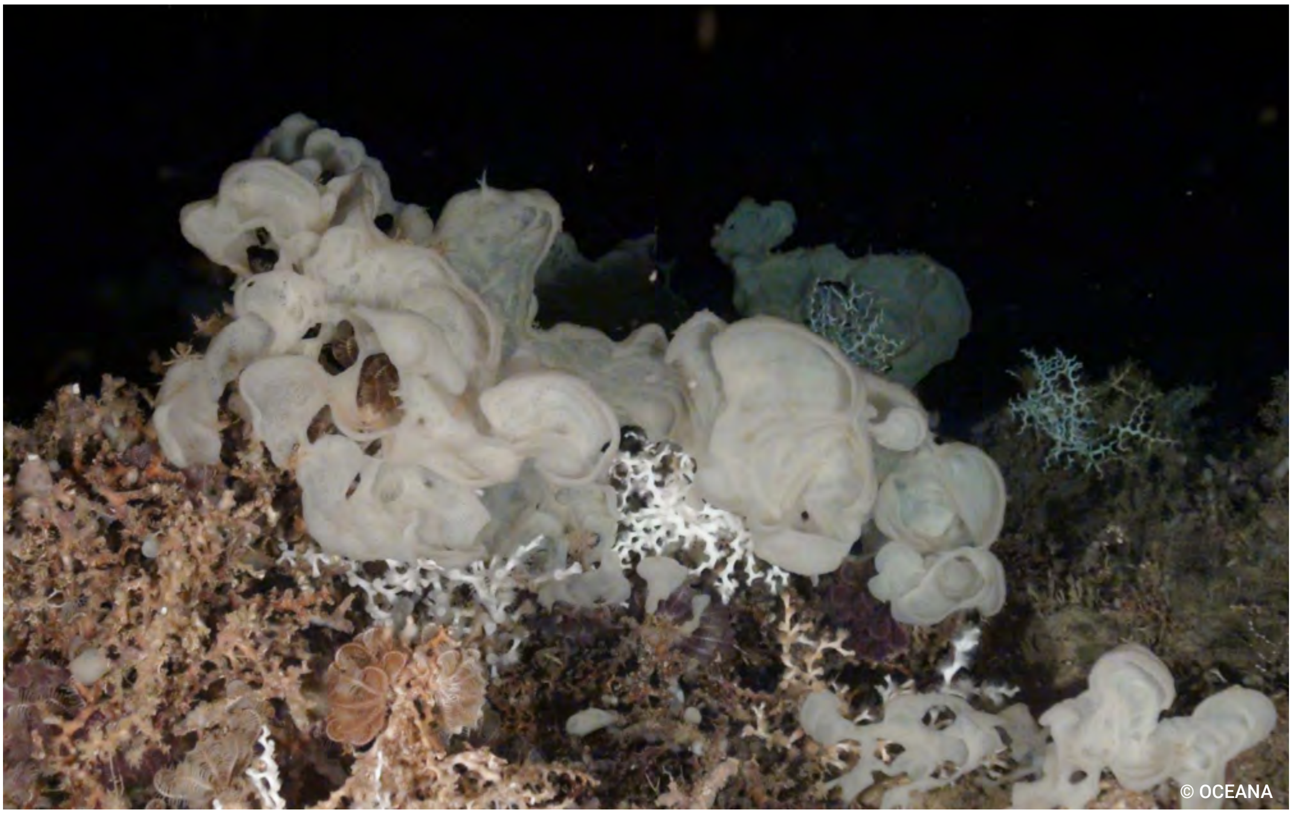


Figure 11. Sponges developing on the corals *Lophelia pertusa* and *Madrepora oculata*, Catifas Bank

Habitats formed by crustaceans

There are two groups of crustaceans that give rise to deep sea habitats in the Mediterranean Sea: the cirripeds and the Ampeliscidae.
In the case of cirripeds, the Balanomorpha *Pachylasma gigantea* is the predominant species, even contributing to deep-sea coral habitats (Angeletti *et al.*, 2011; Schembri *et al.*, 2007), although *Megabalanus spp*. may also create a number of communities of some importance, usually together with mollusks and corals (Aguilar Pers. Obs.).

In the case of the Ampeliscidae, their tubes cover vast extensions of sedimentary bottoms. There are several dozens of species of the genera *Ampelisca, Haploops* and *Byblis* and they have been found on slope edges, on the gentle slopes of escarpments and in canyons and even on seamounts and hydrothermal fields (Bellan-Santini, 1982; Dauvin & Bellan-Santini, 1990; Marín *et al.*, 2014; Esposito *et al.*, 2015; Aguilar Pers. Obs.), in depths that range from the edge of shelf or on the seamount summits to down to more than 700 m.

Figure 12. Ampeliscids fields and *Raja montagui*, Ausias Seamount.

Habitats of bryozoans

The bryozoans usually form mixed aggregations with other benthic invertebrate species, but in some cases they may be dominant, as in the case of large and arborescent species of the genera *Reteporella, Hornera, Pentapora, Myriapora* and *Adeonella*, etc. All of them attach to rocky substrates, but also to gravel or coarse sediment, and their distribution covers the entire Mediterranean.
Also massive species, mostly belonging to the genera *Celleporina* and *Turbicellepora* may form large colonies often associated with gorgonaceans (Rosso Pers. Obs.). Although these species are common in shallow bottoms, they may extend to deeper areas (Bellan-Santini *et al.*, 2002), including escarpments, deep rocky bottoms and seamount summits, etc. (de la Torriente *et al.*, 2014; Aguilar *et al.*, 2010).

Bryozoans are usually found associated with CWC where they are represented by few species, mostly developing small encrusting colonies (Rosso *et al.*, 2010). In the Mediterranean and obviously in the Atlantic, skeletons of deep-sea scleractinians are colonized by bryozoans, for which these skeletons are a great opportunity to get a stable habitat in an elevated position (Zabala *et al.*, 1993). Hidden side of dead shells (from thanatocoenoses or not) are also a privileged habitat of encrusting bryozoans in the deep sea as well as in the coastal zone.
In soft bottoms, from depths of 350/400 m, some stalked species, such as *Kinetoskias sp*. (Maldonado *et al.*,

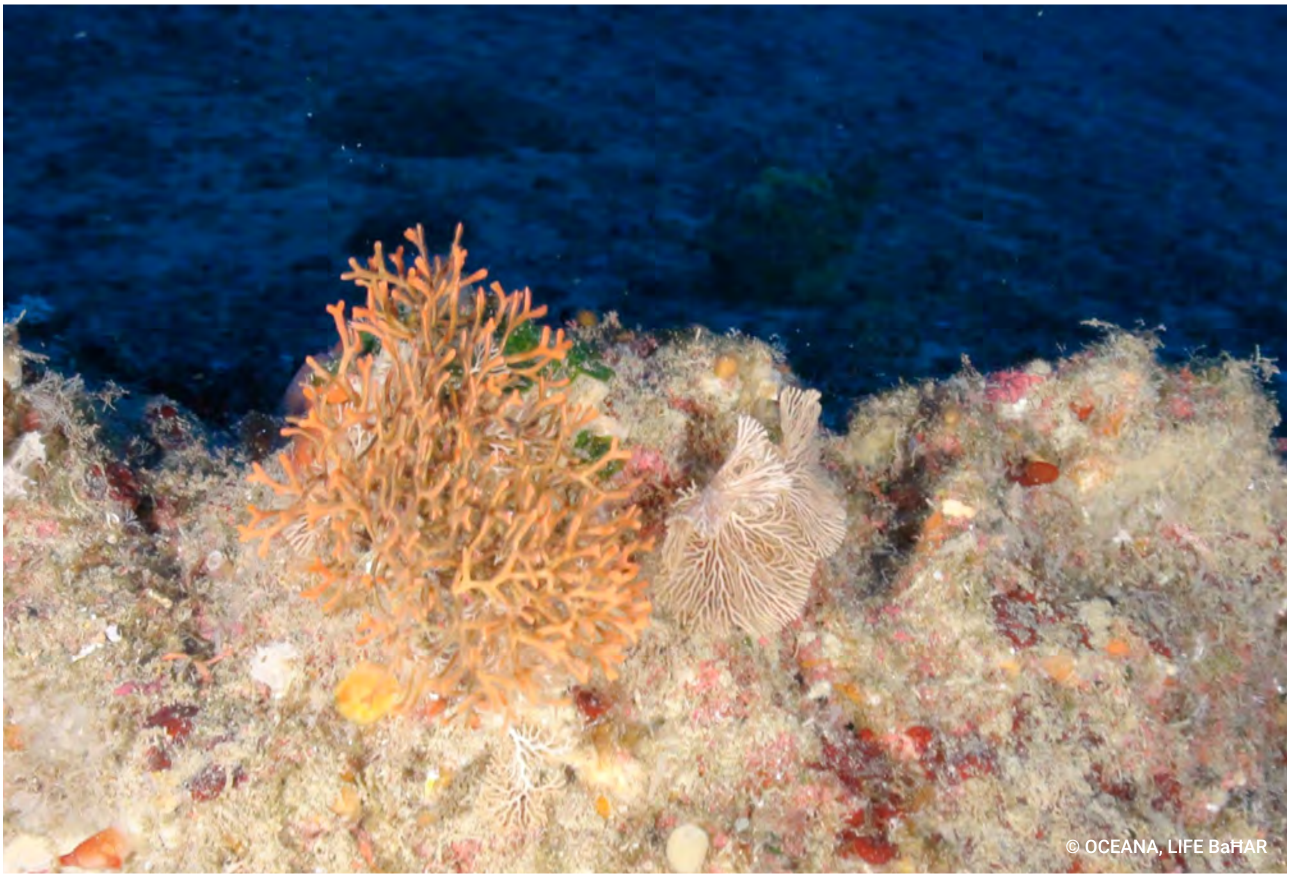


Figure 13. Bryozoans - *Adeonella calveti* and *Hornera frondiculata*, Malta

2015; Aguilar *et al.*, 2013, Harmelin & D'Hondt, 1993, Aguilar Pers. Obs.), or even species from the Candidae family (Aguilar Pers. Obs.), as well as some mm-sized species may begin to appear (Rosso *et al*., 2010). These bryozoans living on muddy bottoms have been found in the Western and Central Mediterranean.

