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Agenda item 3: Guidance on monitoring marine benthic habitats (Common Indicators 1 and 2)

Monitoring protocols of the Ecosystem Approach Common Indicators 1 and 2 related to marine benthic habitats

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UNEP/MAP
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Note by the Secretariat

The 19th Meeting of the Contracting Parties to the Barcelona Convention (COP 19) agreed on the Integrated Monitoring and Assessment Programme (IMAP) of the Mediterranean Sea and Coast and Related Assessment Criteria which set, in its Decision IG.22/7, a specific list of 27 common indicators (CIs) and Good Environmental Status (GES) targets and principles of an integrated Mediterranean Monitoring and Assessment Programme.

During the initial phase of the IMAP implementation (2016-2019), the Contracting parties to the Barcelona Convention updated the existing national monitoring and assessment programmes following the Decision requirements in order to provide all the data needed to assess whether ‘‘Good Environmental Status’’ defined through the Ecosystem Approach process has been achieved or maintained.

In line with IMAP, Guidance Factsheets were developed, reviewed and agreed by the Meeting of the Ecosystem Approach Correspondence Group on Monitoring (CORMON) Biodiversity and Fisheries (Madrid, Spain, 28 February-1 March 2017) and the Meeting of the SPA/RAC Focal Points (Alexandria, Egypt, 9-12 May 2017) for the Common Indicators to ensure coherent monitoring.

Decision IG.23/6 on the 2017 MED QSR (COP 20, Tirana, Albania, 17-20 December 2017) agreed, as general directions towards a successful 2023 Mediterranean Quality Status Report (2023 MED QSR), the following main recommendations:

(i) harmonization and standardization of monitoring and assessment methods;
(ii) improvement of availability and ensuring of long time series of quality assured data to monitor the trends in the status of the marine environment;
(iii) improvement of availability of the synchronized datasets for marine environment state assessment, including use of data stored in other databases where some of the Mediterranean countries regularly contribute; and
(iv) improvement of data accessibility with the view to improving knowledge on the Mediterranean marine environment and ensuring that Info-MAP System is operational and continuously upgraded, to accommodate data submissions for all the IMAP Common Indicators.

Considering evolving needs to fill the gaps, in particular related to the harmonization and standardization of monitoring and assessment methods, the present document provides information on the monitoring protocols of the agreed Ecosystem Approach common indicators 1 and 2 to assess progress towards Good Environmental Status (GES).

The present document is organized along three main monitoring guidelines of benthic marine habitats:

(i) monitoring guidelines of marine vegetation
(ii) monitoring guidelines of coralligenous and other calcareous bioconstructions
(iii) monitoring guidelines of dark habitats

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General premise

The Contracting Parties to the Barcelona Convention have adopted the Ecosystem Approach (EcAp) in 2008 with the Decision IG. 17/6, aimed at reaching "A healthy Mediterranean with marine and coastal ecosystems that are productive and biologically diverse for the benefit of present and future generations" (UNEP/MAP, 2008). This process (EcAp) aims to achieve the Good Environmental Status (GES) through informed management decisions, based on integrated quantitative assessment and monitoring of the marine and coastal environment of the Mediterranean, in order to manage human activities sustainably.

In 2016, during the 19th Meeting of the Contracting Parties to the Barcelona Convention (COP19, Athens, Greece, 9-12 February 2016), an Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP) has also been adopted by the Mediterranean region. The resulting document describes the strategy, objectives and products that the Contracting Parties have to deliver over the second period of the implementation of the EcAp (2016-2021) in the framework of the Mediterranean Action Plan (UNEP/MAP, 2008). The main goal of IMAP is to build and implement a regional integrated monitoring system gathering reliable quantitative and updated data on the status of marine and coastal Mediterranean environment. A list of agreed 27 Common Indicators (CIs), articulated on 11 Ecological Objectives (EO) in synergy with the European Union's Marine Strategy Framework Directive (2008/56/EC), and GES targets of the IMAP have been set in the Decision IG.22/7. In the context of the IMAP, a Common Indicator is defined as "an indicator that summarizes data into a simple, standardised, and communicable figure and is ideally applicable in the whole Mediterranean basin, or at least on the level of sub-regions, and is monitored by all Contracting Parties. A common indicator is able to give an indication of the degree of threat or change in the marine ecosystem and can deliver valuable information to decision makers".

During the initial phase of the IMAP implementation (2016-2019), the Contracting parties to the Barcelona Convention were asked to update the existing national monitoring and assessment programmes in order to provide all the data needed to assess whether the GES defined through the EcAp process has been achieved or maintained. Monitoring programmes at the national level are shared to create a compatible, shared Mediterranean pool of data, usable by each Contracting Party to product common indicator assessment reports in an integrated manner, which ensures comparability across the Mediterranean region.

Among the five EcAp Common Indicators related to "biodiversity" (EO1) fixed by IMAP, two are related to habitats in the Barcelona Convention Decision IG.22/7 (UNEP/MAP, 2008), namely:

- Common Indicator 1: Habitat distributional range, to also consider habitat extent as a relevant attribute
- Common Indicator 2: Condition of the habitat's typical species and communities.

Regarding the assessment of the EO1 "biodiversity", a quantitative definition of GES is difficult, considering the variety of conceptual facets existing around the term "biodiversity" (e.g., genetic diversity, species diversity, and habitat diversity). Thus, the GES boundaries are here defined as "the acceptable deviation from a reference state, which reflects conditions largely free from anthropogenic pressures".

Purpose and aims

The purpose of this document is to elucidate the guidelines for monitoring marine benthic habitats in Mediterranean following common and standardised monitoring programmes, to address the two CIs that specifically relate to habitats, and specifically to those habitats selected by the Parties, i.e. marine vegetation, coralligenous and other calcareous bioconstructions, dark habitats.

Common Indicator 1: Habitat distributional range, to also consider habitat extent as a relevant attribute.

This indicator is aimed at providing information about the geographical area in which the benthic habitat occurs. It reflects the distributional range of benthic habitats that are present on Mediterranean bottoms. The main outputs of the monitoring for this indicator will be maps with the habitat presence and distributional range. Availability of updated and complete maps will allow detecting any important change in the habitat distributional patterns to understand their evolution over time, and measuring their distance from the original, reference status (i.e., the baseline).

Common Indicator 2: Condition of the habitat's typical species and communities.

This indicator is aimed at providing information about the ecological status of the benthic habitat. Assessments should be focused in collecting data on the status of habitats using typical/target species as indicators and/or considering the community composition. Thanks to this indicator any important change in the status of the habitat can be detected, and again availability of long-term data series will allow understanding the trajectories of change experienced by those habitats through time.

The main aim of these guidelines is to provide guidance to managers and decision makers (e.g., environmental authority representatives, researchers, Marine Protected Area - MPA representatives) on field methodologies for long-term monitoring of marine benthic habitats in MPAs, in identified hotspots of biodiversity, or in sites of high conservation relevance (e.g., Natura 2000 sites). These indications should help environmental practitioners in deciding what kind of method to choose at regional and national level to answer the Common Indicators 1 and 2.

In particular, the document is organized along 3 monitoring guidelines for the main benthic habitats:

(1) Guidelines for monitoring marine vegetation

(2) Guidelines for monitoring coralligenous and other calcareous bioconstructions

(3) Guidelines for monitoring dark habitats.

All the three guidelines provide information on the monitoring protocols of the agreed EcAp Common Indicators 1 and 2 towards the GES objective, and address the same common purposes to all monitoring guidelines developed to date:

(i) Harmonisation and standardisation of monitoring and assessment methods

(ii) Assuring the quality of long time series of data to monitor the trends in the status of the marine environment

(iii) Improvement of availability of synchronised datasets for marine environmental state assessment, including data stored in other databases where some of the Mediterranean countries regularly contribute

(iv) Improvement of data accessibility and their continuous upgrading, with the view to improving knowledge on the Mediterranean marine environment, to accommodate data submissions for all the IMAP Common Indicators.

For all the three benthic habitats addressed in these guidelines (i.e., marine vegetation, coralligenous and other calcareous bioconstructions, and dark habitats), available information and existing monitoring protocols have been taken into account, as the base for the updating and harmonisation process. In particular, the following documents represented the starting point of the monitoring guidelines here proposed:

1. Guidelines for standardisation of mapping and monitoring methods of marine Magnoliophyta in the Mediterranean (UNEP/MAP-RAC/SPA, 2015a)[1]
2. Methods for inventorying and monitoring coralligenous and rhodoliths assemblages (UNEP/MAP-RAC/SPA, 2015b)[2]
3. Draft guidelines for inventorying and monitoring of dark habitats (UNEP/MAP-SPA/RAC, 2017)[3].

Also, a lot of scientific papers exist for each of the three benthic habitats. Many of them explain in detail the steps of implementation, the scientific background, and tools requested for their application. Various methods have already been recognised as standard.

In each monitoring guideline here proposed, a global overview of available methods is presented, with the main advantages and disadvantages, the human resources and material requested in order to better estimate the investment needed, and any other practical information. The scale of monitoring is of primary importance for biodiversity assessment, due to the nature of the biodiversity related common indicators, especially the Common Indicator 1 (distributional range, and habitat extent). The assessment scale is expressed as the relevant spatial and temporal resolution of required data. Resolution includes number and location of sampling stations, accuracy of remote indirect surveys, sampling frequencies, and sampling surface, which has to be clearly defined in each monitoring guideline. A balance between accuracy and costs is always required, to ensure a cost-efficiency resolution that will be the correct compromise between very accurate and complete assessment, but more expensive, and partial assessments in accordance with available resources.

All the three documents focus more on the surveying technique for data collection rather than on the following associated analyses. However, a reference to the available recent ecological indices purposely developed for environmental quality assessment is also reported for each habitat. Implementation of rigorous methods to ensure reliability of the data collected in a standardised manner is the fundamental first step to ensure comparability among different regions of the Contracting Parties. Further details on each specific method described and on the most used analyses can be found in the bibliographic references provided.

[1] UNEP/MAP-RAC/SPA. 2015a. Guidelines for standardization of mapping and monitoring methods of Marine Magnoliophyta in the Mediterranean. Pergent-Martini C. (Ed.), RAC/SPA publ., Tunis, 48 p. + Annexes.

[2] UNEP/MAP-RAC/SPA. 2015. Standard methods for inventorying and monitoring coralligenous and rhodoliths assemblages. Pergent G., Agnesi S., Antonioli P.A., Babbini L., Belbacha S., Ben Mustapha K., Bianchi C.N., Bitar G., Cocito S., Deter J., Garrabou J., Harmelin J.-G., Hollon F., Mo G., Montefalcone M., Morri C., Parravicini V., Peirano A., Ramos-Espla A., Relini G., Sartoretto S., Semroud R., Tunesi L., Verlaque M. (Eds), RAC/SPA publ., Tunis, 20 p. + Annex.

[3] UNEP/MAP-SPA/RAC. 2017. Draft guidelines for inventorying and monitoring of dark habitats. Aguilar R., Marín P. (Eds), SPA/RAC publ., Tunis, 58 p.

1. Guidelines for monitoring marine vegetation in Mediterranean

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Introduction

Seagrass meadows are widely recognized as key habitats in tropical and temperate shallow coastal waters of the world (UNEP-MAP-Blue Plan, 2009). They form some of the most productive ecosystems on earth (McRoy and McMillan, 1977), shaping coastal seascapes and providing essential ecological and economic services (Green and Short, 2003; Vassallo et al., 2013). They support high biodiverse associated communities, primary production and nutrient cycling, sediment stabilization and protection of the littoral, and globally significant sequestration of carbon (Waycott et al., 2009 and references therein). A major economic value of over 17000 $ per ha and per annum has been quantified for seagrass meadows worldwide (Costanza et al., 1997).

Seagrass, like all Magnoliophyta, are marine flowering plants of terrestrial origin which returned to the marine environment approx. 120 to 100 million of years. The global species diversity of seagrass is low when compared to any other marine Phylum or Division, with less than sixty species throughout the world. However, they form extensive meadows that extend for thousands of kilometres of coastline between the surfaces to about 50 m depth in very clear marine waters or transitional waters (e.g., estuaries and lagoons). In the Mediterranean region five seagrass species occur: *Cymodocea nodosa*, *Halophila stipulacea* (an invasive Lessepsian species), *Posidonia oceanica*, *Zostera marina*, and *Zostera noltei*. The endemic *Posidonia oceanica* is doubtless the dominant and the most import seagrass species (Green and Short, 2003), and the only one able to build a ‘‘matte’’, a monumental construction resulting from horizontal and vertical growth of rhizomes with entangled roots and entrapped sediment (Boudouresque et al., 2006).

Physical damages resulting from intense human pressures, environmental alterations, climate warming, and reduction of water and sediment quality are causing structural degradation of seagrass meadows worldwide (Orth et al., 2006). An alarming and accelerating decline of seagrass meadows has been reported in the Mediterranean Sea and mainly in the north-western side of the basin, where many meadows have already been lost during last decades (Boudouresque et al., 2009; Waycott et al., 2009; Pergent et al., 2012; Marbà et al., 2014; Burgos et al., 2017).

Concerns about these declines have prompted efforts to protect legally these habitats in several countries. Control and reduction of the full suite of anthropogenic impacts via legislation and enforcement at local and regional scales have been carried out in many countries. *Posidonia oceanica* meadows are defined as priority natural habitats on Annex I of the EC Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora (EEC, 1992), which lists those natural habitat types whose conservation requires the designation of special areas of conservation, identified as Sites of Community Interest (SCIs). Also, the establishment of marine protected areas (MPAs) locally enforces the level of protection on these priority habitats.

Due to their wide distribution, their sedentary habit and their susceptibility to changing environmental conditions, seagrass are habitually used as biological indicators of water quality in accordance with the Water Framework Directive (WFD, 2000/60/EC) and of environmental quality in accordance with the Marine Strategy Framework Directive (MSFD, 2008/56/EC) (Montefalcone, 2009). Due to its recognized ecological importance, *Posidonia oceanica* is considered as the main biological quality element in monitoring programs developed to evaluate the status of marine coastal environment. Standardised monitoring protocols for evaluating and classifying the conservation status of seagrass meadows already exist, which are summarised in the “Guidelines for standardisation of mapping and monitoring methods of marine Magnoliophyta in the Mediterranean” (UNEP/MAP-RAC/SPA, 2015). These monitoring guidelines have been the base for the updating and harmonisation process undertaken in this document.

Detailed spatial information on habitat distribution is a prerequisite knowledge for a sustainable use of marine coastal areas. First step in the prior assessment of the status of any benthic habitat is thus the definition of its geographical distribution and bathymetrical ranges. Seagrass distribution maps are a fundamental prerequisite to any conservation action on these habitats. The available information on the exact geographical distribution of seagrass meadows is still fragmentary on a regional level (UNEP/MAP-RAC/SPA, 2015) and a few extent of the coastline has been mapped, as only 5 States out of the 21 have a mapped inventory covering at least half of their coasts (UNEP/MAP-Blue Plan, 2009). Within the framework of the Action Plan for the Conservation of Marine Vegetation in the Mediterranean, adopted in 1999 by the Contracting Parties to the Barcelona Convention (UNEP/MAP-RAC/SPA, 1999) and during the implementation evaluation of this Action Plan in 2005 (UNEP/MAP-RAC/SPA, 2005), emerged that very few countries were able to set up adequate and standardised monitoring and mapping programs. As a consequence, and following explicit request by managers on the need of practical guides aimed at harmonising existing methods for seagrass monitoring and for subsequent comparison of results obtained by different countries, the Contracting Parties asked the Regional Activity Centre for Specially Protected Areas (RAC/SPA) to improve the existing inventory tools and to propose a standardisation of the mapping and monitoring techniques for these habitats. Thus, the "Guidelines for standardisation of mapping and monitoring methods of marine Magnoliophyta in the Mediterranean" (UNEP/MAP-RAC/SPA, 2015) have been produced, as the result of a number of scientific round tables specifically addressed on this topic.

For mapping seagrass habitats, the previous Guidelines (UNEP/MAP-RAC/SPA, 2015) highlighted the following main findings:

- Several national and international mapping programs have already been carried out
- A standardisation and a clear consensus in the mapping methodology have been reached
- All the methods proposed are usable in all the Mediterranean regions, but some of them are more suitable for a given species (e.g., large-sized species) or particular assemblages (dense meadows)
- Implementation of procedures could be difficult in some regions due to the absence of training, competence and/or specific financing.

For monitoring the condition of seagrass habitats, the previous Guidelines (UNEP/MAP-RAC/SPA, 2015) highlighted the following main findings:

- Several national and international monitoring programs have been successfully implemented in the Mediterranean (e.g., SeagrassNet, Posidonia national monitoring networks)
- Notwithstanding most of the Mediterranean monitoring systems are mainly dedicated to *Posidonia oceanica*, there are some programs (e.g., SeagrassNet) that can be used for almost all seagrass species
- Although the existing monitoring methods are similar, the descriptors used to provide information on the state of the system are quite diverse and cover a vast array of ecological complexity levels (i.e., from the plant to the seascape)
- Some descriptors are used by all the Mediterranean scientific communities (e.g., seagrass density, lower limit depth) but the measuring techniques are often very different, and still require a larger effort to reach precise standardisation
- The different monitoring methods available in the Mediterranean countries seem all feasible when appropriate training is undertaken.

Based on recommendations from the previous CPs group meeting, SPA/RAC has been requested to develop an updated version of the Guidelines for monitoring marine vegetation in Mediterranean (UNEP/MAP-RAC/SPA, 2015), in the context of the IMAP common indicators and in order to ease the task of the MPA managers when implementing their monitoring programs. A reviewing process on the scientific literature, taking into account the latest techniques and the recent works carried out by the scientific community at the international level, has also been carried out.

Monitoring methods

a) COMMON INDICATOR 1: Habitat distributional range and extent

Approach

The CI1 is aimed at providing information about the geographical area in which seagrass meadows occur in the Mediterranean and the total extent of surfaces covered by meadows. The approach proposed for mapping seagrass meadows in the Mediterranean follow the overall procedure established for mapping marine habitats in the north-west Europe within the framework of the European MESH (Mapping European Seabed Habitats) project, ended in 2008. The mapping procedure includes different actions (Fig. 1), that can be synthesised into three main steps:

1) Initial planning
2) Ground surveys
3) Processing and data interpretation

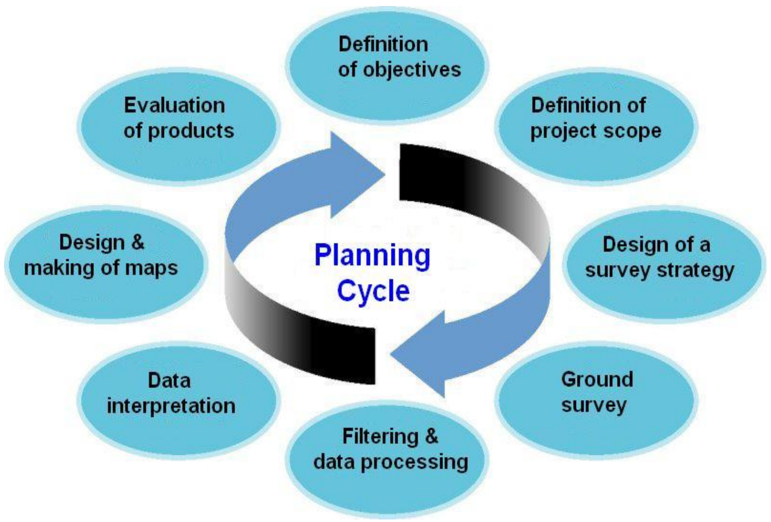

Figure 1: Planning cycle for a habitats' mapping programme (according to the MESH project, 2008).

Initial planning includes the definition of the objectives in order to select the minimum surface to be mapped and the necessary resolution. During this initial phase, tools to be used in the following phases must be defined and the effort (human, material, and financial costs) necessary to produce the mapping evaluated. A successful mapping approach requires the definition of a clear and feasible survey strategy.

Ground survey is the practical phase for data collection. It is often the costliest phase as it generally requires field activities. A prior inventory of the existing data for the area being mapped is recommended, to reduce the amount of work or to have a better targeting of the work to be done.

Processing and data interpretation are doubtlessly the most complex phase, as it requires knowledge and experience, so that the data gathered can be usable and reliable. The products obtained must be evaluated to ensure their coherence and the validity of the results obtained.

Resolution

Selecting an appropriate scale is a critical stage in the planning phase (Mc Kenzie et al., 2001). Even though there is no technical impossibility in using a high precision over large surface areas (or inversely), there is generally an inverse relationship between the precision used and the surface area to be mapped (Mc Kenzie et al., 2001; Fig. 2).

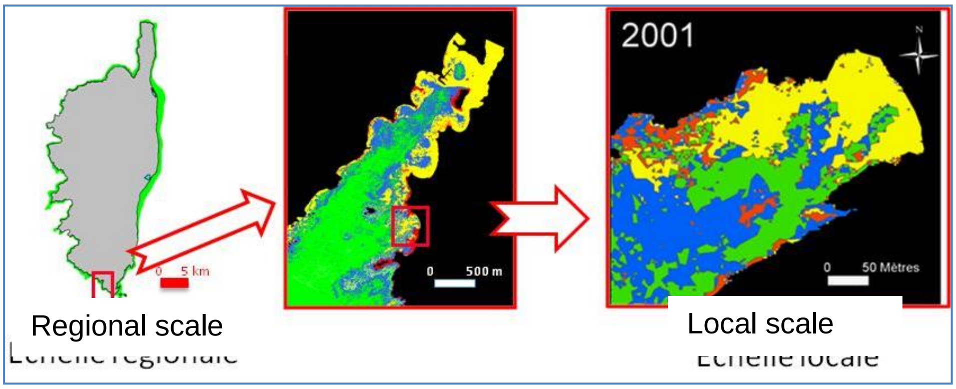

Figure 2: Resolution of a map from regional study to local study (from UNEP/MAP-RAC/SPA, 2015).

When large surface areas have to be mapped and global investigations carried out, an average precision and a lower detail level can be accepted, which means that the habitat distribution and the definition of its extension limits are often only indicative. Measures of the total habitat extent may be subjected to high variability, as the final value is influenced by the methods used to obtain maps and by the resolution during both data acquisition and final cartographic restitution. This type of approach is used for national or sub-regional studies and the minimum mapped surface area is 25 m² (Pergent et al., 1995a). Recently, some global maps showing the distribution of *Posidonia oceanica* meadows in the Mediterranean have been produced (Giakoumi et al., 2013; Telesca et al., 2015) (Fig. 3). These maps, however, are still incomplete being the available information highly heterogeneous due to the high variability in the mapping and monitoring efforts across the Mediterranean basin. This is especially true for the southern and the eastern coasts of the Mediterranean, where data are scarce, often patchy and can be difficultly found in literature. In data-poor regions, availability of high-quality mapping information on benthic habitat distribution is practically inexistent, due to limited resources. However, these low-resolution global maps can be very useful for an overall knowledge of the bottom areas covered by the plant, and to evaluate where surveys must be enforced in the future to collect missing data. Also, those maps are important to highlight specific areas subjected to a declining trend, where monitoring and management actions must be implemented to reverse the observed trend and to ensure proper conservation.