Habitats of polychaetes

Many polychaetes form associations with species such as anthozoans, sponges, bryozoans, and brachiopods, etc., on rocky substrates of escarpments and mountains, in canyons and caves, etc., but may also occur in single-species aggregates or as a dominating species on soft bottoms.
Sabellids and serpulids are among the most widely distributed tube polychaetes. They have been found forming dense aggregates in deep sedimentary bottoms around Alboran Island, as in the case of *Sabella pavonina* (Gofas *et al.*, 2014); creating small reefs together with corals, as for *Serpula vermicularis* in the Bari Canyon (Sanfilippo *et al.*, 2013); or in great numbers occupying extensive areas in detritic beds on the slopes of seamounts, the continental slope or submarine canyons heads, as in the case of *Filograna implexa* (Würtz & Rovere, 2015) that can also collaborate in deep-sea coral reef forming (D'Onghia *et al.*, 2015) such as the Eunicidan *Eunice norvegica* (Taviani *et al.*, 2016b).

As for the terebellids, the sand mason worm (*Lanice conchilega*) creates colonies in sandy bottoms and sandy muds of the circalittoral and bathyal zones, and has been found in great densities in seamounts such as the Chella Bank in the Alboran Sea or canyons such as La Fonera in Catalonia. No studies have been carried out on their abundance and distribution in the Mediterranean, but data from the North Sea records densities of several hundreds or thousands of individuals per square meter, forming structures with functions similar to those of some biogenic reefs (Rabaut *et al.*, 2007).

The siboglinids, meanwhile, generate important aggregations in mud volcanoes, hypersaline lakes and other structures with chemosynthetic communities, such as the Amsterdam mud volcano, between the Anaximenes and Anaxagoras marine ranges in the Eastern Mediterranean (Shank *et al.*, 2011).

Figure 14. Polychaetes - *Lanice conchilega*, Emile Baudot Seamount.

Habitats of mollusks

The main aggregations, concretions and mollusk reefs in deep bottoms are those formed by oysters of the Gryphaeidae family. *Neopycnodonte cochlear* can be found in the photic zone, but it also creates beds in the deep-sea, whether on rocky or detritic bottoms, on escarpments and seamounts, and in canyons, etc. (Fabri *et al.*, 2014; de la Torriente *et al.*, 2014). *Neopycnodonte zibrowii* is found only on rocky bottoms, also belonging to escarpments, seamounts and canyons, but its distribution is at a greater depth, from 350 m down to depths of more than 1,000 m (Beuck *et al.*, 2016; Taviani *et al.*, 2016b). The large limid *Acesta excavata* contributes to hard bottom communities in the Gulf of Naples associated with *N. zibrowii* and the stony corals *M. oculata, L. pertusa, D. dianthus* and *Javania cailleti* (Taviani *et al.*, 2016c). There are also other species of mollusks, such as *Spondylus gussoni* and *Asperarca nodulosa*, which can occur in large numbers, sometimes co-occurring with deep sea corals (Foubert *et al.*, 2008; Rosso *et al.*, 2010; Taviani *et al.*, 2016b). Their facies may be dominant in some seabeds or be part of other deep-sea dwelling communities, on the rocky bottoms of escarpments and canyons, together with brachiopods or other bivalves.

Figure 15. Mollusks - Reef of Vermetids, Lebanon.

Other habitats

Brachiopods such as *Megerlia truncata, Terebratulina retusa, Argyrotheca spp., Megathyris detruncata, Novocrania anomala*, etc., form part of many marine habitats and microhabitats on rocky bottoms, including underwater canyons and stony coral bathyal habitats (Madurell *et al.*, 2012; Angeletti *et al.*, 2015; Taviani *et al.*, 2016b). However, there is another species that forms important facies in soft bottoms, with a wide bathymetric range, although the higher concentrations are usually found in detritic areas on the edge of the shelf and the beginning of the continental slope. This species is *Gryphus vitreus* (Madurell *et al.*, 2012; Aguilar *et al.*, 2014; EC, 2006).
In other cases, the dominant species are the Ascidiacea such as *Diazona violacea* (UNEP-MAP-RAC/SPA, 2013) and/or different species of solitary ascidians belonging to the families Molgulidae, Ascidiidae, Pyuridae and Styelidae (Templado *et al.*, 2012). These aggregations may occur on seamounts or in slope areas, on detritic muddy bottoms (Pérès and Picard, 1964) or rocky bottoms heavily covered by sediments.
Worthy of note within the non-sessile species are the communities formed by echinoderms that play a key role in the structuring of soft and hard bottoms. The habitats formed by large aggregations of crinoids (*Leptometra spp*.) are recognised as sensitive because of the abundance of associated species and their importance for some commercial species (Colloca *et al.*, 2014). However, *Leptometra phalangium* is not exclusively restricted to soft bottoms, but can also occur in equal numbers on rocky bottoms (Marín *et al.*, 2011; 2011b) or even on coral reefs (Pardo *et al.*, 2011; Aguilar Pers. Obs.).

It is also important to note the occurrence of this type of aggregation on soft bottoms involving urchins such as *Gracilechinus acutus* and *Cidaris cidaris* (Templado *et al.*, 2012; Aguilar Pers. Obs.), holothurians such as *Mesothuria intestinalis* and *Penilpidia ludwigi* (Cartes *et al.*, 2008; Pagès *et al.*, 2007), ophiuroids such as *Amphiura spp.*, etc., and also on some rocky bottoms and reefs, with an abundance of specimens of *Ophiothrix* spp. and *Holothuria forskali*, etc. (Templado *et al.*, 2012).

Equally important are the Archaean communities and microbial mats (Pachiadaki & Kormas, 2013; Pachiadaki *et al.*, 2010; Giovannelli *et al.*, 2016) together with their associated chemosymbiotic mollusks (e.g. Lucinidae, Vesicomyidae, Mytilidae, Thyasiriidae) or polychaetes (*Lamellibrachia* sp., *Siboglinum* sp.), and ghost shrimps (*Calliax* sp.) which inhabit areas rich in sulphur and methane (Taviani, 2014). Most sites refer to cold seepage and occur in the Eastern Mediterranean, at the Napoli mud volcano in the abyssal plain between Crete and North Africa (revised by Olu-Le Roy *et al.*, 2004; Taviani, 2011), or in the Osiris and Isis volcanoes in the fluid seepage area in the Nile deep-sea fan (Dupré *et al.*, 2007; Southward *et al.*, 2011), and the Eratosthenes seamount south of Cyprus (Taviani, 2014), but they are also known in the Gela Basin pockmark field to the south of Sicily (Taviani *et al.*, 2013), and in the Jabuka-Pomo area in the Adriatic (Taviani, 2014). Hydrothermal communities are rarer and documented on submarine volcanic apparatuses in the Tyrrhenian and Aegean Seas (Taviani, 2014). These chemosynthetic communities usually occur at great depths, down to more than 2,000 m.

Figure 16. Other habitats - Brachiopods *Gryphus vitreus*, Emile Baudot Escarpment

Figure 17. Other habitats - Crinoids *Leptometra phalangium*, Algarrobo Bank

Thanatocoenoses

The fossil or subfossil remains of many marine species generate thanatocoenoses (assemblages of dead organisms or fossils) which provide habitats of great importance in dark habitats. These can have very diverse origins, but continue to constitute biogenic structures that act as reefs or three-dimensional formations, and which also provide substrate for the settlement of multiple species.

Among these formations are the thanatocoenoses dominated by ancient remains and bioconstructions of coral, mollusks, brachiopods, polychaetes and sponges. These bottoms are found on seamounts, bathyal plateaus, escarpments, and in canyons, etc. They include the compacted seabeds of old aggregations of *Gryphus vitreus* (Aguilar Pers. Obs.); reefs and rubble of *Madrepora oculata, Lophelia pertusa, Desmophyllum dianthus, Dendrophyllia cornigera*, oysters (*Neopycnodonte zibrowii*), etc. (Županović, 1969; Taviani & Colantoni, 1979; Zibrowius & Taviani, 2005; Taviani *et al.*, 2005b; Malinverno *et al.*, 2010 Rosso *et al.*, 2010; Fourt *et al.*, 2014b; 2011b; Bo *et al.*, 2014c); beds of *Modiolus modiolus* shells (Gofas *et al.*, 2014; Aguilar *et al.*, 2013; subfossil reefs of polychaetes such as *Pomatoceros triqueter* (Domínguez-Carrió *et al.*, 2014); fossilised structures of old sponge aggregations such as *Leiodermatium sp*. (Aguilar Pers. Obs.); concentrations of hexactinellid spicules; bryozoan remains (Di Geronimo *et al.*, 2001); and even accumulations of algae and plants such as rhizomes and leaves of *Posidonia oceanica* transported from superficial areas to deep-sea bottoms.

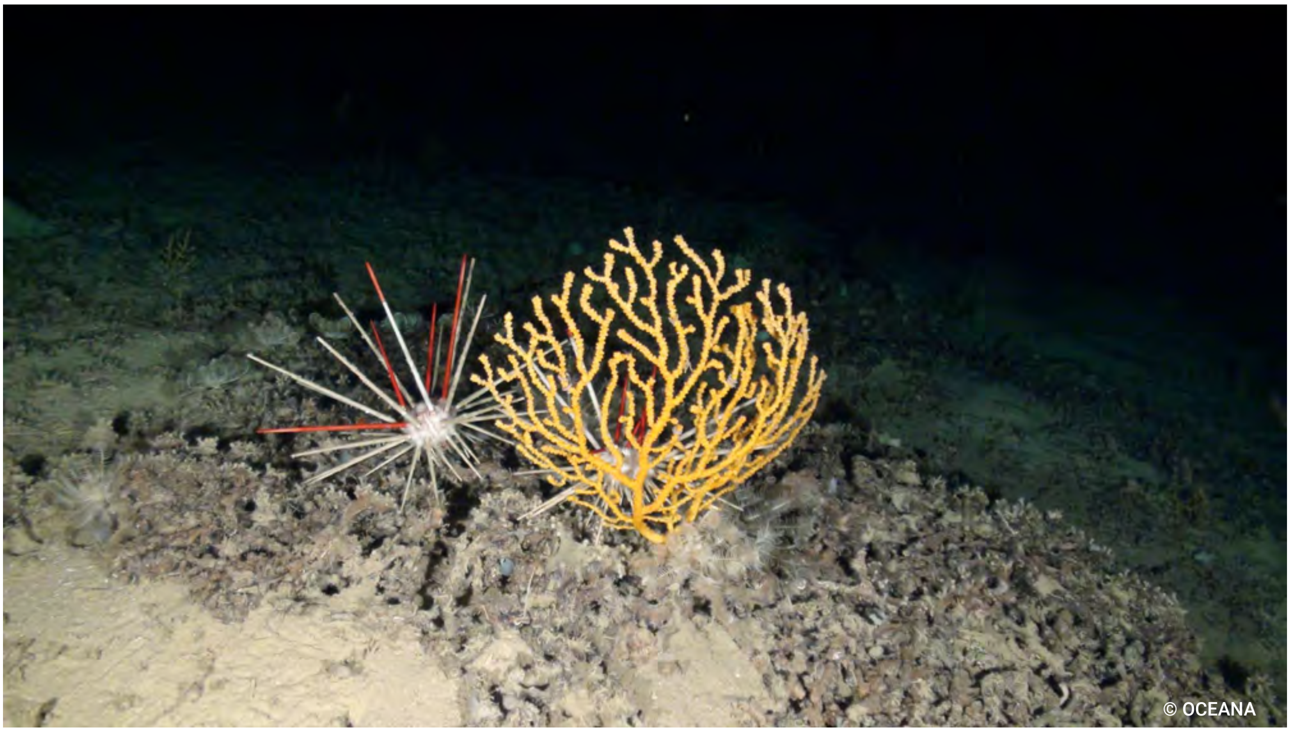


Figure 18. Sea urchins (*Cidaris cidaris*), gorgonian (*Acanthogorgia hirsuta*) and crinoids (*Leptometra phalangium*) living on coral thanatocoenoses

II.2. METHODOLOGIES FOR THE STUDY OF DEEP-SEA HABITATS

Methodologies to study deep-sea dark habitats include a wide array of technologies and equipment. Acoustic, visual and extractive means must be combined to acquire the most accurate information.

Multibeam sonar, side scan sonar and sub-bottom profilers like TOPAS (Topographic parametric sonar) provide an important overview of the seabed, making it possible to identify and locate the presence of seamounts, canyons, mud volcanoes, pockmarks, carbonated mounds, reefs, etc., and also providing key information for the detection of potential areas where other dark habitats, more difficult to detect using acoustic methods, might occur.

The use of ROVs, bathyscaphes, submarines, landers, dropping cameras, etc., provide visual and georeferenced information on the geological formations and benthic communities on these seabeds, allowing the verification of information provided by other methods, and providing greater certainty, facilitating real data about the presence of species, distribution patterns, estimates of densities, biological associations, etc. In the case of the ROVs and submarines, these allow the completion of transects and the selective collection of samples, which greatly facilitates the identification of key species in the habitat formation, as well as the species associated with them.

The use of grabs allows more extensive sampling in large areas, also providing information on species of infauna and on small organisms that it is not possible to detect/identify with other methods.

The use of AUVs, CTDs and other methods to analyse the water column provides complementary information on water masses, currents, physicochemical data, etc., which combined with all the other information allows a better interpretation of the deep ecosystems. Regarding AUVs, those equipped with multibeam echosounder (or Side Scan Sonar) and cameras are widely used to explore and map large areas in deep-sea environments. The initial costs of these instruments usually prevent their use by small research institutes, but the large amount of data collected and the large area surveyed makes them a very vantageous approach with respect to use of large ship for several days.

New techniques of DNA analysis, besides providing information on populations, species, etc., can shed light on the species inhabiting the area that have not been detected with other methods and can also supply information on their abundance.

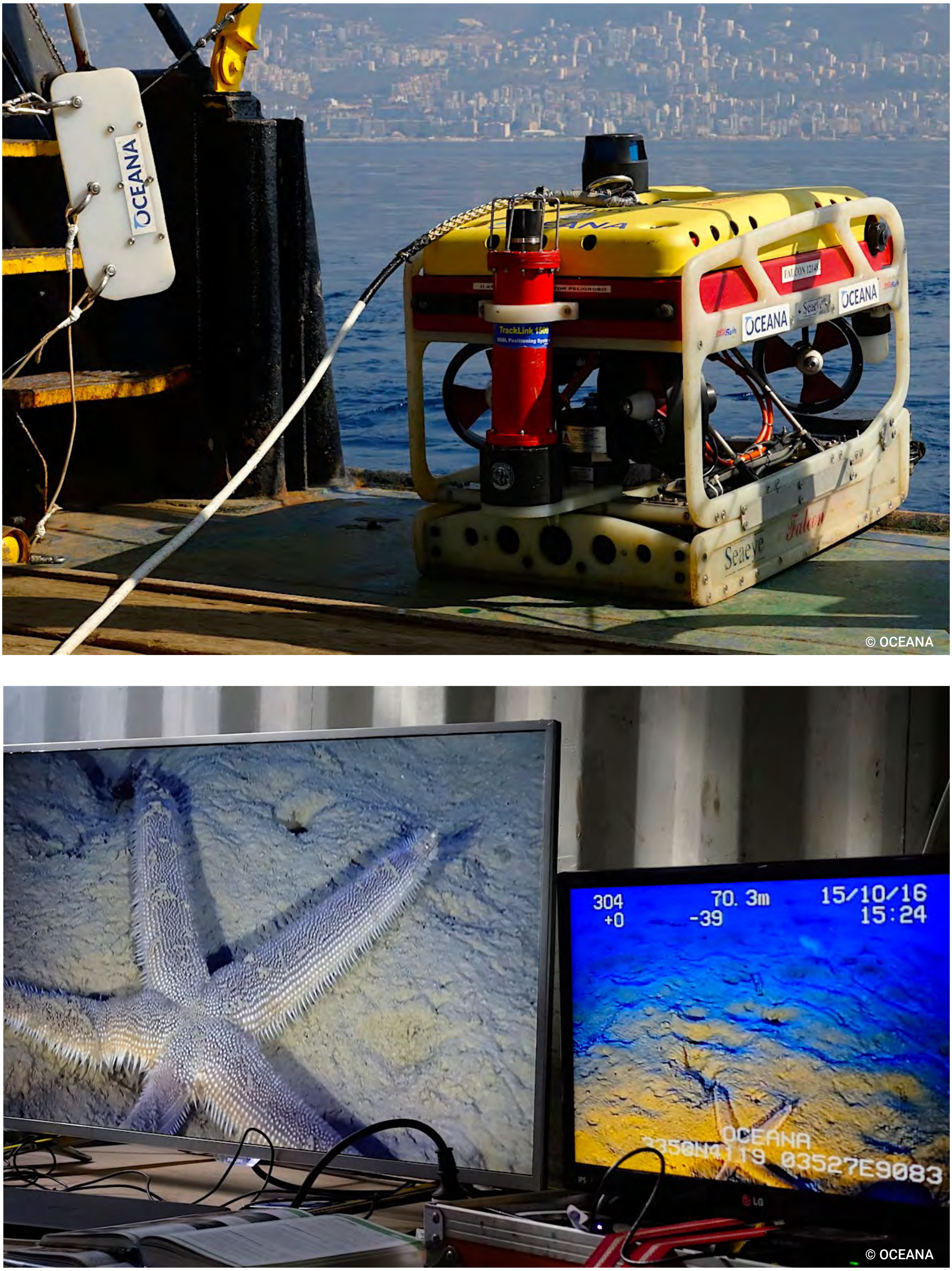


Figure 19. Example of ROV used for the exploration of dark habitats

Figure 20. CTD sensors for water column analysis

II.3. MONITORING: COMMON INDICATORS FOR MONITORING DEEP-SEA HABITATS

Having in mind the overarching principles guiding the development of the Integrated Monitoring and Assessment Programme (IMAP), the following list of indicators has been selected for monitoring dark habitats from the initial list of common and candidate indicators (agreed as core of the IMAP). As suggested by IMAP, selected indicators need to be reliable, reproducible and (as far as possible) inter-comparable between operators across the Mediterranean (or subregions). Selection (in blue) has been made according to criteria of relevancy and/or applicability to deep-sea habitats:

Table 1. Common Indicators selected for monitoring Deep-sea Habitats (related Ecological Objectives[6] are also included in the first column)

Ecological Objective	INDICATORS	Relevancy/ Applicability
EO1	1. Habitat distributional range (EO1) to also consider habitat extent as a relevant attribute	
	2. Condition of the habitat's typical species and communities (EO1)	
	3. Species distributional range (EO1 related to marine mammals, seabirds, marine reptiles)	NOT APPLICABLE
	4. Population abundance of selected species (EO1, related to marine mammals, seabirds, marine reptiles)	NOT APPLICABLE
	5. Population demographic characteristics (EO1, e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates related to marine mammals, seabirds, marine reptiles)	NOT APPLICABLE
EO2	6. Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species, particularly invasive, non-indigenous species, notably in risk areas (EO2, in relation to the main vectors and pathways of spreading of such species)	Maybe relevant in the Levantine basin
EO3	7. Spawning stock Biomass (EO3)	NOT APPLICABLE
	8. Total landings (EO3)	NOT APPLICABLE
	9. Fishing Mortality (EO3)	NOT APPLICABLE
	10. Fishing effort (EO3)	NOT APPLICABLE
	11. Catch per unit of effort (CPUE) or Landing per unit of effort (LPUE) as a proxy (EO3)	NOT APPLICABLE
	12. Bycatch of vulnerable and non-target species (EO1 and EO3)	
EO5	13. Concentration of key nutrients in water column (EO5)	NOT RELEVANT
	14. Chlorophyll-a concentration in water column (EO5)	NOT RELEVANT
EO7	15. Location and extent of the habitats impacted directly by hydrographic alterations (EO7) to also feed the assessment of EO1 on habitat extent	NOT APPLICABLE
EO8	16. Length of coastline subject to physical disturbance due to the influence of man-made structures (EO8) to also feed the assessment of EO1 on habitat extent	NOT APPLICABLE

[6] See Appendix III

EO9	17. Concentration of key harmful contaminants measured in the relevant matrix (EO9, related to biota, sediment, seawater)	NOT APPLICABLE
	18. Level of pollution effects of key contaminants where a cause and effect relationship has been established (EO9)	NOT APPLICABLE
	19. Occurrence, origin (where possible), and extent of acute pollution events (e.g. slicks from oil, oil products and hazardous substances) and their impact on biota affected by this pollution (EO9)	NOT APPLICABLE
	20. Actual levels of contaminants that have been detected and number of contaminants which have exceeded maximum regulatory levels in commonly consumed seafood (EO9)	NOT APPLICABLE
	21. Percentage of intestinal enterococci concentration measurements within established standards (EO9)	NOT APPLICABLE
EO10	22. Trends in the amount of litter washed ashore and/or deposited on coastlines (including analysis of its composition, spatial distribution and, where possible, source) (EO10)	NOT APPLICABLE
	23. Trends in the amount of litter in the water column (including microplastics) and on the seafloor (EO10)	
CANDIDATE INDICATORS		
EO10	24. Candidate Indicator: Trends in the amount of litter ingested by or entangling marine organisms focusing on selected mammals, marine birds and marine turtles (EO10)	NOT APPLICABLE
EO8	25. Candidate Indicator: Land use change (EO8)	NOT APPLICABLE
EO11	26. Candidate indicator: Proportion of days and geographical distribution where loud, low, and mid-frequency impulsive sounds exceed levels that are likely to entail significant impact on marine animals (EO11)	NOT APPLICABLE
	27. Candidate Indicator: Levels of continuous low frequency sounds with the use of models as appropriate (EO11)	NOT APPLICABLE