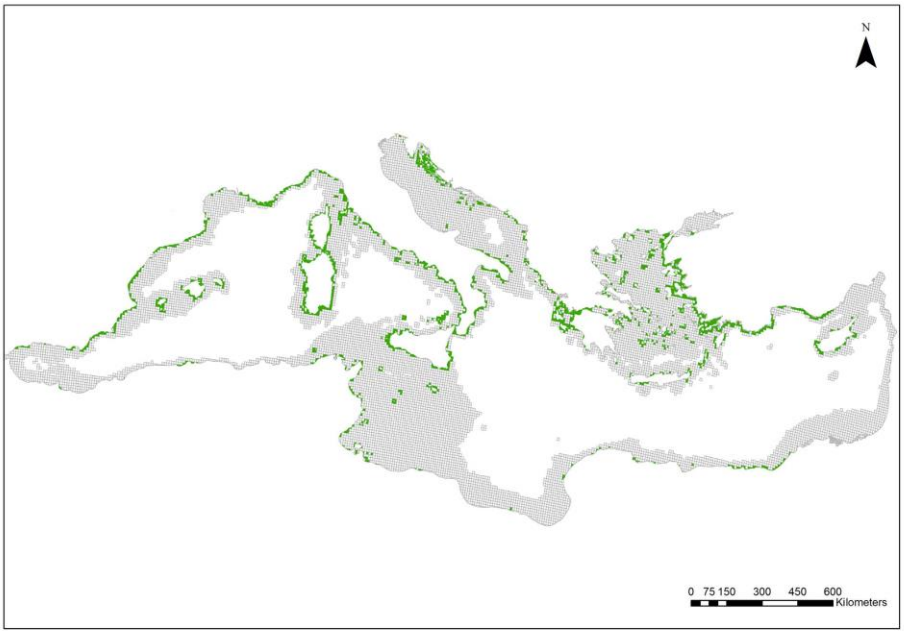

Figure 3: Distribution of *Posidonia oceanica* meadows in the Mediterranean Sea (green areas) (from Giakoumi et al., 2013).

On the contrary, when smaller areas have to be mapped, a much higher precision and resolution level is required and is easily achievable thanks to the high-resolution mapping techniques available to date. However, obtaining detailed maps is time consuming and costly, thus practically impossible when time or resources are limited (Giakoumi et al., 2013). The minimum surface area can be lower or equal to 1 m^2 in local scale studies (Pergent et al., 1995a). These detailed maps provide an accurate localisation of the habitat distribution and a precise definition of its extension limits and total habitat extent, all features necessary for future control and monitoring purposes over a period of time. These high-resolution scales are also used to select remarkable sites where monitoring actions must be concentrated. As highlighted by the MESH project (2008), most of the environment management and marine spatial planning activities require a range of habitat maps between these two extremes.

Methods

Maps of seagrass distribution and extent can be obtained by using indirect instrumental mapping techniques and/or direct field visual surveys (Tab. 1). In the last 50 years the technology in benthic habitat mapping has increased a lot, and several instrumental mapping techniques have been successfully applied to seagrass meadows (see synthesis in Pergent et al., 1995a; McKenzie et al., 2001; Dekker et al., 2006; Hossain et al., 2015). To map shallow meadows (from 0 to about 10-15 m depth, depending on water transparency and weather conditions), it is possible to use optical sensors (e.g., satellite telemetry, multi or hyper spectral imaging, aerial photography). For meadows in deeper waters (down to 10-15 m depth), the acoustic techniques (e.g., side scan sonar, multi-beam echosounder) are recommended. Sampling methods involving blind grabs, dredges and box corers or direct field visual surveys by scuba diving observations (using transects or permanent square frames), Remotely Operated Vehicles (ROVs), and underwater video recordings allow to ground-truthing the remote sensing data, and provide very high-resolution maps of meadows over small spatial scales (Montefalcone et al., 2006). All these techniques are, however, time consuming, expensive and provide only sporadic information. The simultaneous use of two or more methods makes it possible to optimize the results being the information obtained complementary. Four parameters can be mapped from remote sensing data: presence/absence, percentage cover, species, and biomass. The selection of the most relevant parameter in the scientific literature depended on the area mapped, the availability of ground truth data, and the specific target of each study (Topouzelis et al., 2018).

The use of remote sensing allows characterising extensive coastal areas for assessment of the spatial patterns of seagrass meadows, and simultaneously can be used to reveal temporal patterns due to the high frequency of the observation. Remote sensing covers a variety of technologies from satellite telemetry, aerial photography, and vessel acoustic systems. The power of remote sensing techniques has been highlighted by Mumby et al. (2004), who highlighted that 20 s of airborne acquisition time would equal 6 days of a field survey. However, all indirect mapping techniques are intrinsically affected by uncertainties due to manual classification of spectral or acoustic signatures of seagrass meadows on the images and sonograms, respectively. Errors in images or sonograms interpretation may arise when two habitat types are not easily distinguished by the observer (e.g., shallow seagrass meadows or dense patch of canopy-forming macroalgae). Interpretation of remote sensing data requires extensive field calibration and the ground-truthing process remains essential (Pergent et al., 2017). As the interpretation of images/sonograms is also a time-consuming and tedious task, several image processing techniques were proposed in order to rapidly automate the interpretation of sonograms and make this interpretation more reliable (Montefalcone et al., 2013 and references therein). These methods allow a good discrimination between soft sediments and seagrass meadows, between continuous and patchy seagrass, between a dense seagrass meadow and one exhibiting only limited bottom cover. Human eye, however, always remains the final judge.

Satellite telemetry is a valuable tool providing a cost-effective way to easily acquiring large-scale and high-resolution seagrass distribution information in shallow waters. Landsat images have been used successfully for regional mapping of seagrass distribution in many Mediterranean countries. The wide area coverage of satellite imaging might reveal large-scale patterns; however, mapping seagrass meadows from space on a large scale cannot provide the same levels of accuracy and detail of a direct field visual survey. Coupling a high-resolution digital camera with side scan sonar for acquiring underwater videos in a continuous way has recently proved to be a non-destructive and cost-effective method for ground-truthing in seagrass habitats mapping (Pergent et al., 2017).

Despite the increasing number of studies on seagrass mapping with remote sensing instruments, datasets are not often available in the geographic information systems (GIS) platform. As a final remark, only recently some modelling approaches have been developed to obtain estimation of the potential distribution of seagrass meadows in the Mediterranean. The probability of presence of the species in a given area has been modelled using: i) a binomial generalised linear model as a function of the bathymetry and water transparency, dissolved organic matter, sea surface temperature and salinity, mainly obtained from satellite data (Zucchetta et al., 2016); ii) morphodynamics features, i.e. wave, climate and seafloor morphology, to predict the seaward and landward boundaries of meadows (Vacchi et al., 2012, 2014).

Table 1: Synthesis of the main survey tools used for defining the Common Indicator 1_Habitat distributional range and extent for seagrass meadows. When available, the depth range, the surface area mapped, the spatial resolution, the efficiency (expressed as area mapped in km^2 per hour), the main advantages or the limits of each tool are indicated, with some bibliographical references.

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Satellite images	From 0 to 10-15 m	From few km^2 to large areas (over 100 km^2)	From 0.5 m	Over 100 km^2/hour	• A global and large-scale coverage of virtually all coastal areas • Availability of free digital images, usable without authorization, from the web (e.g., Google Earth) • High geometric resolution	• Limited to shallow waters characterization • Good weather conditions required (no clouds and no wind) • Possible errors in image interpretation among distinct habitats • Possible errors in image interpretation due to bathymetric variations	Kenny et al. (2003)
Multispectral and/or hyperspectral images	From 0 to 25 m, with an optimum up to 15 m	From 50 km^2 to 5000 km^2	From 1 m		• High resolution allowing to distinguish seagrass species • Possibility to collect data even during bad weather conditions	• Complex acquisition and processing procedures requiring the presence of specialists • Necessary to validate the observations with field data • Difficulty in habitat identification in the case of very patchy populations	Mumby and Edwards (2002); Mumby et al. (2004); Dekker et al. (2006); Gagnon et al. (2008);

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Aerial images	From 0 to 10-15 m	Adapted to small areas (10 km²), but it can be used for areas over 100 km²	From 0.3 m	Over 10 km²/hour	• Very high resolution • Manual, direct and easy interpretation of the images • Availability of libraries with chronological series of images (often free) • Good identification of boundaries between populations	• Same limits as for satellite images • Difficulty in geometrical corrections and strong deformations if verticality is not respected or if image covers a small area (low altitude view) • Difficulty in obtaining authorisations for imaging in some countries	Frederiksen et al. (2004); Kenny et al. (2003); Diaz et al. (2004)
Side scan sonar	Below 8 m	From large to medium areas (50-100 km²)	From 0.1 m	0.8 to 3.5 km²/hour	• Very high resolution • Realistic representation of the seafloor • Good identification of boundaries between populations • Good identification between meadows of different density • Quick execution	• Small patches (smaller than 1 m²) or low-density meadows cannot be distinguished • Loss of definition at image edge, requiring adjustments between adjacent profiles • Possible errors in image interpretation due to large signal amplitude variations (levels of grey)	Paillard et al. (1993); Kenny et al. (2003); Clabaut et al. (2006)
Single-beam acoustic sonar	Below 10 m		From 0.5 m	1.5 km²/hour	• Good geo-referencing • Quick execution	• Low discrimination between habitats • Lower reliability compared to satellite techniques	Kenny et al. (2003); Riegl and Purkis (2005)

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Multi-beam acoustic sonar	Below 2-8 m	From large to medium areas (50-100 km^2)	From 1 m	0.2 km^2/hour	• Possibility to obtain 3 D image of meadows • Data on biomass per surface area unit can be obtained	• Huge amount of rough data collected, needing very efficient computer systems for processing and archiving data • Complex data processing • Possible errors in image interpretation	Kenny et al. (2003); Komatsu et al. (2003)
Transect or permanent square frames (quadrates)	Depths easily accessible by scuba diving (0-40 m)	Small areas, usually between 25 m^2 to 100 m^2 for permanent square	From 0.1 m	0.01 km^2/hour	• Very high resolution and detail in the information collected • Possibility to identify small structures (patches) and to localize population boundaries • Ground-truthing of the remote sensing data • Possibility to do simultaneous monitoring	• Many working hours • Small areas mapped • Necessity of numerous observers to cover larger areas	Pergent et al. (1995a); Montefalcone et al. (2006)
Video camera (ROV or towed camera)	Whole bathymetric range of seagrass distribution	Small areas, usually under 1 km^2	From 0.1 m	0.2 km^2/hour	• Very high resolution • Easy to use • Possibility to record seafloor images for later interpretation	• Long time to gain and process data • Positioning errors due to gap between the vessel position and the camera when towed	Kenny et al. (2003); Diaz et al. (2004)

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Laser-telemetry	Depths easily accessible by scuba diving (0-40 m)	Small areas, under 1 km²	Some centimetres	0.01 km²/hour	• Very accurate localization of population boundaries or remarkable structures • Possibility to do simultaneous monitoring	• Range limited to 100 m in relationship to the base, and thus no possibility to work over large areas • Necessity for markers on seafloor for positioning of the base when monitoring over time is requested • Possible acoustic signal perturbation due to large variations in temperature or salinity • Specific training on the equipment is requested	Descamp et al. (2005)
GIB (GPS intelligent buoy)	Depths easily accessible by scuba diving (0-40 m)	Small areas, under 1 km²			• Same characteristics as for laser-telemetry, but with a greater range (1.5 km)	• Quite difficult technique • Need of many related equipments, and of team of divers	Descamp et al. (2005)

Once the surveying is completed, data collected needs to be organised so that it can be used in the future by everyone and can be appropriately archived and easily consulted. Resulting dataset can be integrated with similar data from other sources, providing a clear definition of all metadata (MESH project, 2008).

1) Optical data

Satellite images are gained from satellites in orbit around the earth. Data is obtained continuously and today it is possible to buy data that can reach a very high resolution (Tab. 2). It is also possible to ask for a specific programming of the satellite (programmed to pass over an identified sector with specific requirements), but this will require much higher costs.

The rough data must undergo a prior geometrical correction to compensate for errors due to the methods the images are obtained (e.g., errors of parallax, inclination of the satellite) before it can be used. Images already geo-referenced should also be obtained even if their cost is much higher than the rough data.

Table 2: Types of satellites and resolution of the sensors used for mapping seagrass meadows. n.a. = data not available.

Satellite	**Resolution**	**References**
Landsat 8	15 m	Dattola et al. (2018)
SPOT 5	2.5 m	Pasqualini et al. (2005)
IKONOS (HR)	1.0 m	Fornes et al. (2006)
QuickBird	0.7 m	Lyons et al. (2007)
Geoeyes	0.5 m	Amran (2017)

In view of the changes of the light spectrum depending on the depth, satellite telemetry can be used for mapping shallow meadows (see Tab. 1). In clear waters the maximum depths reached can be:

- With the blue channel up to approx. 20-25 m depth
- With the green channel up to 15-20 m
- With the red channel up to 5-7 m
- Channel close to the infra-red approx. from tens of centimetres up to 20 m.

Multispectral or hyperspectral imaging is based on images collected simultaneously and composed of numerous close and contiguous spectral bands (generally 100 or more). There is a wide variety of airborne sensors (e.g., CASI1[1], Deaedalus Airborne Thematic Mapper; Godet et al., 2009), which provide data in real time and also during unfavourable lighting conditions (Tab. 1). It is possible to create libraries with specific spectral responses, so that measured values can be compared to distinct component species and appraise the vegetation cover (Ciraolo et al., 2006; Dekker et al., 2006).

Aerial images obtained through various means (e.g., airplanes, drones, ULM) may have different technical characteristics (e.g., shooting altitude, verticality, optical quality). Even though it is more expensive, shooting films from a plane that is equipped with an altitude and verticality control system and using large size negatives (24 x 24) allows for high quality results (i.e., increase in the geometrical resolution). For example, on a photo at the scale 1/25000 the surface area covered is 5.7 km × 5.7 km

[1] CASI: Compact Airborne Spectrographic Imager

(Denis et al., 2003). In view of the progress made in the last few decades in terms of shooting (e.g., the quality of the film, filters, lens) and in following processing (e.g., digitalization, geo-referencing), aerial photographs represents today one of the most preferred surveying methods for mapping seagrass meadows (Mc Kenzie et al., 2001).

2) Acoustic data

Sonar provides images of the seafloor through the emission and reception of ultrasounds. Among the main acoustic mapping techniques, Kenny et al. (2003) distinguish: (1) wide acoustic beam systems like the side scan sonar (SSS), (2) single beam sounders (3) multiple narrow beam bathymetric systems, and (4) multi-beam sounders.

Side scan sonar tow-fish (transducer), with its fixed recorder, emits acoustic signals. The obtained images, or sonograms, visualize the distribution and the boundaries of the different entities over a surface area of 100 to 200 m along the pathway (Clabaut et al., 2006; Tab. 1). The resolution of the final map partly depends on the means of positioning used by the vessel (e.g., radio localisation or satellite positioning). The existence of a sonogram atlas (Clabaut et al., 2006) could be helpful in interpreting the data. Although this method has strong limitations in shallow waters (Tab. 1), a side scan sonar array able to efficiently map seagrass beds residing in 1 m or less of water has been recently developed (Greene et al., 2018).

Single-beam sounder is based on the simultaneous emission of two frequencies separated by several octaves (38 kHz and 200 kHz) to obtain the seafloor characterisation. The sounder’s acoustic response is different depending on whether the sound wave is reflected by an area covered or not covered by vegetation.

Multi-beam sounder may precisely and rapidly provide: (i) topographical images of the seafloor (bathymetry), (ii) sonar images representing the local reflectivity of the seafloor as a consequence of its nature (backscatter). The instrument simultaneously measures the depth in several directions, determined by the system’s receiver beams. These beams form a beam perpendicular to the axis of the ship. The seafloor can thus be explored over a wide band (5 to 7 times the depth) with a high degree of resolution. 3D structure of the seafloor is also obtained, where meadows can be visualized and the biomass can be evaluated (Komatsu et al., 2003).

3) Samplings and visual surveys

Field samples and direct observations provide discrete punctual data (sampling of distinct points regularly spread out in a study area). They are vital for ground-truthing the instrumental surveys, and for the validation of continuous information (complete coverage of surface areas) obtained from data on limited portions of the study area or along the pathway. Field surveys must be sufficiently numerous and distributed appropriately to obtain the necessary precision and also in view of the heterogeneity of the habitats. In the case of meadows of *Cymodocea nodosa*, *Posidonia oceanica*, *Zostera marina* or *Zostera noltei*, destructive sampling (using dredger buckets, core samplers, trawls, dredgers) are forbidden in view of the protected character of these species (UNEP/MAP, 2009) and direct underwater samples (e.g., shoot samples) should be limited as much as possible.

Observations from the surface can also be made by observers on a vessel using, for instance, a bathyscope, or by using imagery techniques such as photography and video. Photographic equipment and cameras can be mounted on a vertical structure (sleigh) or within remotely operated vehicle (ROV). The camera on a vertical structure is submerged at the back of the vessel and is towed by the vessel that advances very slowly (under 1 knot), whilst the ROVs have their own propulsion system and are remotely controlled from the surface.

The use of towed video cameras (or ROVs) during surveys makes it possible to see the images on the screen in real time, to identify specific features of the habitat and to evaluate any changes in the habitat or any other characteristic element of the seafloor, and this preliminary video survey may be also useful to locate sampling stations. Recorded images are then reviewed to obtain a cartographical restitution on a GIS platform for each of the areas surveyed. To facilitate and to improve the results obtained with the camera, joint acquisition modules integrating the depth, images of the seafloor and geographical positioning have been developed (UNEP/MAP-RAC/SPA, 2015).

In situ direct underwater observations by scuba diving represent the most reliable, although time-consuming, surveying technique. Surveys can be done along lines (transect), or over small surface areas (permanent square frames, i.e. quadrates) positioned on the seafloor and located to follow the limits of the habitat. The transect consists of marked lines wrapped on a rib and laid on the bottom from fixed points and in a precise direction, typically perpendicular or parallel with respect to the coastline (Bianchi et al., 2004). Any changes in the habitat and in the substrate typology, within a belt at both sides of the line (considering a surface area of about 1-2 m per side), are recorded on underwater slates (Fig. 4). The information registered allows precise and detailed mapping of the sector studied (Tab. 1).

Marking the limits of a meadow also allows obtaining a distribution map. Laser-telemetry is a useful technique for highly precise mapping surveying over small surface areas (Descamp et al., 2005). The GIB system (GPS Intelligent Buoys) consists of 4 surface buoys equipped with DGPS receivers and submerged hydrophones. Each of the hydrophones receives the acoustic impulses emitted periodically by a synchronized pinger installed on-board the underwater platform and records their times of arrival. Knowing the moment of emission of these signals and the sound propagation speed in the water, the distances between the pinger and the 4 buoys is directly calculated. The buoys communicate via radio with a central station (typically on-board a support vessel) where the position of the underwater target is computed and displayed. The depth is also indicated by the pressure sensor (Alcocer et al., 2006). To optimize meadows mapping operations, the pinger can be also fixed on a submarine scooter driven by a diver. The maximum distance of the pinger in relationship to the centre of the polygon formed by the 4 buoys can be approx. 1500 m (UNEP/MAP-RAC/SPA, 2015).

Free diving monitoring with a differential GPS can also be envisaged to locate the upper limits of the meadows. The diver follows precisely the contours of the limits and the DGPS continuously records the diver’s geographical data. The mapping data is integrated on a GIS platform using the route followed. The acquisition speed is 2-3 km/hour; the sensor precision can be sub metric (UNEP/MAP-RAC/SPA, 2015).

In situ direct underwater observations by scuba diving along transect.

Data interpretation

The MESH project (2008) identified four important stages for the production of a habitat map:

1. Processing, analysis and classification of the biological data, through a process of interpretation of acoustic and optical images when available
2. Selecting the most appropriate physical layers (e.g., substrate, bathymetry, hydrodynamics)
3. Integration of biological data and physical layers, and use of statistical modelling to predict seagrass distribution and interpolate information
4. The map produced must then be evaluated for its accuracy, i.e. its capacity to represent reality, and therefore its reliability.

During <u>the processing analysis and classification stage</u>, the updated list of benthic marine habitat types for the Mediterranean region should be consulted (UNEP/MAP-SPA/RAC, 2019) to recognize any specific habitat type (i.e., seagrass species). As seagrass assemblages are often small in size, they can only be identified with high (metric) precision mapping. The updated list identifies the specific “seagrass meadow” habitats that are also listed in the annex of the Habitats Directive (Directive 92/43/EEC), and which must be taken into consideration within the framework of the NATURA 2000 programs. A complete description of these habitats and the criteria for their identification are also available in Bellan-Santini et al. (2002). Habitats that must be reported on maps are the following (UNEP/MAP-SPA/RAC, 2019):

LITTORAL

MA3.5 Littoral coarse sediment

MA3.52 Mediolittoral coarse sediment

MA3.521 Association with indigenous marine angiosperms

MA3.522 Association with *Halophila stipulacea*

MA4.5 Littoral mixed sediment

MA4.52 Mediolittoral mixed sediment

MA4.521 Association with indigenous marine angiosperms

MA4.522 Association with *Halophila stipulacea*

MA5.5 Littoral sand

MA5.52 Mediolittoral sands

MA5.521 Association with indigenous marine angiosperms

MA5.522 Association with *Halophila stipulacea*

MA6.5 Littoral mud

MA6.52 Mediolittoral mud

MA6.52a Habitats of transitional waters (e.g. estuaries and lagoons)

MA6.521a Association with halophytes (*Salicornia* spp.) or marine angiosperms (e.g. *Zostera noltei*)

INFRALITTORAL

MB1.5 Infralittoral rock

MB1.54 Habitats of transitional waters (e.g. estuaries and lagoons)

MB1.541 Association with marine angiosperms or other halophyta

MB2.5 Infralittoral biogenic habitat

MB2.54 *Posidonia oceanica* meadows

MB2.541 *Posidonia oceanica* meadow on rock

MB2.542 *Posidonia oceanica* meadow on matte

MB2.543 *Posidonia oceanica* meadow on sand, coarse or mixed sediment

MB2.544 Dead matte of *Posidonia oceanica*

MB2.545 Natural monuments/Ecomorphoses of *Posidonia oceanica* (fringing reef, barrier reef, atolls)

MB2.546 Association of *Posidonia oceanica* with *Cymodocea nodosa* or *Caulerpa* spp.

MB2.547 Association of *Cymodocea nodosa* or *Caulerpa* spp. with dead matte of *Posidonia oceanica*

MB5.5 Infralittoral sand

MB5.52 Well sorted fine sand

MB5.521 Association with indigenous marine angiosperms

MB5.522 Association with *Halophila stipulacea*

MB5.53 Fine sand in sheltered waters

MB5.531 Association with indigenous marine angiosperms

MB5.532 Association with *Halophila stipulacea*

MB5.54 Habitats of transitional waters (e.g. estuaries and lagoons)

MB5.541 Association with marine angiosperms or other halophyta

MB6.5 Infralittoral mud sediment

MB6.51 Habitats of transitional waters (e.g. estuaries and lagoons)

MB6.511 Association with marine angiosperms or other halophyta

The selection of physical layers to be shown on maps and to be used for following predictive statistical analyses may be an interesting approach within the general framework of mapping seagrass habitats, and it would reduce the processing time, but it is still of little use for the Mediterranean meadows as only few of the classical physical parameters (e.g., substrate type, depth, salinity) are able to clearly predict the distribution of species (Fig. 5).

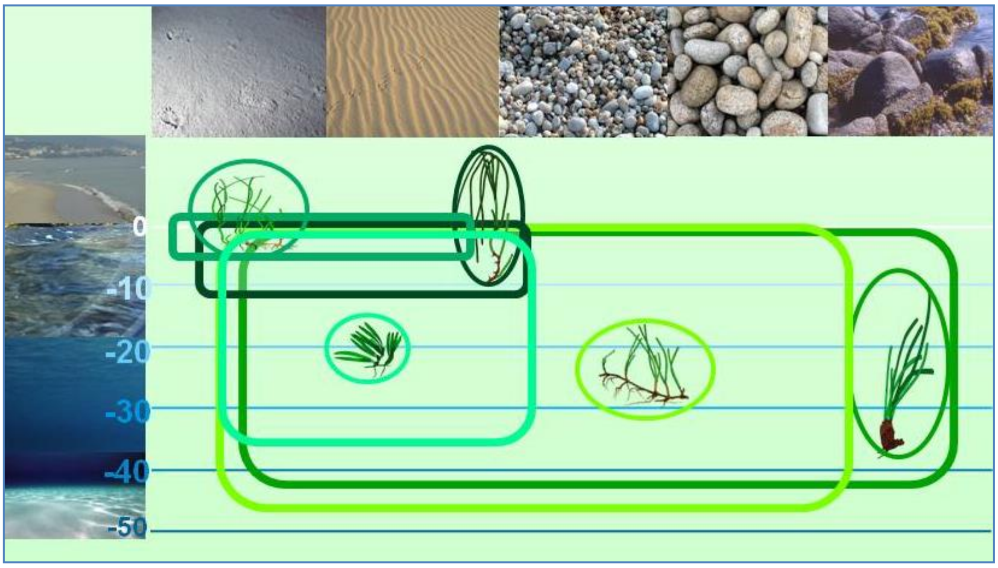

Figure 5: Distribution of seagrass species depending on the nature of the substrate and the depth in the Mediterranean (from UNEP/MAP-RAC/SPA, 2015).