Assessment guidelines and general considerations for selected Common Indicators are developed in the tables below based on IMAP Guidance:

Box 1. Monitoring guidelines for Deep-sea Habitats (based on IMAP UNEP(DEPI)/MED IG.22/Inf.7)

RELATED ECOLOGICAL OBJECTIVE	SELECTED COMMON INDICATORS and KEY CONCEPTS	GENERAL CONSIDERATIONS	ASSESSMENT GUIDELINES
EO1 [Biological diversity]	**COMMON INDICATOR 1. Habitat distributional range** **Operational objective: key deep-sea habitats are not lost.** • **Parameter/metric:** surface area of lost habitat for each habitat type aiming at mapping products. • **Target**, the lost area per habitat type could be set as to not exceed an acceptable percentage of the baseline value[7]. • For Protected Habitats (e.g. under SPA/BD) target could be set as habitat loss stable or decreasing and not greater than the baseline value (as an example, as regards the EU guidance for the assessment of conservation status under the Habitats Directive, Member States have generally adopted a 5% tolerance above the baseline to represent 'stable'.	Constraints for monitoring: • Slow growth rates and long-lived history in most of the communities and species associated to dark habitats; however, results from aquaria experiments conducted in the last two decades are a very helpful instrument to estimate changes and monitor populations (Lartaud *et al.*, 2014; Movilla *et al.*, 2014) • Limitations to obtain new data: high costs of Research Vessels (R/V) and the necessary technology for research and expeditions (e.g. ROV, submersibles, underwater cameras, etc.). • Sea-bed irregularities make difficult to explore sea bottom geomorphological features (seamounts, submarine canyons, caves) by using traditional sampling methods for mapping or monitoring (quadrates, transects) difficulties to replicate experiences. • High resolution bathymetric maps (e.g. produced by multibeam echosonar) are very useful tools for location and description of deep-sea habitats; however, they are not usually available.	**Selection of sites:** on the basis of a risk-based approach in order to prioritize or identify additional monitoring effort required and keep the monitoring effort cost-effective - Special consideration to habitats considered as essential for (1) the early developmental stages of mostly mobile fauna (e.g. spawning, feeding grounds) or (2) benthic assemblages consider to be key components of the deep-sea as engineer species and to assure a proper ecosystem functioning: habitat-bio-engineering species, specific deep-sea assemblages, etc; full monitoring programs for habitat types under protective regulations and/or those whose species are categorized as threatened (acc. Red List) must also bear in mind. - Marine Protected Areas. - Based on general geomorphological data: to predict the location of a potential habitat type on the basis of the substrate (biological communities is expected to be different depending on seabed nature (muddy, rocky, sandy, etc.) and physical parameters (depth, currents, etc.). - Maybe also useful species distribution models (e.g. MEDISEH[8]– Mediterranean Sensitive Habitats). **Periodicity** (six-yearly) - According to the rates of change in natural and anthropogenic influences in the Region/sub-region.

[7] as an example, this target was derived from OSPAR to not exceed 15% of the baseline value and was similarly proposed by HELCOM
[8] MEDISEH report: Mediterranean Sensitive Habitats (Giannoulaki et al., 2013)

	However, in some cases a more stringent <1% tolerance has been attached to the maintenance of habitat extent). • For habitats protected that have historically been reduced, the target should be that the area increases towards the size of the baseline.	**Sampling** • **Methods:** hard substrates are preferably monitored using optical, non-destructive methods (underwater-video, ROV) - living specimens can be collected by ROV arm; infaunal communities are sampled using standardized grabs or corers (see Appendix VI). • **Design:** in accordance with international or national guidelines but always considering the use of non-invasive methods or models that may reduce the effort and/or expense on a long-term basis. NOTE: Regarding the use of non-destructive sampling technics, sometimes benthic trawling has been recommended as appropriate for sampling benthic habitats, however despite they can provide useful data, these methods are not recommended for assessment of highly sensitive habitats to the impact of physical damage (e.g. biogenic reefs, maërl beds, soft bottom communities dominated by long-lived species as for instance large sponges, bamboo corals among others). Same problem applies to towed video, henceforth neither of them is recommended to identify deep-sea habitats extent. **Spatial basis:** it should be according to the Mediterranean biogeographic sub-areas in order to reflect changes in the biological character of each habitat type across the Mediterranean and its sub-regions.	- Evidence collection is sufficient to distinguish the effects of anthropogenic disturbance from natural and climatic variability. - Assess cost-efficiency: some pressure elements upon biodiversity maybe monitored more frequently that the actual state of biodiversity (e.g. fishing activity). **Data sources:** - mainly derived from "regular" existing activities such as Environmental Impact Assessments, VMS or fisheries log book data; data from targeted surveys (e.g. research activities or relevant scientific projects) can also be considered. - information provided by maps can be refined using scan sonar, multibeam bathymetric survey, etc. and will need to be validated in the field by direct sampling of sediment and/or biota (grab/core sampling) or by remote observation (footage and pictures taken by ROV. **Data gathering:** • Develop common computing methodologies, sampling concepts and mapping instructions, specifying the accuracy (spatial resolution or grid) of the determination of extent (area) a priori. • Determine the appropriate assessment scales in detail. • Develop standardized data flows for spatial pressure data. **Zero-state:** Identify extent (area) baselines for each habitat type.

EO1 [Biological diversity]	**COMMON INDICATOR 2. Condition of the habitat's typical species** **Operational Objective:** key coastal and marine habitats remain in natural condition, in terms of structure and functions. **Target:** to reach a ratio of typical and/or characteristic species similar to baseline conditions for all considered communities. **Calculation:** it involves simple comparison of typical and/or characteristic species per habitat and sub-region with respect to baseline conditions for all considered communities. **Reference state[9]:** an acceptable deviation from baseline conditions would need to be defined. This deviation might be implemented by setting a certain percentage value to define GES.	**Typical species:** this concept emerges from the conservation status of natural habitats to their long-term natural distribution, structure and functions, as well as to the long-term persistence of their typical species within the territory. Typical species composition should be near/close to natural conditions for their habitat to be considered in natural condition **Reference list of habitats:** not defined yet (in process). **Methods:** • Depend on the habitat type (and selected species) to be addressed. Large attached epibenthic species on hard substrates are preferably monitored using optical, non-destructive methods, such as underwater-video. Endobenthic communities are sampled using standardized grabs or corers. • Several specific benthic biotic indices have been developed and have become operational (see IMAP guidance, 2016), in particular to fulfill MED GIG (Mediterranean Geographical Intercalibration Group) requirements. They are all well methodologically defined but the way to combine these parameters in sensitivity/tolerance classification or depending on structural, functional and physiological attributes is heterogeneous, depending on the issue (pressure type), habitat types or sub-region. NOTE: Information about the typical and/or characteristic species of some habitats and their past state/conditions is often unavailable for southern and eastern sub-regions of the Mediterranean. The limited data availability may restrict the number of habitats that can be assessed with sufficient statistical confidence at present. Although benthic biotic indices are conceptually applicable in all sub-regions, adjustments might be required in order to cover biogeographic heterogeneity.	**Typical species lists:** to be defined per sub-region (or bioregion) to allow for the consistent assessment of state/condition. Long-lived species and species with high structuring or functional value for the community should preferably be included; however, it should also contain small and short-lived species if they characteristically occur in the habitat under natural conditions as they can also be functionally very important for the community. **Periodicity:** This list should be updated every six years. **Resources required:** • Research vessels, suited to work in bathyal zones (below 150-200 m depth). • Adequate equipment (box core samplers, grabs, underwater camera systems, etc.) for sample collection. • Laboratory infrastructure to analyze samples (e.g. microscopes, weighing scales). • Qualified personnel for data processing, analysis and interpretation. • Good taxonomy skills are essential for the adequate assessment of this indicator.

[9] The definition of a reference state of Mediterranean Sea habitats may be problematic and the use of past state may be more appropriate.

EO2 [Non-indigenous species]	**COMMON INDICATOR 6. Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species, particularly invasive, non-indigenous species, notably in risk areas** Monitoring should preferably take place in the period of the year where most species would be expected to be present.	**Non-indigenous species** (NIS; synonyms: alien, exotic, non-native, allochthonous) are 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 is due to intentional or unintentional introduction resulting from human activities. **Invasive alien species** (IAS) are a subset of established NIS which have spread, are spreading or have demonstrated their potential to spread elsewhere, and have an effect on biological diversity and ecosystem functioning (by competing with and on some occasions replacing native species), socio-economic values and/or human health in invaded regions. **Data collection method:** in order to be most cost efficient, existing monitoring and surveying programmes should be adapted, for each specific case. It is recommended that Contracting Parties make an inventory of existing marine biological monitoring programmes, surveys, and datasets which may be used (adapted) to report findings of IAS. NOTE: Although invasive alien species may be responsible for high ecological impact in particular for reducing the population of some native species, some NIS, particularly crustaceans and fish have become an important fishery resource. The migration of Lessepsian (meaning through the Suez Canal) NIS appears to play an important role for fisheries, particularly in the Levantine basin. In such a case, management of these fisheries corresponds to GFCM. This is a matter of concern included in the GFCM "Mid-term	**Selection of sites:** • Generally should start on a localized scale, such as "hot-spots" and "stepping stone areas" for alien species introductions. Such areas include ports and their surrounding areas, docks, marinas, aquaculture installations, heated power plant effluents sites, offshore structures. • In the event that MPA does not exist, sampling or findings maybe opportunistic due to the high cost of operations in deep-sea areas. Monitoring at "hot-spots" would typically involve more intense monitoring effort, sampling at least once a year at ports and their surrounding areas. • The number of monitoring stations would also be expected to vary depending on the type of area of introductions (i.e. whether large ports and their immediate surroundings or aquaculture sites, offshore structures). **Selection of species to target:** through existing national, regional and international information networks and databases: • Marine Mediterranean Invasive Alien Species (MAMIAS) database developed for RAC/SPA with information up to 2012. • "Andromeda" invasive species database for the Mediterranean and Black Sea is developed under the PERSEUS Project. • European Alien Species Information Network (EASIN) developed by the Joint Research Centre of the European Commission facilitates the exploration of non-indigenous species information in Europe (and the entire Mediterranean), from distributed resources through a network of interoperable web services, following internationally recognized standards and protocols