The data integration and modelling stage will differ depending on the survey tools and acquisition strategy used. Due to its acquisition rapidity, aerial techniques usually allow to cover completely littoral and shallow infralittoral zones and this greatly reduces interpolation of data. On the contrary, surveys from vessels are often limited because of time and costs involved, and only rarely allow to obtain a complete coverage of the area. Coverage under 100% automatically means that it is impossible to obtain high resolution maps and therefore interpolation procedures have to be used, so that from partial surveys a lower resolution map can be obtained (MESH project, 2008; Fig. 6). Spatial interpolation is a statistical procedure for estimating data values at unsampled sites between actual data collection locations. Elaborating the final meadow distribution map on a GIS platform allows using different spatial interpolation tools (e.g., Inverse Distance Weighted, Kriging) provided by the software. Even though this is rarely mentioned, it is important to provide information on the number and the percentage of data acquired on field and the percentage of interpolations run.

An “overlapping” survey strategy combining a partial coverage of a large surface area and a more detailed coverage of smaller zones of particular interest could be an interesting compromise. Sometimes it might be enough to have a precise and detailed map only of the extension limits (upper and lower) of the meadow, and the presence between these two limits could be reduced to occasional field investigations leaving the interpolation to play its part (Pasqualini et al., 1998).

The processing and digital analysis of data (optical or acoustic) on GIS allows to creating charts where each tonality of grey is associated to a specific texture representing a type of population/habitat, also on the basis of in situ observations for ground-truthing. A final map is thus created where it is possible to identify the bare substrate, hard substrates and seagrass meadows. Specific processing (e.g., analysis of the roughness, filtering and thresholding) make additional information accessible, such as the seagrass cover or the presence of anthropogenic signs (Pasqualini et al., 1999).

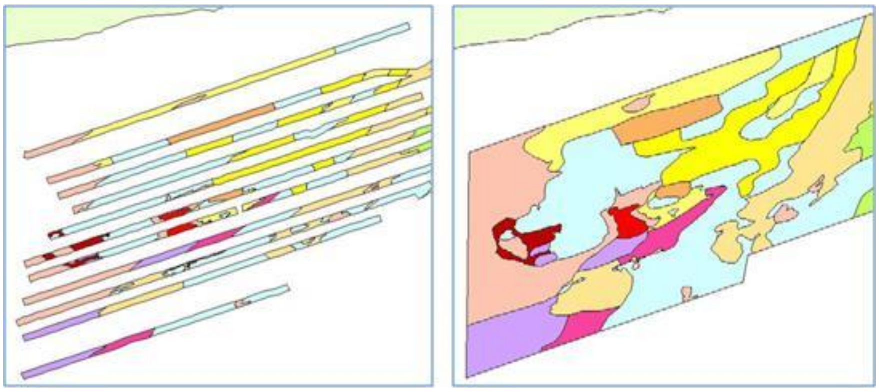

Figure 6: Example of partial coverage survey (left) and the output of the final map produced through interpolation (right). The area surveyed is about 20 km wide (from UNEP/MAP-RAC/SPA, 2015).

To facilitate a comparison among maps, standardised symbols and colours should be used for the graphic representation of the main seagrass assemblages (Meinesz and Laurent, 1978; Fig. 7). When the cartographical detail is good enough, it is possible to indicate also the discontinuous meadows that are characterised by a cover below 50% or the two main species that constitute a mixed meadow (the colour of the patches allows identification of the species concerned). To represent some typical forms of *Posidonia oceanica* meadows (e.g., striped, atolls) no specific symbols are available being these forms (bands and circular structures, respectively) easily identifiable on map.

On the resulting maps the seagrass habitat distributional range and its total extent (expressed in square meters or hectares) can be defined. These maps can be also compared with previous historical available data from literature to evaluate any changes experienced by meadow over a period of time (Mc Kenzie et al., 2001). Using the overlay vector methods on GIS, a diachronic analysis can be done, where temporal changes are measurements in term of percentage gain or loss of the meadow extension, through the creation of concordance and discordance maps (Barsanti et al., 2007).

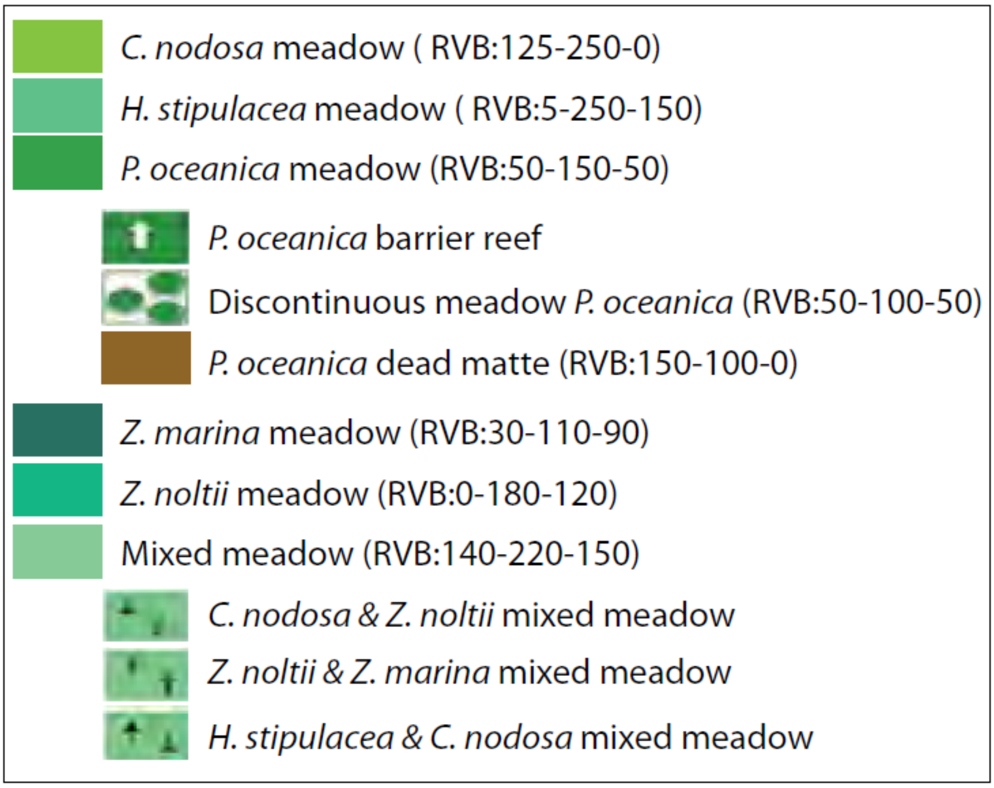

Figure 7: symbols and colours used for the graphic representation of the main seagrass assemblages. RVB: values in red, green and blue for each type of meadow (from UNEP/MAP-RAC/SPA, 2015).

The reliability of the map produced should also be evaluated. Several evaluation scales of reliability have already been proposed and may be useful for seagrass meadows. Pasqualini (1997) proposes a reliability scale in relation to the image processing of the aerial photos, which can also be applied to satellite images, or another scale in relation to the processing of sonograms (UNEP/MAP-RAC/SPA, 2015). Reliability lower than or equal to 50% means that the author should try to improve the reliability of the data (for example increasing the number of segments during image processing) or maybe that the scale needs to be adapted.

Denis et al. (2003) propose a reliability index of the cartographic data based on the map scale (scale of 5), the positioning system (scale of 5) and the acquisition method (scale of 10) (UNEP/MAP-RAC/SPA, 2015). The reliability index ranges from 0 to 20 and can vary from one point to another of the map, depending on the bathymetry or the technique used.

Leriche et al. (2001) proposed a reliability index rated from 0 to 50, which weighs three parameters: (i) the initial scale of the map (source map) and the working scale (target map), (ii) the method of data acquisition (e.g., dredges, grabs, aerial photography, side scan sonar, scuba diving), and (iii) the method of data georeferencing.

b) COMMON INDICATOR 2: Condition of the habitat's typical species and communities

Approach

Seagrasses have been used as biological indicators of the water quality according to the European Water Framework Directive (WFD, 2000/60/EC), and as indicators of the environmental quality (i.e., condition of the habitat) according to the MSFD (2008/56/EC) and the EcAp CI2 fixed by IMAP and related to "biodiversity" (EO1). The CI2 is aimed at providing information about the condition (i.e., ecological status) of seagrass meadows.

Monitoring the ecological status of seagrass meadows is today mandatory and is even an obligation for numerous Mediterranean countries due to the fact that:

- Four out of the five species present in the Mediterranean (*C. nodosa*, *P. oceanica*, *Z. marina*, and *Z. noltei*) are listed in the Annex 2 (list of endangered or threatened species) of the Protocol concerning Specially Protected Areas and Biological Diversity (Decision of the 16th Ordinary meeting of the Contracting Parties, Marrakech, 3-5 November 2009; UNEP/MAP, 2009)
- Three species (*C. nodosa*, *P. oceanica*, and *Z. marina*) are listed in the Annex 1 (strictly protected flora species) of the Bern Convention concerning the Mediterranean geographical region
- Seagrass meadows constitute are defined as priority natural habitats by the European Directive No. 92/43 (EEC, 1992).

This regulatory "recognition" also means that efficient management measures and conservation practices are required to ensure that these priority habitats, their constituent species and their associated communities are and remain in a satisfactory ecological status. The good state of health of seagrasses will then reflect the Good Environmental Status (GES) pursued by the Contracting Parties to the Barcelona Convention under the Ecosystem Approach (EcAp) and under the Marine Strategy Framework Directive (MSFD).

A defined and standardised procedure for monitoring the status of seagrass meadows, comparable to that provided for their mapping, should follow these three main steps:

1. Initial planning
2. Setting-up the monitoring system
3. Monitoring over time and analysis.

<u>The initial planning</u> is required to define the objective(s), determine the duration, identify the sites to be monitored, choose the descriptors to be evaluated with their acquisition modalities (i.e., the sampling strategy), and evaluate the human, technical and financial needs to ensure implementation and sustainability. This initial phase is therefore very important.

<u>The setting-up phase</u> is the concrete operational phase, when the monitoring program is set-up (e.g., positioning fixed markers) and realised. This phase may turn out to be most expensive, including costs for going out to sea during field activities, equipment for sampling, and human resources, especially under difficult weather conditions. Field activities must thus be planned during a favourable season, also because some of the parameters chosen for monitoring purposes must be collected during the same period. This phase might be quite long especially if numerous sites have to be monitored.

<u>Monitoring over time and data analysis</u> phase seem to be easy being the data acquisition a routine operation, with no major difficulties if the previous two phases had been carried out correctly. Data analysis needs clear scientific competence. Duration of the monitoring, in order to be useful, must be medium-time at least. This phase often constitutes the key element of the monitoring system as it makes it possible to:

- Interpret the acquired data
- Demonstrate its validity and interest
- Check that the monitoring objectives have been attained.