		strategy (2017–2020) towards the sustainability of Mediterranean and Black Sea fisheries". Invasive species Pterois miles (common lionfish) and Lagocephalus sceleratus (silver-cheeked toadfish) have been considered in the list of priorities for which scientific advice should be provided.	• MedMIS project which is an online information system for monitoring invasive non-native species in MPAs developed by IUCN.
EO3 [Harvest of Commercially exploited fish and shellfish]	**COMMON INDICATOR 12. Bycatch of vulnerable and non-target species (EO1 and EO3)** **Operational objective:** incidental catches of vulnerable species are minimized.	Standardized information on bycatch for deep-sea fisheries is also scarce in the Mediterranean Sea and sources of data are currently limited to the reporting obligation under the GFCM regulatory framework (bycatch of vulnerable and non-target species). Monitoring of bycatch has been commonly associated to pelagic species (e.g. cetaceans and turtles), although responding to the needs and reality of Mediterranean fisheries it has been extended to other vulnerable species. **Sampling methods:** • Direct observation a) At sea monitoring of commercial catches (by observers on board); Due to the lack of data GFCM is working on a strategy aimed at developing a regional pioneer sampling programme with observers on board. b) Dedicated survey. c) Fishers (by self-sampling) can sample their own bycatch in order that surveys could be made more representative of the whole fleet segment without having to have too many observers. • Conducting direct dialogues with fishers (by questionnaires), collecting also perspectives on the bycatch issue, which is meant to complement the on board observations data analyses, and to provide an integrated approach toward management.	**GFCM reporting system:** entered into force in 2016 and must be done through the Data Collection Reference Framework (DCRF) Task 3 "Incidental catch of vulnerable species". Purposes of the DCRF are quantification of incidental catches by fleet segment and assessment of the impact of fisheries on species of conservation concern. Task 3 refers to the specific reporting of incidental catch of: • sea turtles, seals, cetaceans, sharks and rays species as identified in a number of GFCM recommendations (see Appendix IV-1 and IV-2); and • species included in the Annex II "List of Endangered or Threatened Species" and Annex III "List of species whose exploitation is regulated" of the Protocol SPA/BD. GFCM mandatory data for incidental catches of vulnerable species (see Appendix V). NOTE: In addition to the reporting from DCRF-Task 3 should be also considered that GFCM is currently developing measures for protection of Vulnerable Marine Ecosystems (VME). According to UNGA Resolutions and Deep-sea Fisheries Guidelines (FAO, 2009) areas where VME occur or likely occur should be close to deep-sea fisheries. This closure areas established as spatial management measures may serve as selected areas for monitoring. In addition, reporting of potential encounter protocols should be also considered for monitoring purposes.

<table>
<tr>
<td>EO10 [Marine litter]</td>
<td>COMMON INDICATOR 23. Trends in the amount of litter on the seafloor

Target: The sampling strategy should enable the generation of good detail of data, in order to assess most likely sources, the evaluation of trends and the possibility of evaluating the effectiveness of measures.</td>
<td>Sampling
• The most common approach to evaluate seafloor litter distribution is to use opportunistic sampling, which is, at the same time, the most cost-efficient method.
• Procedures for determining seafloor litter distributions are similar to those used for benthic and biodiversity assessments, the use of submersibles or Remotely Operated Vehicles (ROVs) is a possible approach for deep sea areas although this requires expensive equipment.
• Sampling is usually coupled with fisheries surveys or professional bottom trawling operations, monitoring in MPAs, offshore platforms, etc. or regular programmes on biodiversity.
• Sampling units should be stratified relative to sources (urban, rural, close to riverine inputs) and impacted offshore areas (major currents, shipping lanes, fisheries areas, etc.); and it should be placed more emphasis on the abundance and nature of items (e.g. bags, bottles, pieces of plastics) rather than their mass.

Methods:
Monitoring programmes for demersal fish stocks (e.g. MEDITS) operate at large regional scale (mainly in the continental shelf) and provide data using a harmonized protocol, which may provide a consistent support for monitoring litter at regional scale on a regular basis. Thus, it seems to be the most suitable for large scale evaluation and monitoring. However, as mentioned for Common Indicator 1, trawling is not recommended as sampling strategy, so opportunistic sampling during ROV surveys might be the best approach, even in rocky areas where trawling is not possible because of limitations due to geomorphological reasons (see appendix VII).</td>
<td>Strategy
• To be determined by each Contracting Party at national level. Large-scale evaluations of marine litter in the deep sea-floor are scarce because of available resources to collect data.
• Monitoring will depend on affected areas, but previous results indicate that priority should be given to coastal canyons. As litter accumulates and degrades slowly in deep sea waters, a multiyear evaluation will be sufficient.

Protocol
• Based on (1) existing trawling surveys; and (2) video imagery and support harmonization at regional level, if applied trans-nationally (see Appendix VII).
• It is derived from the MEDITS protocol (see the protocol manual, Bertran et al., 2007).
• The hauls are positioned following a depth stratified sampling scheme with random drawing of the positions within each stratum. The number of positions in each stratum is proportional to the surface of these strata and the hauls are made in the same position from year to year. The following depths (10 – 50; 50 – 100; 100 – 200; 200 – 500; 500 - 800 m) are fixed in all areas as strata limits. The total number of hauls for the Mediterranean Sea is 1385; covering the shelves and slopes from 11 countries in the Mediterranean. The haul duration is fixed at 60 minutes at depths over 200 m (defined as the moment when the vertical net opening and door spread are stable), using the same GOC 73 trawl with 20 mm mesh nets (Bertran et al, 2007) and sampling between May and July, at 3 knots between 20 and 800 m depth.</td>
</tr>
</table>

			Templates for data recording have been integrated in MEDITS Manuals. Data on litter should be collected on these templates using items categories such as those listed for Sea-floor prepared by TSG-ML (for sea floor surveys categories are including: plastic, paper and cardboard, wood (processed), metal, glass and ceramics, cloth (textile), rubber, others).

II.4. CASE STUDIES (DEEP SEA LEBANON, MEDSECAN-CORSECAN, LIFE BAĦAR, INDEMARES)

Different scientific projects in the Mediterranean have made use of different combinations of technologies to detect and describe the benthic communities and dark habitats. Examples include the LIFE+ INDEMARES project in seamounts, canyons and deep seabeds in Spanish waters, the MEDSEACAN-CORSECAN project carried out in the submarine canyons of the French Mediterranean, the LIFE+ Bahar project in the deep waters and trench of Malta, and the Deep-Sea Lebanon project to study the submarine canyons of Lebanon. A number of important studies have also been carried out in specific locations that have made use of technologies for the study of dark habitats, including: the coral province of Santa Maria di Leuca in the Ionian Sea; the seamounts of the Tyrrhenian Sea; the Eratosthenes Seamount to the south of Cyprus; the underwater volcanoes in the Nile deep-sea fan in Egypt; the seamounts and underwater banks in the Alboran Sea; the escarpments around the Hellenic Arc; the Jabuka depression and South Adriatic Pit in the Adriatic Sea; the submarine volcanoes between the Anaximenes and Anaxagoras seamounts; the Afiq and Achziv canyons in Israel; and the Emile Baudot escarpment and seamounts of the Balearic Islands.

All of this work has involved the combination of different technologies. In the case of the two LIFE+ projects (BAĦAR and INDEMARES), topobathymetric studies have been carried out by means of multibeam sonar in order to create a three-dimensional map that allows the identification of potential sites with deep habitats, especially reefs and aggregations of corals and sponges. The use of ROVs and submarines has led to the characterisation of benthic communities and the selective collection of individuals from the most characteristic aggregations as well as species that may be new to science or "rare" in the Mediterranean.

In both cases the studies have been combined with grab sampling to study infauna, identify the type of bottom and substrate, the granulometry, the organic matter content, etc.

The results obtained have made it possible to map communities of gorgonians and corals on seamounts and in canyons, sponge aggregations, coral and polychaete reefs, deep underwater caves, communities of pennatulaceans and bamboo corals, and many other dark habitats.

Niskin bottles, CTDs and hydroacoustic methods have also been used at times to collect water column information.

In France, the MEDSECAN-CORSECAN studies have focused on submarine canyons in the Gulf of Lion and Corsica, providing information collected using ROVs, submarines and side scan sonar at depths of between 35 and about 1,000 m. This has made it possible to study dozens of canyons, as well as terraces, banks and rocky bottoms with a wide diversity of dark habitat communities, such as reefs, gorgonian gardens, soft bottoms with pennatulaceans, and sponge aggregations, etc.

ROVs, grabs and CTDs have also been used to study Lebanon's submarine canyons in the Deep-Sea Lebanon project, including the heads of the canyons and the adjacent shelf at depths of between 50 and 1,050 m. The data obtained have provided new information on the presence of coral and gorgonian communities in the Levantine Sea, important areas with thanatocoenoses of corals and polychaetes, sponge aggregations, and seabeds with pennatulaceans, etc.

These projects also provide information on the presence/absence of species and habitats, abundance and conservation statuses, including threats such as the colonisation of exotic species, accumulation of rubbish and other waste of anthropogenic origin, remnants of fishing gear, the impact of fishing methods on the benthos, and mining.

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B. RECOMMENDATIONS

Inventorying and monitoring dark habitats in the Mediterranean constitutes a unique challenge given the ecological importance of their communities and the threats that hang over their continued existence. Long neglected due to their remote location and the limited means to investigate these areas, today these habitats must be the subject of priority programs and a thematic workshop that brings together the main specialists who work on monitoring their communities.

The first Mediterranean Symposium on the conservation of Dark Habitats (Portorož, Slovenia, October 2014) drew attention on the necessity to improve knowledge of dark habitats and their distribution in the Mediterranean in order to establish international cooperation networks and also to facilitate sharing of experiences among Mediterranean countries. During the different sessions, a great effort was made in order to collect for the first time existing scientific information on the distribution of dark habitats in canyons, caves and escarpments, their biodiversity, community functioning and connectivity aspects. Nevertheless, there were still obvious gaps of knowledge with regard to the distribution and diversity of dark habitats from the eastern and southern parts of the Mediterranean Sea. Particular attention was also given to the pressures on these habitats and the possibility for evaluating their impact. Several recommendations were outlined:

- Encourage the Contracting Parties to elaborate their own National Action Plans for the conservation of dark habitats taking into account the specificities of the areas concerned within their jurisdictions.
- Suggest appropriate legislative measures, particularly as regards impact studies for coastal development and to investigate the activities that can affect these communities.
- Support inventories on the distribution, diversity, community structure and function of dark habitats.
- Set up, update and integrate the available scientific databases.
- Promote education and awareness for the public, actors and decision-makers, aiming at highlighting the vulnerability and importance to preserve dark habitats.
- Establish conservation initiatives in areas supporting dark habitats that are significant for the Mediterranean marine environment.

From a regional perspective RAC/SPA shall support CPs in order to:

- Set up collaborative tools and/or platforms to help scientists in exchanging data and experience.
- Launch awareness campaigns on the importance of protecting dark habitats along with training and capacity-building sessions.
- Start addressing the assessment of associated ecosystem services.
- Begin the process for designation of new protected areas aiming at conservation of deep-sea areas.

Additional measures can be also considered such as:

- Enforcement of existing regulatory measures, particularly those addressing to avoid the impact of destructive fishing practices over identified deep-sea sensitive habitats, vulnerable marine ecosystems or essential fish habitats (spawning and nursery grounds).
- Support to better fishing practices (including small-scale artisanal fisheries) in areas where ecosystem engineers develop (e.g. implementation of pelagic doors in benthic fishing gears, switch from bottom trawling to long line fishing gears / gillnets / traps).


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C. APPENDICESS

Appendix I. List of the most common species in Mediterranean marine caves

* Endangered or threatened species (Annex II: SPA/BD PROTOCOL) or species whose exploitation is regulated (Annex III: SPA/BD PROTOCOL)

Foraminiferans

Miniacina miniacea (Pallas, 1766)

Sponges

Aaptos aaptos (Schmidt, 1864)
Acanthella acuta Schmidt, 1862
Agelas oroides (Schmidt, 1864) – more abundant in the Eastern Mediterranean
Aplysilla rosea (Barrois, 1876)
Aplysina cavernicola (Vacelet, 1959) *
Axinella damicornis (Esper, 1794)
Axinella verrucosa (Esper, 1794)
Chondrosia reniformis Nardo, 1847 – often discoloured
Clathrina coriacea (Montagu, 1818)
Clathrina clathrus (Schmidt, 1864)
Cliona viridis (Schmidt, 1862)
Cliona schmidti (Ridley, 1881)
Cliona celata Grant, 1826
Crambe crambe (Schmidt, 1862)
Dendroxea lenis (Topsent, 1892)
Diplastrella bistellata (Schmidt, 1862)
Dysidea avara (Schmidt, 1862)
Dysidea fragilis (Montagu, 1818)
Erylus discophorus (Schmidt, 1862)
Fasciospongia cavernosa (Schmidt, 1862)
Geodia cydonium (Jameson, 1811)
Haliclona (Halichoclona) fulva (Topsent, 1893)
Haliclona (Reniera) cratera (Schmidt, 1862)
Haliclona (Rhizoniera) sarai (Pulitzer-Finali, 1969)
Haliclona (Soestella) mucosa (Griessinger, 1971)
Hemimycale columella (Bowerbank, 1874)
Ircinia dendroides (Schmidt, 1862)
Ircinia oros (Schmidt, 1864)
Ircinia variabilis (Schmidt, 1862)
Jaspis johnstoni (Schmidt, 1862)
Myrmekioderma spelaeum (Pulitzer-Finali, 1983)
Oscarella spp.
Penares euastrum (Schmidt, 1868)
Penares helleri (Schmidt, 1864)
Petrobiona massiliana Vacelet & Lévi, 1958 * – more common in the Western Mediterranean
Petrosia (Petrosia) ficiformis (Poiret, 1789) – often discoloured
Phorbas tenacior (Topsent, 1925)
Plakina spp.
Pleraplysilla spinifera (Schulze, 1879)
Scalarispongia scalaris (Schmidt, 1862)
Spirastrella cunctatrix Schmidt, 1868
Spongia (Spongia) officinalis Linnaeus, 1759 *
Spongia (Spongia) virgultosa (Schmidt, 1868)
Terpios gelatinosa (Bowerbank, 1866)

Cnidarians

Arachnanthus oligopodus (Cerfontaine, 1891)
Astroides calycularis (Pallas, 1766) * – in southern areas of the Western Mediterranean
Caryophyllia (Caryophyllia) inornata (Duncan, 1878)
Cerianthus membranaceus (Spallanzani, 1784)
Corallium rubrum (Linnaeus, 1758) *
Eudendrium racemosum (Cavolini, 1785)
Eunicella cavolini (Koch, 1887) – more common in the Western Mediterranean
Halecium spp.
Hoplangia durotrix Gosse 1860
Leptopsammia pruvoti Lacaze-Duthiers 1897
Madracis pharensis (Heller, 1868) – more abundant in the Eastern Mediterranean
Obelia dichotoma (Linnaeus, 1758)
Paramuricea clavata (Risso, 1826) – more common in the Western Mediterranean
Parazoanthus axinellae (Schmidt, 1862) – more common in the Adriatic and the Western Mediterranean occidentale
Phyllangia americana mouchezii (Lacaze-Duthiers, 1897)
Polycyathus muellerae (Abel, 1959)

Decapods

Athanas nitescens (Leach, 1814)
Dromia personata (Linnaeus, 1758)
Eualus occultus (Lebour, 1936)
Galathea strigosa (Linnaeus, 1761)
Herbstia condyliata (Fabricius, 1787)
Lysmata seticaudata (Risso, 1816)
Palaemon serratus (Pennant, 1777)
Palinurus elephas (Fabricius, 1787) *
Plesionika narval (Fabricius, 1787) – more common in the Eastern Mediterranean

Scyllarides latus (Latreille, 1802) *
Scyllarus arctus (Linnaeus, 1758) *
Stenopus spinosus Risso, 1826

Mysids

Harmelinella mariannae Ledoyer, 1989
Hemimysis lamornae mediterranea Bacescu, 1936
Hemimysis margalefi Alcaraz, Riera & Gili, 1986
Hemimysis speluncola Ledoyer, 1963
Siriella jaltensis Czerniavsky, 1868

Polychaetes

Filograna implexa Berkeley, 1835
Filogranula annulata (O. G. Costa, 1861)
Filogranula calyculata (O.G. Costa, 1861)
Filogranula gracilis Langerhans, 1884
Hermodice carunculata (Pallas, 1766) – more common in the Eastern Mediterranean
Hydroides pseudouncinata Zibrowius, 1968
Janita fimbriata (Delle Chiaje, 1822)
Josephella marenzelleri Caullery & Mesnil, 1896
Metavermilia multicristata (Philippi, 1844)
Protula tubularia (Montagu, 1803)
Semivermilia crenata (O. G. Costa, 1861)
Serpula cavernicola Fassari & Mollica, 1991
Serpula concharum Langerhans, 1880
Serpula lobiancoi Rioja, 1917
Serpula vermicularis Linnaeus, 1767
Spiraserpula massiliensis (Zibrowius, 1968)
Spirobranchus polytrema (Philippi, 1844)
Vermiliopsis labiata (O. G. Costa, 1861)
Vermiliopsis infundibulum (Philippi, 1844)
Vermiliopsis monodiscus Zibrowius, 1968

Mollusks

Lima lima (Linnaeus, 1758)
Lithophaga lithophaga (Linnaeus, 1758) *
Luria lurida (Linnaeus, 1758) *
Neopycnodonte cochlear (Poli, 1795)
Peltodoris atromaculata Bergh, 1880
Rocellaria dubia Pennant, 1777

Bryozoans

Adeonella calveti (Canu & Bassler, 1930) – mainly in the Western Mediterranean
Adeonella pallasii (Heller, 1867) – endemic to the Eastern Mediterranean
Celleporina caminata (Waters, 1879)
Corbulella maderensis (Waters, 1898)
Crassimarginatella solidula (Hincks, 1860)
Hippaliosina depressa (Busk, 1854) – more common in the Eastern Mediterranean
Myriapora truncata (Pallas, 1766)
Onychocella marioni (Jullien, 1882)
Puellina spp.
Reteporella spp.
Schizomavella spp.
Schizotheca spp.
Turbicellepora spp.

Brachiopods

Argyrotheca cistellula (Searles-Wood, 1841)
Argyrotheca cuneata (Risso, 1826)
Joania cordata (Risso, 1826)
Megathiris detruncata (Gmelin, 1790)
Novocrania anomala (O. F. Müller, 1776)
Tethyrhynchia mediterranea Logan & Zibrowius, 1994

Echinoderms

Amphipholis squamata (Delle Chiaje, 1828)
Arbacia lixula (Linnaeus, 1758)
Centrostephanus longispinus (Philippi, 1845) *
Hacelia attenuata Gray, 1840
Holothuria spp.
Marthasterias glacialis (Linnaeus, 1758)
Ophioderma longicauda (Bruzelius, 1805)
Ophiothrix fragilis (Abildgaard, 1789)
Paracentrotus lividus (de Lamarck, 1816) *

Ascidians

Cystodytes dellechiajei (Della Valle, 1877)
Didemnum spp.
Aplidium spp.
Halocynthia papillosa (Linnaeus, 1767)
Microcosmus spp.
Pyura spp.