The objectives of the monitoring can cover the conservation of seagrass meadows and also their use as an ecological indicator of the quality of the marine environment. The main aims of seagrass monitoring are generally:

- Preserve and conserve the heritage of the priority habitats, with the aim of ensuring that the meadows are in a satisfactory ecological status (GES) and also identify as early as possible any degradation of these priority habitats or any changes in their distributional range and extent. Assessment of the ecological status of meadows allows to measure the effectiveness of local or regional policies in terms of management of the coastal environment
- Build and implement a regional integrated monitoring system of the quality of the environment, as requested by the Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP) during the implementation of the EcAp in the framework of the Mediterranean Action Plan. The main goal of IMAP is to gather reliable quantitative and updated data on the status of marine and coastal Mediterranean environment
- Evaluate effects of any coastal activity likely to impact seagrass meadows during environmental impact assessment procedures. This type of monitoring aims to establish the condition of the habitat at the time "zero" before the beginning of activities, then monitor the state of health of the meadows during the development works phase or at the end of the phase, to check for any impacts.

The objective(s) chosen will influence the choices in the following steps (e.g., duration, sites to be monitored, descriptors, sampling methods; Tab. 3). In general, and irrespective of the objective advocated, it is judicious to focus initially on a small number of sites that are easily accessible and that can be regularly monitored after short intervals of time (Pergent and Pergent-Martini, 1995; Boudouresque et al., 2000). The sites chosen must be: i) representative of the portion of the coastal area investigated (e.g., nature of the substrate), ii) cover most of the possible range of environmental situations, and iii) include sensitive zones, stable zones or reference zones. Then, with the experience gained by the surveyors and the means (funds) available, this network could be extended to a larger number of sites.

Table 3: Monitoring criteria depending on the objectives.

Monitoring objective	**Sites to be monitored**	**Descriptors**	**Monitoring duration and interval**
Heritage conservation	Sites with low anthropogenic pressures or reference sites (i.e., MPAs, Sites of Community Interest) to get information on the natural evolution of the environment	• Extent of the meadow and depths of their limits • Descriptors of the state of health of meadow (e.g., cover, shoot density)	• Medium and long term (min. 10 years) • Data acquisition at least annually for non-persistent species and 2-3 years for perennial species
Monitoring environmental quality	Identify the main anthropogenic pressures likely to affect the quality of the environment and initiate monitoring in at least 3 sites, 2 reference/control	• Descriptors of the quality of the environment (e.g., turbidity, depth of lower limit, enhancement in nutrients, nitrogen	• Medium term (5 to 8 years) • Data acquisition is variable depending on the species concerned (1-3 years)

	sites and 1 impacted site, all representative of the coastal area	content of leaves, chemical contamination, trace metals in plant)	
Environmental impact assessment	The site subject to coastal development or interventions. The selection of 2 reference/control sites might be also useful	• Specific descriptors to be defined depending on the possible consequences of human activities	• Short term (generally 1-2 years) • Initiate before the impact ("zero" time), it can be continued during, or just after the conclusion. A further control can be made one year after the conclusion

To ensure the sustainability of the monitoring system the following final remarks must be taken into account:

- Identify the partners, competences and means available
- Planning the partnership modalities (who is doing what? when? and how?)
- Ensure training for the stakeholders so that they can set up standardised procedures to guarantee the validity of the results, and so that comparisons can be made for a given site and among sites
- Individuate a regional or national coordinator depending on the number of sites concerned for monitoring and their geographical distribution
- Evaluate the minimum budget necessary for running the monitoring network (e.g., costs for permanent operators, temporary contracts, equipment, data acquisition, processing and analysis).

Methods

Descriptors basically provide information on the state of health of a meadow. A great number of descriptors has been proposed to assess the ecological status of seagrass meadow (e.g., Pergent-Martini et al., 2005; Foden and Brazier, 2007; Montefalcone, 2009; Orfanidis et al., 2010). Some of the most common descriptors (Tab. 4) use a standardised sampling method, especially for *P. oceanica* (Pergent-Martini et al., 2005), but there are still many disparities among data acquisition methods despite efforts to propose a common approach (Short and Coles, 2001; Buia et al., 2004; Lopez y Royo et al., 2010a). For each descriptor listed in Table 4, some bibliographic references are provided, where detailed descriptions of sampling tools and methodologies can be found.

The available descriptors work at each of the different ecological complexity levels of seagrass (Montefalcone, 2009): the population (i.e., the meadow), the individual (i.e., the plant), the physiological or cellular, and the associated community (especially leaves epiphytes). Some ecological indices (see next section) have been developed to work at the seascape ecological level (CI, Moreno et al., 2001; SI and PSI, Montefalcone et al., 2007; PI, Montefalcone et al., 2007) or at the ecosystem level (EBQI; Personnic et al., 2014). Some recent ecological indices integrate different ecological levels (e.g., PREI, Gobert et al., 2009; POMI, Romero et al., 2007).

Descriptors listed in Tab. 4 can be obtained using different methodologies and sampling approaches: i) on maps resulting from remote sensing surveys or visual inspections (e.g., meadow extent and depths of the limits); ii) in situ observation by scuba diving (e.g., lower limit type, cover, and rhizome baring); iii) direct sampling of plants (e.g., phenological descriptors). All methods requiring the direct sampling of plants for subsequent laboratory analyses are destructive, and thus the impact of the sampling procedure must be taken into account during the initial planning phase (Buia et al., 2004). Not-destructive procedures should be always preferred, especially in the case of protected species (e.g., *Posidonia* *oceanica*) and when the monitoring is carried out within MPAs. An effective monitoring should be done at intervals over a period of time, even if it could mean a reduced number of sites and a reduced number of descriptors being monitored. Number of adopted descriptors should be adequate enough to avoid errors of interpretation, but sufficiently reduced to ensure permanent monitoring. Simultaneous application of various descriptors working at different ecological complexity levels is the best choice to understand most of the possible responses of the system to environmental alterations (Montefalcone, 2009). The nature of the descriptors is less important than reproducibility, reliability and the precision of the method used for its acquisition.

In situ observation and samples must be done over defined and, possibly, standardised surface areas, and the number of replicates must be adequate for the descriptor involved and high enough to catch the heterogeneity of the habitat. The analyses at the individual (the plant), physiological or cellular, and most of the analyses associated at the community level (the associate organisms of leaves and rhizomes) require collection of shoots. For *P. oceanica* the mean number of sampled and measured shoots ranges between a minimum of 10 to a maximum of 20 shoots collected at each sampling station (Pergent-Martini et al., 2005). For measuring *P. oceanica* shoot density, a standardised surface area is settled at 40 cm × 40 cm with a minimum of 5 replicated counts per station. An adequate number of stations must be localised randomly within the meadow, and usually in correspondence of the meadow upper limit, the meadow lower limit and at intermediate depths, in a number of 2 to 3 sampling stations per depth. To assess the overall ecological condition of the meadow, samples of shoots can be performed only at the intermediate meadow depth, which is usually at about 15 m depth, where the meadow is expected to find the optimal conditions for its development (Buia et al., 2004) and during late spring or early summer season (Gobert et al., 2009).

Among all the descriptors listed in Table 4, the shoot density can be viewed as the most adopted standardised not-destructive descriptor in the *P. oceanica* monitoring programs (Pergent-Martini et al., 2005) (Fig. 8), because it provides important information about vitality and dynamic of the meadow and proves effective in revealing environmental alterations (Montefalcone, 2009). Following the requirements of the WFD in the European countries, the existing scales for its classification have been adapted with the creation of five classes (bad, poor, moderate, good, and high; Annex 1). This scale provides a tool to classify the ecological status of the meadow that can be used in the frame of the IMAP under the EcAp. Evaluating depth and typology of both the upper and the lower limits of the meadow and monitoring over time their positions with permanent marks (i.e., *balises*) are commonly adopted procedures to assess the evolution of the meadow in term of stability, improvement or regression that is linked to water transparency, hydrodynamic regimes, sedimentary balance and human activities along the coastline (Fig. 8). The classification scale of the lower limit depth (Annex 1) is another valid tool, although this scale could require some adaptations according to the specific geographical area and the morphodynamics setting of the site. For instance, in many *P. oceanica* meadows in the Ligurian Sea (NW Mediterranean) the lower limit rarely reaches depths greater than 20-25 m, due to natural constrains (e.g., substrate typology, seafloor topography). In all these cases, meadows would be classified from moderate to bad ecological status, even without or very few human pressures.

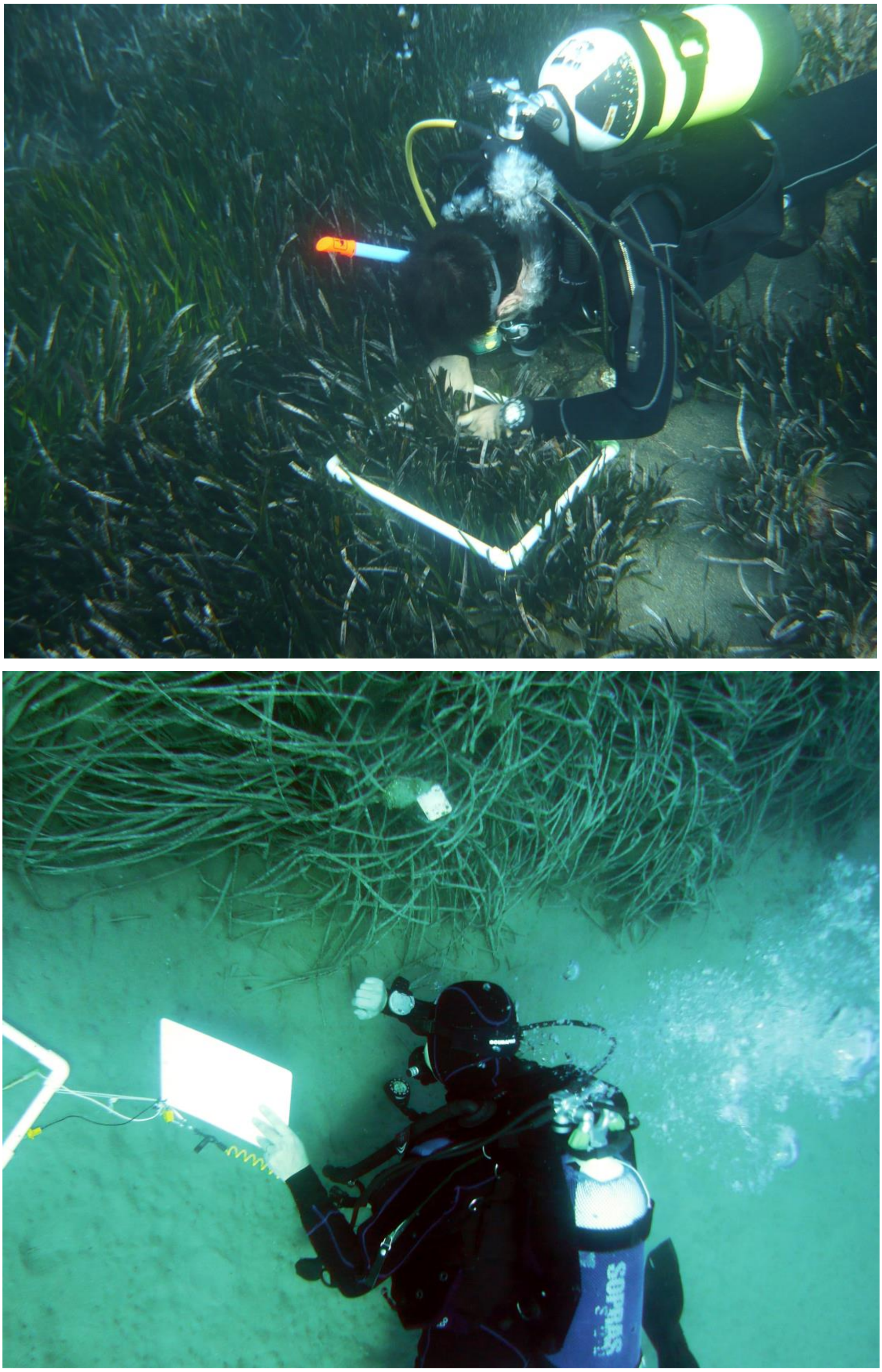

Figure 8: In situ measurement of *Posidonia oceanica* shoot density using the standard square frame of 40 cm × 40 cm (upper image) and monitoring over time of the meadow lower limit position with permanent marks (lower image).