Pisces

Apogon (Apogon) imberbis (Linnaeus, 1758)
Conger conger (Linnaeus, 1758)
Corcyrogobius liechtensteini (Kolombatovic, 1891)
Didogobius splechtnai Ahnelt & Patzner, 1995
Gammogobius steinitzi Bath, 1971
Gobius spp.
Grammonus ater (Risso, 1810)
Parablennius spp.
Phycis phycis (Linnaeus, 1766)
Sciaena umbra Linnaeus, 1758*
Scorpaena maderensis Valenciennes, 1833 – more common in the Eastern Mediterranean
Scorpaena notata Rafinesque, 1810
Scorpaena porcus Linnaeus, 1758
Scorpaena scrofa Linnaeus, 1758
Serranus cabrilla (Linnaeus, 1758)
Serranus scriba (Linnaeus, 1758
Thorogobius ephippiatus (Lowe, 1839)

PRELIMINARY LIST OF THE PRINCIPAL SPECIES TO BE CONSIDERED ON THE MONITORING

* Endangered or threatened species (Annex II: SPA/BD PROTOCOL) or species whose exploitation is regulated (Annex III: SPA/BD PROTOCOL)

Sponges

Agelas oroides (Schmidt, 1864)
Aplysina cavernicola (Vacelet, 1959) *
Axinella damicornis (Esper, 1794)
Axinella verrucosa (Esper, 1794)
Ircinia spp.
Petrobiona massiliana Vacelet & Lévi, 1958 *
Petrosia (Petrosia) ficiformis (Poiret, 1789)
Spongia (Spongia) officinalis Linnaeus, 1759 *

Cnidarians

Astroides calycularis (Pallas, 1766) *
Caryophyllia (Caryophyllia) inornata (Duncan, 1878)
Cerianthus membranaceus (Spallanzani, 1784)
Corallium rubrum (Linnaeus, 1758) *
Eunicella cavolini (Koch, 1887)
Hoplangia durotrix Gosse 1860
Leptopsammia pruvoti Lacaze-Duthiers 1897
Madracis pharensis (Heller, 1868)
Paramuricea clavata (Risso, 1826)
Phyllangia americana mouchezii (Lacaze-Duthiers, 1897)
Polycyathus muellerae (Abel, 1959)

Decapods

Palinurus elephas (Fabricius, 1787) *
Plesionika narval (Fabricius, 1787)
Scyllarides latus (Latreille, 1802) *
Scyllarus arctus (Linnaeus, 1758) *
Stenopus spinosus Risso, 1826

Mysids

Hemimysis margalefi Alcaraz, Riera & Gili, 1986
Hemimysis speluncola Ledoyer, 1963

Polychaetes

Serpula cavernicola (Fassari & Mollica, 1991)
Serpula vermicularis (Linnaeus, 1767)
Protula tubularia (Montagu, 1803)
Hyalopomatus spp.

Mollusks

Lima lima (Linnaeus, 1758)
Luria lurida (Linnaeus, 1758) *

Bryozoans

Adeonella spp.
Myriapora truncata (Pallas, 1766)
Reteporella spp.
Schizotheca spp.

Ascidians

Halocynthia papillosa (Linnaeus, 1767)
Hermania momus (Savigny, 1816) – non-indigenous species reported in Eastern Mediterranean caves
Microcosmus spp.

Pisces

Apogon (Apogon) imberbis (Linnaeus, 1758)
Grammonus ater (Risso, 1810)
Thorogobius ephippiatus (Lowe, 1839)

Appendix II. Modified example of fill-in sheet developed in the context of monitoring studies by V. Gerovasileiou (HCMR).

The form was based on the approach for the evaluation of the ecological quality of marine cave habitats developed by Rastorgueff *et al*. (2015). In addition to the species data included in the form, photoquadrats covering a total surface of 1-4 m^2 should be acquired for the study of sessile communities.

Area :	**Date :**	**Observer :**	
Latitude :		**Longitude :**	
Submersion level : Submerged / Semi-submerged		**Cave morphology :** Blind cave / Tunnel – No. of entrances: …	
Total length of cave: …	**Maximum water depth: …**	**Minimum water depth: …**	
Entrance A' – Max depth (m): …	Height (m): …	Width (m): …	Orientation: …
Entrance B' – Max depth (m): …	Height (m): …	Width (m): …	Orientation: …
Other topographic features :	Internal beach /	Air pockets /	Speleothems / …
Micro-habitats :			

Detritivorous / omnivorous species (number of species and individuals observed at 5 min interval)				
Herbstia condyliata	1–2	3–4	5–10	>10
Galathea strigosa	1–2	3–4	5–10	>10
Scyllarus arctus	1–2	3–4	5–10	>10
. . .	1–2	3–4	5–10	>10
. . .	1–2	3–4	5–10	>10
. . .	1–2	3–4	5–10	>10
. . .	1–2	3–4	5–10	>10
. . .	1–2	3–4	5–10	>10
Mysids	0	few		swarm

Fish species observed / cave zone (CE: entrance, SD: semi-dark zone, DZ: dark zone)	**Decapod species observed / cave zone** (CE: entrance, SD: semi-dark zone, DZ: dark zone)
… /	… /
… /	… /
… /	… /
… /	… /
… /	… /
… /	… /
… /	… /
… /	… /

***Cerianthus membranaceus* (number of individuals)**	0	1-2	>2
***Arachnanthus oligopodus* (number of individuals)**	0	1-2	>2

Other typical and/or protected species	**Threats and pressures**	
	Broken bryozoans	…
	Air bubbles	…
	Marine litter	…
	Non-indigenous species	…
	…	…
	…	…
	Other comments	

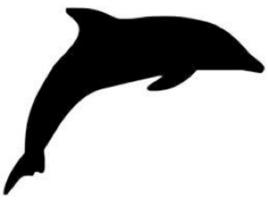
Appendix III. EcAp Ecological Objectives

EO 1: Biological diversity Biological diversity is maintained or enhanced. The quality and occurrence of coastal and marine habitats and the distribution and abundance of coastal and marine species are in line with prevailing physiographic, hydrographic, geographic, and climatic conditions.	The biological diversity is «the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems». The term 'maintained' is key to the quantification of GES for EO 1. This condition has three determining factors: a. no further loss of the diversity within species, between species and of habitats/communities and ecosystems at ecologically relevant scales; b. any deteriorated attributes of biological diversity are restored to and maintained at or above target levels, where intrinsic conditions allow; c. where the use of the marine environment is sustainable.
EO 2: Non-indigenous species Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystem	Non-indigenous species are species, subspecies or lower taxa introduced outside of their natural range 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. In the Mediterranean, marine invasive species are regarded as one of the main causes of biodiversity loss potentially modifying all aspects of marine and other aquatic ecosystems.
EO 3: Harvest of Commercially exploited fish and shellfish Populations of selected commercially exploited fish and shellfish are within biologically safe limits, exhibiting a population age and size distribution that is indicative of a healthy stock.	The level of exploitation under EO 3 should be that of 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.
EO 4: Marine food webs Alterations to components of marine food webs caused by resource extraction or human-induced environmental changes do not have long-term adverse effects on food web dynamics and related viability.	A healthy marine ecosystem requires a well-functioning of its food web. This ecological objective is the same as EO 3, but also includes species which are not commercially exploited.
EO 5: Eutrophication Human-induced eutrophication is prevented, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algal blooms, and oxygen deficiency in bottom waters.	Eutrophication is a process driven by enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, leading to: increased growth, primary production and biomass of algae; changes in the balance of nutrients causing changes to the balance of organisms; and water quality degradation. The consequences of eutrophication are undesirable if they appreciably degrade ecosystem health and/or the sustainable provision of goods and services. These changes may occur due to natural processes. Management concern begins when they are attributed to anthropogenic sources. GES with regard to eutrophication is achieved when the biological community remains well-balanced and retains all necessary functions in the absence of undesirable disturbance associated with eutrophication.

EO 6: Sea floor integrity Sea-floor integrity is maintained, especially in priority benthic habitats.	Many human activities (e.g. trawling, dredging, seabed mining, drilling) cause physical damage to seabed. However, given the serious consequences of their impacts, especially on vulnerable habitats and those with a low capacity of restoration, stronger measures to minimize the physical deterioration of the seabed should be implemented.
EO 7: Hydrography Alteration of hydrographic conditions does not adversely affect coastal and marine ecosystems.	Hydrographical conditions are characterized by the physical characteristics of seawater such as bathymetric data, seafloor topography, current velocity, wave exposure, turbulence, turbidity, temperature and salinity. These characteristics play a crucial role in the dynamics of marine ecosystems and can be altered by human activities, especially in coastal areas. Alterations to hydrographical conditions can occur due to the construction of physical structures or through excavation of navigational channels.
EO 8: Coastal ecosystems and landscapes The natural dynamics of coastal areas are maintained and coastal ecosystems and landscapes are preserved.	Coastal zones play a key role in the economic development of regions and nations as they are a significant source of various goods and services. Mediterranean coastal areas are threatened by coastal development that modifies the coastline through the construction of buildings and infrastructures. However, there has not been systematic monitoring, in particular not quantitatively based monitoring or any major attempt to systematize characteristics of coastal ecosystems on a wider Mediterranean basis. The status assessment of EO 8 aims to fill this gap because it reflects the aim of the Barcelona Convention to include coastal areas in the assessment.
OE 9: Pollution Contaminants cause no significant impact on coastal and marine ecosystems and human health.	GES under EO 9 is achieved when contaminants cause no significant impact on coastal and marine ecosystems and human health.
EO 10: Marine litter Marine and coastal litter does not adversely affect coastal and marine environments.	Marine litter is a problem not just along coastlines but also in the high seas, as the waste from human activities is often degraded directly which can cause health as well as aesthetic problems. In particular the breakdown at sea of plastic into tiny particles or microplastics can be detrimental to marine environmental quality.
EO 11: Energy including underwater noise Noise from human activities causes no significant impact on marine and coastal ecosystems.	Anthropogenic energy introduced by human activities into the marine environment includes sound, light and other electromagnetic fields, heat and radioactive energy. Among these, the most widespread and pervasive is underwater sound. Sources of marine noise pollution include ship traffic, geophysical exploration and oil and gas exploitation, military sonar use and underwater detonations. Such activities are growing throughout the Mediterranean Sea and marine organisms can be adversely affected both on short and long timescale. Management concern is primarily associated to the negative effects of noise on sensitive protected species, such as some species of marine mammals, though there is growing awareness that an ecosystem-wide approach also needs to be considered.

Appendix IV-1. Vulnerable Species to be reported as by-catch

NOTE: Most of the species in the tables below frequently occur with pelagic fisheries; however incidental catches of these species might eventually occur with fisheries targeting deep-sea species (birds are not considered in this Annex since they do not apply to deep-sea). In addition to the species below, Species from Annexes II and III from the Protocol SPA/BD shall be considered.