Table 4: Synthesis of main descriptors used in seagrass monitoring used for defining the Common Indicator 2_Condition of the habitat. When available, the measuring/sampling method, the expected response in the case of increased human pressure and the main factors likely to affect the descriptor, the destructive nature of the method (Destr.), the target species, the advantages and limits, and some bibliographical references are provided. The target species are: Cn = *Cymodocea nodosa*, Hs = *Halophila stipulacea*, Po = *Posidonia oceanica*, Zm = *Zostera marina*, Zn = *Zostera noltei*. The complexity level at which each descriptor works is also indicated (i.e., population, plant, physiological or cellular, community).

Descriptor	Method	Expected response/factors	Destr.	Target species	Advantages	Limits	References
Population							
Meadow extent (i.e. surface area)	Mapping (Cf. Part “a” of this document) and/or identification of the position of limits	• Reduction of the total meadow extent • Coastal development, turbidity, mechanical impacts	No	All	• Informative of many aspects of the meadow • Usable everywhere in view of the many techniques available • Cover the whole depth range of meadow distribution	• For slow growing species (Po) needs of pre-positioning markers to evaluate change in meadow extent, and long response time (several years) • Sampling must be done during the season of maximum distribution for species with marked seasonal growth (generally in summer)	Foden and Brazier (2007)
Bathymetric position of meadow upper limit of (in m) and its morphology	A detailed mapping of seagrass extension limit landward (Cf. Part “a” of this document) or placing fixed markers (e.g., permanent blocks, acoustic system)	• Shift of the upper limit at greatest depths • Coastal development	No	All	• Easily measured (also by scuba diving) • Morphology of this limit may reflect environmental conditions	• For Cn, Hs and Zn, strong seasonal variability necessitating periodical monitoring or observations at the same season for all sites • Fixed markers might disappear if site is strongly frequented	Pergent et al. (1995); Montefalcone (2009)
Descriptor	**Method**	**Expected response/factors**	**Destr.**	**Target species**	**Advantages**	**Limits**	**References**

Bathymetric position of meadow lower limit of (in m)	A detailed mapping of seagrass extension limit seaward (Cf. Part "a" of this document) or placing fixed markers (e.g., permanent blocks, acoustic system)	• Shift of the lower limit landward at shallower depths • Turbidity	No	All	• Easily measured (also by scuba diving) • Classification scale available for Po	• For Cn, Hs and Zn, strong seasonal variability necessitating periodical monitoring or observations at the same season for all sites • Beyond 30 m depth, acquisition is difficult and costly (limited diving time, need for experienced divers, numerous dives requested) • Fixed markers (balises) might disappear (e.g., trawling) • For slow growing species (Po) long time required to see any progress (several years)	Pergent et al. (2008); Annex 1
Meadow lower limit type	In situ observations	• Change in morphology • Turbidity, mechanical impacts (e.g., trawling)	No	Po	• Well known descriptor • Several types described • Classification scale for Po	• Good knowledge of Po meadows necessary to identify some of the types • Difficult and costly the assessment at great depths (>30 m)	Boudouresque and Meinesz (1982); Pergent et al. (1995); Montefalcone (2009); Annex 1
Presence of inter-matte channels and dead matte areas	Highly detailed mapping of the area (Cf. Part "a" of this document, permanent square frames) and/or in situ observations	• Increase in the extent • Mechanical impacts (e.g., anchoring, fishing gear)	No	Po	• Easy to measure • Surface areas can be measured on maps	• Dead matte areas are natural components intrinsic to some types of meadows (e.g., striped meadows) and do not reflect systematically human influence	Boudouresque et al. (2006)

Descriptor	Method	Expected response/factors	Destr.	Target species	Advantages	Limits	References
Density (shoots · m^{-2})	No. of shoots counted within a square frame (fixed dimension and depth) by divers. The square size depends on the species meadow density. For *P. oceanica* is 40 cm x 40 cm	• Reduction • Turbidity, mechanical impacts (e.g., anchoring)	No	All	• Easy to measure • Low-cost • Can be measured at all depths • Classification scale available for Po	• Strong variability with depth • Long acquisition time for densities over 800 shoots • Many replicates necessary to evaluate meadow heterogeneity • Considerable risk of error if: a) surveyor is inexperienced; b) high density; c) small sized species. In this latter case in situ counting can be replaced by sampling over a given area and the counting can be done in the lab. (destructive technique)	Duarte and Kirkman (2001); Pergent-Martini et al. (2005); Pergent et al. (2008); Annex 1
Cover (in %)	Average percentage of the surface area occupied (in vertical projection) by meadow in relation to the surface area observed. Various methods to measure the cover in situ by divers or in lab. (photos or video, visual estimation). Variable observation surface area (0.16 to 625 m²), visualised by quadrate or transparent plate	• Reduction • Turbidity	No	All	• Rapid • On photos, possibility of comparison over time and less errors due to subjectivity • All depths • Estimated also from aerial images or sonograms at large scale	• Strong seasonal and bathymetric variability • Comparison of data obtained using different methods and different observation surface areas is not always reliable due to the fractal nature of cover • Sampling strategy and design must include proper spatial variability • High subjectivity of in situ estimations	Buia et al. (2004); Pergent-Martini et al. (2005); Boudouresque et al. (2006); Romero et al. (2007); Montefalcone (2009)
Descriptor	**Method**	**Expected response/factors**	**Destr.**	**Target species**	**Advantages**	**Limits**	**References**

Percentage of plagiotropic rhizomes	Counting of plagiotropic rhizomes in a given surface area (e.g., 40 cm x 40 cm, which can be visualised by a quadrate)	• Increase • Mechanical impacts (e.g., anchoring, fishing gear)	No	Cn, Po	• Easy, rapid and low-cost • Classification scale available for Po	• Mainly used at shallow depths (0-20 m)	Boudouresque et al. (2006); Annex 1
Plant							
Leaves surface area (cm² · shoot), and other phenological measures	Counting and measuring the length and width of different types of leaves in each shoot (10 to 20 shoots)	• Reduction of leaves surface area (Po) for overgrazing and human impacts • Increase in the length of leaves (Po, Cn) for nutriments enhancement	Yes	All	• Easy, rapid and low-cost • Possibility to measure the length of adult leaves (most external leaves) in situ to avoid sampling • Classification scale available for Po	• Strong seasonal variability • Strong individual variability and necessity to measure (and sample) an adequate number of shoots	Giraud (1977, 1979); Lopez y Royo et al. (2010b); Orfanidis et al. (2010); Annex 1
Necrosis on leaves (in %)	Percentage of leaves with necrosis, through observation in lab.	• Increase • Increased contaminants concentration	Yes	Po	• Easy, rapid and low-cost	• Necrosis is very rare in some sectors of the Mediterranean (e.g., Corsica littoral)	Romero et al. (2007)
State of the apex	Percentage of leaves with broken apex	• Increase • Overgrazing, mechanical impacts (e.g., anchoring)	No	Po	• Easy, rapid and low-cost • Specific marks of the bit of some animals are easily recognisable	• Not informative of the grazing pressure in the case of strong hydrodynamism and on old leaves	Boudoresque and Meinesz (1982)

Descriptor	Method	Expected response/factors	Destr.	Target species	Advantages	Limits	References
Foliar production (in mg dry weight · shoot^{-1}yr^{-1})	For Po possibility, thanks to lepidochronology, to reconstruct number of leaves produced in one year, at present or in the past. For other species, measuring leaves through markings or by using the relationship bases length/leaves growth (Zm)	• Reduction • Nutrients deficit, increase in interspecific competition	Yes/ No (Zm)	All	• For Po lepidochronology allows assessments at all depths • Classification scale available • For Zm the relationship bases length/leaves growth allows in situ non destructive measuring	• Long time to acquire • Monthly monitoring, or at least for 4 seasons is necessary	Pergent (1990); Gaeckle et al. (2006); Pergent et al. (2008)
Rhizome production (in mg dry weight · shoot^{-1} yr^{-1}) or elongation (in mm yr^{-1})	For Po possibility, thanks to lepidochronology, to reconstruct rate of growth or biomass per year	• Increase • Accumulation of sediments due to coastal development	Yes	Po	• Independent from season • Classification scale available for Po	• Interpretation sometimes difficult as rhizome production increase can be also observed in reference sites in the absence of human impacts	Pergent et al. (2008); Annex 1
Burial or baring of the rhizomes (in mm)	Measuring the degree of burial or baring of rhizomes in situ, or the percentage of buried or bared shoots on a given surface area	• Increase in burial for increased sedimentation (e.g., coastal development, dredging) • Increase in baring for deficit in the sediment load	No	All	• Easy to measure in situ • Not destructive and low-cost • Independent from season		Boudoresque et al. (2006)

Descriptor	Method	Expected response/factors	Destr.	Target species	Advantages	Limits	References
Physiological or cellular							
Nitrogen and phosphorus content in plant (in % dry weight)	Dosage through mass spectrometry and plasma torch in different plant tissues after acid mineralisation (e.g., rhizomes for Po)	• Increase • Nutriments enhancement	Yes	All	• Short response time to environmental changes • Classification scale for Po	• Very expensive • Analytical equipment and specific competence necessary	Romero et al. (2007); Annex 1
Carbohydrate content (in % dry weight) in plant and sediments	Dosage through spectrophotometry after alcohol extraction in different plant tissues (e.g., rhizomes for Po)	• Reduction • Human impacts	Yes	All	• Short response time to environmental changes • Classification scale for Po	• Very expensive • Analytical equipment and specific competence necessary	Alcoverro et al. (1999, 2001); Romero et al. (2007); Annex 1
Trace metal content (in $\mu g \cdot g^{-1}$)	Dosage through spectrometry in different plant tissues after acid mineralisation	• Increase • Increased concentration of metallic contaminants	Yes	All	• Short response time to environmental changes • Classification scale for Po	• Very expensive • Analytical equipment and specific competence necessary	Salivas-Decaux (2009); Annex 1
Nitrogen isotopic relationship ($d^{15}N$ in ‰)	Dosage through mass spectrometer in different plant tissues after acid mineralisation (e.g., rhizomes for Po)	• Increase for nutriments enhancement from farms and urban effluents • Reduction for nutriments enhancement from fertilizers	Yes	Po	• Short response time to environmental changes	• Very expensive • Analytical equipment and specific competence necessary	Romero et al. (2007)
Sulphur isotopic relationship ($d^{34}S$ in ‰)	Dosage through mass spectrometer in different plant tissues (e.g., rhizomes of Po)	• Reduction • Human impacts	Yes	Po	• Short response time to environmental changes	• Very expensive	Romero et al. (2007)

						• Analytical equipment and specific competence necessary	
Descriptor	**Method**	**Expected response/factors**	**Destr.**	**Target species**	**Advantages**	**Limits**	**References**
Community							
Epiphytes biomass (in mg dry weight · shoots^{-1} or % dry weight · shoots^{-1}) and epiphytes cover (in %) of leaves	• Measure of biomass (µg · shoots-1) after scraping, drying and weighing • Measure of nitrogen content (in % dry weight) • Measure using simple CHN analyser • Estimate the epiphytes cover on leaves under a binocular • Indirect estimation of biomass from epiphytes cover	• Increase • Nutriments enhancement from rivers, high touristic frequentation	Yes	All	• Easy to measure • Low-cost (biomass and cover) • Classification scale available for Po • Early-warning indicator	• Time-consuming • Strong seasonal and spatial variability • Specific analytical equipment (nitrogen content) necessary	Morri (1991); Pergent-Martini et al. (2005); Romero et al. (2007); Fernandez-Torquemada et al. (2008); Giovannetti et al. (2008, 2015)

The setting-up phase is the concrete operational phase of the monitoring program that starts with the data acquisition. The observations and samplings during the acquisition phase or data validation of the cartographical surveys, could also constitute an output of a monitoring system (Kenny et al., 2003), and cartography could also represent a monitoring tool (Tab. 4; Boudouresque et al., 2006).