Group of vulnerable species	Family	Species	Common name
Cetaceans 	Balaenopteridae	*Balaenoptera acutorostrata*	Common minke whale
		Balaenoptera borealis	Sei whale
		Balaenoptera physalus	Fin whale
		Megaptera novaeangliae	Humpback whale
	Balenidae	*Eubalaena glacialis*	North Atlantic right whale
	Physeteridae	*Physeter macrocephalus*	Sperm whale
		Kogia simus	Dwarf sperm whale
	Phocoenidae	*Phocoena phocoena*	Harbour porpoise
	Delphinidae	*Steno bredanensis*	Rough-toothed dolphin
		Grampus griseus	Risso's dolphin
		Tursiops truncatus	Common bottlenose dolphin
		Stenella coeruleoalba	Striped dolphin
		Delphinus delphis	Common dolphin
		Pseudorca crassidens	False killer whale
		Globicephala melas	Long-finned pilot whale
		Orcinus orca	Killer whale
	Ziphiidae	*Ziphius cavirostris*	Cuvier's beaked whale
		Mesoplodon densirostris	Blainville's beaked whale
Seals	Phocidae	*Monachus monachus*	Mediterranean monk seal
Sharks, Rays, Chimae-ras 	Alopiidae	*Alopias vulpinus*	Common thresher
	Carcharhinidae	*Carcharias taurus*	Sand tiger
		Carcharhinus plumbeus	Sandbar shark
		Carcharodon carcharias	Great white shark
		Prionace glauca	Blue shark
	Centrophoridae	*Centrophorus granulosus*	Gulper shark
	Cetorhinidae	*Cetorhinus maximus*	Basking shark
	Gymnuridae	*Gymnura altavela*	Spiny butterfly ray
	Hexanchidae	*Heptranchias perlo*	Sharpnose sevengill shark
	Lamnidae	*Isurus oxyrinchus*	Shortfin mako
		Lamna nasus	Porbeagle
	Myliobatidae	*Mobula mobular*	Devil fish
	Odontaspididae	*Odontaspis ferox*	Small-tooth sand tiger shark
	Oxynotidae	*Oxynotus centrina*	Angular rough shark
	Pristidae	*Pristis pectinata*	Smalltooth sawfish
		Pristis pristis	Common sawfish

	Rajidae	*Dipturus batis*	Common skate
		Leucoraja circularis	Sandy ray
		Leucoraja melitensis	Maltese skate
		Rostroraja alba	Bottlenose skate
	Rhinobatidae	*Rhinobatos cemiculus*	Blackchin guitarfish
		Rhinobatos rhinobatos	Common guitarfish
	Sphyrnidae	*Sphyrna lewini*	Scalloped hammerhead
		Sphyrna mokarran	Great hammerhead
		Sphyrna zygaena	Smooth hammerhead
	Squatinidae	*Squatina aculeata*	Sawback angel shark
		Squatina oculata	Smoothback angel shark
		Squatina squatina	Angel shark
	Triakidae	*Galeorhinus galeus*	School/Tope shark
Sea turtles 	Cheloniidae	*Caretta caretta*	Loggerhead turtle
		Chelonia mydas	Green turtle
		Eretmochelys imbricata	Hawksbill Turtle
		Lepidochelys kempii	Kemp's ridley sea turtle
	Dermochelyidae	*Dermochelys coriacea*	Leatherback sea turtle
	Trionychidae	*Trionyx triunguis*	African softshell turtle

Appendix IV-2. Vulnerable Species to be reported as by-catch: Rare elasmobranch species

This list reports elasmobranchs species that are considered rare but are present in the Mediterranean and the Black Sea (source: GFCM-DCRF – version 2016.2)

Group of rare species	Family	Species	Common name
Sharks, Rays, Chimaeras 	Alopiidae	*Alopias superciliosus*	Bigeye thresher
	Hexanchidae	*Hexanchus nakamurai*	Bigeye sixgill shark
	Echinorhinidae	*Echinorhinus brucus*	Bramble shark
	Squalidae	*Squalus megalops*	Shortnose spurdog
	Centrophoridae	*Centrophorus uyato*	Little gulper shark
	Somniosidae	*Centroscymnus coelolepis*	Portugese dogfish
		Somniosus rostratus	Little sleeper shark
	Lamnidae	*Isurus paucus*	Longfin mako
	Scyliorhinidae	*Galeus atlanticus*	Atlantic catshark
	Carcharhinidae	*Carcharhinus altimus*	Bignose shark
		Carcharhinus brachyurus	Bronze whaler shark
		Carcharhinus brevipinna	Spinner shark
		Carcharhinus falciformis	Silky shark
		Carcharhinus limbatus	Blacktip shark
		Carcharhinus melanopterus	Blacktip reef shark
		Carcharhinus obscurus	Dusky shark
		Galeocerdo cuvier	Tiger shark
		Rhizoprionodon acutus	Milk shark
	Torpedinidae	*Torpedo nobiliana*	Great torpedo
		Torpedo sinuspersici	Variable torpedo ray
	Rajidae	*Dipturus nidarosiensis*	Norwegian skate
		Leucoraja fullonica	Shagreen skate
		Leucoraja naevus	Cuckoo skate
		Raja africana	African skate
		Raja brachyura	Blonde skate
		Raja montagui	Spotted skate
		Raja polystigma	Speckled skate
		Raja radula	Rough skate
		Raja undulata	Undulate skate
	Dasyatidae	*Dasyatis centroura*	Roughtail stingray
		Dasyatis marmorata	Marbled stingray
		Dasyatis pastinaca	Common stingray
		Dasyatis tortonesei	Tortonese's stingray
		Himantura uarnak	Honeycomb whipray
		Taeniura grabata	Round fantail stingray
	Myliobatidae	*Pteromylaeus bovinus*	Bullray
	Rhinopteridae	*Rhinoptera marginata*	Lusitanian cownose ray
	Sphyrnidae	*Sphyrna tudes*	Smalleye hammerhead

Appendix V. Fishing Gears

NOTE: Just fishing gears impacting deep-sea habitats should be considered (source: GFCM – Data Collection Reference Framework - Version 2016.2)

Gear name	Code
Purse seine without purse lines (lampara)	LA
Purse seine with purse lines (purse seines)	PS
One boat-operated purse seines	PS1
Two boat-operated purse seines	PS2
Beach seines	SB
Danish seines	SDN
Pair seines	SPR
Scottish seines	SSC
Boat or vessel seines	SV
Seine nets (not specified)	SX
Otter trawls (not specified)	OT
Bottom otter trawls	OTB
Midwater otter trawls	OTM
Otter twin trawls	OTT
Pair trawls (not specified)	PT
Bottom pair trawls	PTB
Midwater pair trawls	PTM
Bottom trawls	TB
Bottom beam trawls	TBB
Bottom nephrops trawls	TBN
Bottom shrimp trawls	TBS
Midwater trawls	TM
Midwater shrimp trawls	TMS
Other trawls (not specified)	TX
Boat dredges	DRB
Hand dredges	DRH
Lift nets (not specified)	LN
Boat-operated lift nets	LNB
Portable lift nets	LNP
Shore-operated stationary lift nets	LNS
Cast nets	FCN

Nom de l'engin	Code
Falling gear (not specified)	FC
Gillnets and entangling nets (not specified)	GEN
Gillnets (not specified)	GN
Encircling gillnets	GNC
Driftnets	GND
Fixed gillnets (on stakes)	GNF
Set gillnets (anchored)	GNS
Combined gillnets-trammel nets	GTN
Trammel nets	GTR
Aerial traps	FAR
Traps (not specified)	FIX
Stationary uncovered pound nets	FPN
Pots	FPO
Stow nets	FSN
Barrier, fences, weirs, etc.	FWR
Fyke nets	FYK
Handlines and pole-lines (mechanized)	LHM
Handlines and pole-lines (hand operated)	LHP
Longlines (not specified)	LL
Drifting longlines	LLD
Set longlines	LLS
Trolling lines	LTL
Hooks and lines (not specified)	LX
Harpoons	HAR
Pumps	HMP
Mechanized dredges	HMD
Harvesting machines (not specified)	HMX
Miscellaneous gear	MIS
Recreational fishing gear	RG
Gear not known or not specified	NK

Appendix VI. Quick guidance on survey methods useful to locate, determine extent and assess biodiversity in deep-sea habitats (adapted from IMAP Guidance, 2016).

Type of data	HABITAT		
	Hard beds associated with Coralligenous biocoenosis	Bathyal rocks with Anthozoa	Infralittoral and circalittoral detritic bottoms dominated by *Leptometra spp*
Remote Methods			
Side scan sonar[10]	Locate, extent	Locate, extent	Locate, extent
Multibeam bathymetry[10]	Locate, extent	Locate, extent	Locate, extent
AGDS[10] **(Acoustic Ground Discrimination Systems)**	Locate, extent	Locate, extent	Locate, extent
AUV (Autonomous underwater vehicle)	Locate, extent	Locate, extent	Locate, extent
Direct sampling or observation methods			
Grab/core sampling	Biodiversity (not recommended)	Biodiversity (not recommended)	Biodiversity
Towed video	Extent (not recommended)	Extent (not recommended)	Extent (not recommended)
Drop-down video / photography / ROV	Extent / Biodiversity	Extent / Biodiversity	Extent / Biodiversity
Epibenthic trawls / dredges	Not recommended	Not recommended	Not recommended

[10] For all remote sensing, distinguishing habitats from each other and from the surrounding seabed depends on the resolution of the sampling method – higher resolution will provide better data to distinguish habitats, but covers smaller areas and is more expensive to collect and process than lower resolution data

Appendix VII. Summary of methods available for litter evaluation on sea floor (according to MSFD GES Technical Subgroup on Marine Litter, 2011)

Component	Shallow waters	Continental shelves and canyon bottoms	Deep sea floor
Depth	0 – 40 m	40 – 800 m	200 - 2500 m
Areas to be monitored	Coastal	Shelves	Priorities must be considered and given to deep sea areas close to sources (costal, urban, affected by litter).
Approach	Diving	Trawling	Submersibles (ROVs - Autonomous or manned submersibles)
Existing program	E.g. Project AWARE dive against debris, NGO initiative	MEDITS related programs (including Black Sea), IBTS related (IBTS, EVHOE, CGFS, ...) Cruises (OSPAR/ICES)	Irregular dives (France)
Areas not concerned			Baltic countries, North Sea countries, North Adriatic, etc.
Areas largely concerned	All Mediterranean countries, Baltic	Any shelf	Mediterranean (Spain, France, West and south east Italy, Greece, Cyprus), Portugal, England (Partly)
Sample size	10-2000 m2	1-5 ha	0.1-2 km routes / dive
Units	Density (items/ha)	Density (items/ ha , per categories)	Items (per categories) / km route
Categories	Plastic, paper and cardboard glass and ceramics, metal, leather/ clothes, others, fishing gear	Plastic, paper and cardboard glass and ceramics, metal, leather/ clothes, others fishing gear	Plastic, paper and cardboard glass and ceramics, metal, leather/ clothes, others, fishing gears
	Compatible among indicators	Compatible among indicators	Compatible among indicators
Frequency	Every year	Every 1-3 years	Every 5-10 years
Inter calibration	Possible	Possible	Difficult
Research needed			Search for accumulation areas

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