At the regional spatial scale, two main monitoring systems have been developed: 1) the seagrass monitoring system (SeagrassNet), which was established at the worldwide scale at the beginning of the year 2000 and covers all the seagrass species (Short et al., 2002); and 2) the “Posidonia” monitoring network started at the beginning of the 1980s in the Mediterranean (Boudouresque et al., 2006), which is specific to *Posidonia oceanica* but can be adapted to other Mediterranean species and to the genus *Posidonia* worldwide. The “Posidonia” monitoring network is still used today, with a certain degree of variability from one country to another and even more from a region to another, in at least nine Mediterranean countries and in over 350 sites (Buia et al., 2004; Boudouresque et al., 2006, Romero et al., 2007; Fernandez-Torquemada et al., 2008; Lopez y Royo et al., 2010a). After the work carried out within the framework of the Interreg IIIB MEDOCC programme “Coherence, development, harmonization and validation of evaluation methods of the quality of the littoral environment by monitoring the *Posidonia oceanica* meadows”, and the “MedPosidonia” programme set up by RAC/SPA, an updated and standardised approach for the *P. oceanica* monitoring network has been tested and validated (UNEP/MAP-RAC/SPA, 2009). The main differences between the former two monitoring systems are:

- Within the framework of SeagrassNet, monitoring is done along three permanent transects, laid parallel to the coastline and positioned respectively (i) in the most superficial part of the meadow, (ii) in the deepest part and (iii) at an intermediate depth between these two positions. The descriptors chosen (Short et al., 2002; Tab. 5) are measured at fixed points along each transect and every three months.
- Within the framework of the “Posidonia” monitoring network, measurements are taken (i) in correspondence of fixed markers placed along the lower limit of the meadow, (ii) at the upper limit, and (iii) at the intermediate and fixed depth of 15 m. The descriptors (Tab. 5) are measured every three years only if, after visual surveys, no visible changes in the geographical position of the limits are observed.

SeagrassNet allows to comparing the data obtained in the Mediterranean with the data obtained in other regions of the world, having world coverage of over 80 sites distributed in 26 countries (www.seagrassnet.org). However, this monitoring system is not suitable for large-size species (such as *Posidonia* genus) and for meadows where lower limit is located beyond 25 m depth. This monitoring system has been set up only for one site in the Mediterranean (Pergent et al., 2007). The “Posidonia” monitoring network, in view of the multiplicity of descriptors identified (Tab. 5), allows to compare different meadows in the Mediterranean and also to evaluate the plant’s vitality and the quality of the environment in which it grows. Other monitoring systems, such as permanent transect with seasonal monitoring, or acoustic surveys, can be used in particular situations like the monitoring of lagoons environments (Pasqualini et al., 2006) or for the study of relict meadows (Descamp et al., 2009).

The sampling technique and the chosen descriptors define the nature of the monitoring (e.g., monitoring of chemical contamination of the environment, discharge into the sea from a treatment plant, effects of beach nourishments, general evaluation of the meadow state of health) (Tab. 4). There are no ideal methods for mapping or universal descriptors for the monitoring of seagrass meadows, but rather a great diversity of efficient and complementary tools. They must be chosen depending on the objectives, the species present and the local context. Independently from the descriptors selected, particular attention must be paid to the validity of the measurements made (acquisition protocol, precision of the measurements, reproducibility; Lopez y Royo et al., 2010a). The following data processing and interpretation phase is thus fundamental to ensure the good quality of the monitoring programme.

Table 5: Descriptors measured within the framework of the SeagrassNet, the “Posidonia” monitoring Network and the MedPosidonia monitoring programs (Pergent et al., 2007).

Descriptors	**SeagrassNet**	**“Posidonia” monitoring Network**	**MedPosidonia**
Light	x		
Temperature	x		x
Salinity	x		
Lower limit	Depth	Depth, type and cartography	Depth, type and cartography
Upper limit	Depth	Depth, type and cartography	Cartography
Density	12 measurements along each transect	Measurement at each of the 11 markers	Measurement at each of the 11 markers
% Plagiotropic rhizomes		Measurement at each of the 11 markers	Measurement at each of 11 markers
Baring of rhizomes		Measurement at each of the 11 markers	Measurement at each of the 11 markers
Cover	12 measures along transect	At each marker using video (50 m)	Measurement at each of the 11 markers
Phenological analysis	12 measures along transect	20 shoots	20 shoots
Lepidochronological analysis		10 shoots	10 shoots
State of the apex		20 shoots	20 shoots
Biomass (g DW)	Leaves		
Necromass	Rhizome and scales		
Granulometry of sediments		1 measurement	1 measurement
% organic material in sediment		1 measurement	1 measurement
Trace-metal content			Ag and Hg

Data processing and interpretation

Measurements made in situ must be analyzed and archived. Samples collected during field activities must be properly stored for following laboratory analyses. Data interpretation needs expert judgment and evaluation and can be made by comparing the measured data with the data available in the literature, either directly or through scales. Checking that the results obtained respond to the monitoring objectives (reliability and reproducibility of the results, valid interpretations and coherence with the observations made) is another important step to validate monitoring effectiveness.

The huge increase of studies on *Posidonia oceanica* (over 2400 publications indexed in the Web of Science) means that in the last few decades a growing number of interpretation scales have been set up for the most widely used descriptors for monitoring this species (e.g., Giraud, 1977; Meinesz and Laurent, 1978; Pergent et al., 1995b; Pergent-Martini et al., 2005; Montefalcone et al., 2006, 2007; Salivas-Decaux et al., 2010; Montefalcone, 2009; Tab. 4).

As for cartography, an integration of the monitoring data into a geo-referenced information system (GIS), which can be freely consulted (like MedGIS implemented by RAC/SPA), is to be recommended and should be encouraged, so that the data acquired becomes available to the wider public and can be of benefit to the maximum number of users.

Ecological indices

Ecological synthetic indices are today widespread for measuring the ecological status of ecosystems in view of the Good Environmental Status (GES) achievement or maintenance. Ecological indices succeed in ‘‘capturing the complexities of the ecosystem yet remaining simple enough to be easily and routinely monitored’’ and may therefore be considered ‘‘user-friendly’’ (Montefalcone, 2009 and references therein). They are anticipatory, integrative, and sensitive to stress and disturbance. Many ecological indices had been employed in the seagrass monitoring programs, e.g. the Leaf Area Index (Buia et al., 2004), the Epiphytic Index (Morri, 1991). Following the requirements of the WFD in the European countries, many synthetic indices have been set up to provide, on the basis of a panel of different descriptors, a global evaluation of the environmental quality based on the “seagrass” biological quality element. The most adopted indices in the regional/national monitoring programs are the following (Table 6):

- POSWARE (Buia et al., 2005)
- POMI (Romero et al., 2007)
- POSID (Pergent et al., 2008)
- Valencian CS (Fernandez-Torquemada et al., 2008)
- PREI (Gobert et al., 2009)
- BiPo (Lopez y Royo et al., 2009)
- Conservation Index (CI) (Moreno et al., 2001)
- Substitution Index (SI) (Montefalcone et al., 2007)
- Phase Shift Index (PSI) (Montefalcone et al., 2007)
- Patchiness Index (PI) (Montefalcone et al., 2010)
- EBQI (Personnic et al., 2014)

Most of the ecological indices integrate different ecological levels (Table 6). The POSWARE index is based on 6 descriptors working at the population and individual level. The multivariate POMI index is based on a total of 14 structural and functional descriptors of *Posidonia oceanica*, from cellular to community level. The POSID index is based on 8 descriptors working at the community, population, individual and cellular level. Some of the descriptors working at the cellular level and used for computing the POMI and the POSID index are very time-consuming (such as the chemical and biochemical composition and the contaminants), thus showing little usage in the *P. oceanica* monitoring programs (Pergent-Martini et al., 2005). The Valencian CS index integrates 9 descriptors from individual to community level. The PREI index is based on 5 descriptors working at the population, individual and community level. The BiPo index is based only on 4 non-destructive descriptors at the population and individual level and is particularly well suited for the monitoring of protected species or within MPAs.

Some not-destructive ecological indices have been developed to work at the seascape ecological level, such as the CI (Moreno et al., 2001) the SI and PSI (Montefalcone et al., 2007) and the PI (Montefalcone et al., 2007). The CI measures the proportional abundance of dead matte relative to living *P. oceanica* and can be used as a perturbation index (Boudouresque et al., 2006), although dead matte areas may also originate from natural causes (e.g., hydrodynamism). The SI has been proposed for measuring the amount of replacement of *P. oceanica* by the other common native Mediterranean seagrass *Cymodocea nodosa* and by the three species of green algae genus *Caulerpa*: the native *Caulerpa prolifera* and the two alien invaders *C. taxifolia* and *C. cylindracea*. The SI, applied repeatedly in the same meadow, can objectively measure whether the substitution is permanent or progressive or, as hypothesized by Molinier and Picard (1952), will in the long term facilitate the reinstallation of *P. oceanica*. While the application of the CI is obviously limited to those seagrass species that form a matte, the SI can be applied to all cases of substitution between two different seagrass species and between an alga and a seagrass. PSI is another synthetic ecological index that identifies and measures the intensity of the phase shift occurring within the seagrass ecosystem; it provides a synthetic evaluation of the irreversibility of changes undergone by a regressed meadow. The biological characteristics and the reproductive processes of *P. oceanica* are not conducive to a rapid re-colonisation of dead matte (Meinesz et al., 1991). If a potentiality of recovery still exists in a meadow showing few and small dead matte areas, a large-scale regression of *P. oceanica* meadow must therefore be considered almost irreversible on human-life time scales. All these indices are useful tools for assessing the quality of coastal environments in their whole, not only for assessing the quality of the water bodies. The PI has been developed to evaluate the level of fragmentation of the habitat and used the number of patches for measuring the fragmentation of seagrass meadows.

One of the most recently proposed index works at the ecosystem level (EBQI; Personnic et al., 2014). This index has been developed on the basis of a simplified conceptual model of the *P. oceanica* ecosystem, where a set of 17 representative functional compartments have been identified. The quality of each functional compartment is then evaluated through the selection of one or two specific descriptors (most of them not-destructive) and the final index value integrates all compartment scores. Being an ecosystem-based index, it complies with the MSFD and the EcAp requirements. However, its complete and thus complex formulation makes this index more time-consuming when compared to other indices.

Intercalibration trials between the POMI and the POSID indices have shown that there is coherence in the classification of the five sites studied (with the Corsican sites showing a higher classification than the Catalonia sites) (Pergent et al., 2008). Applying the BIPO index to 9 Mediterranean sites yields an identical classification of the Catalonia sites as the classification obtained with the POMI index (Lopez y Royo et al., 2010c). Finally, using both the POSID and the BiPo indices within the framework of the “MedPosidonia” programme a similar classification of the meadows studied was found (Pergent et al., 2008). A recent exercise to compare a number of descriptors and ecological indices at different ecological levels (individual, population, community and seascape) in 13 *P. oceanica* meadows of the Ligurian Sea (NW Mediterranean) showed a low consistency among the four levels, and especially between the plant (e.g., leaves surface) and the meadows (e.g., shoot density, lower limit depth) descriptors. Also, the PREI index showed inconsistency with most of the descriptors (Karayali, 2017). In view of this result, the combined use of more descriptors and indices, covering different levels of ecological complexity, should be preferred in any monitoring program.

At the present state of knowledge, it is difficult to prefer one or another of these synthetic indices, as it has not yet been possible to compare all of them on a single site. As a general comment, those indices based on a high number of descriptors imply excessive costs in terms of acquisition time and the budget required (Fernandez-Torquemada et al., 2008).

Table 6: Descriptors used in the synthetic ecological indices mostly adopted in the regional/national monitoring programs to evaluate environmental quality based on the "seagrass" biological quality element.

Index	Cellular	Individual	Population	Community	Ecosystem	Seascape
POSWARE		Width of the intermediate leaves; leaves production; rhizomes production and elongation	Shoot density; meadow cover			
POMI	P, N and sucrose content in rhizomes; $\delta^{15}N$ and $\delta^{34}S$ isotopic ratio in rhizomes; Cu, Pb, and Zn content in rhizomes	Leaves surface; percentage foliar necrosis	Shoot density; meadow cover; percentage of plagiotropic rhizomes	N content in epiphytes		
POSID	Ag, Cd, Pb, and Hg content in leaves	Leaves surface; Coefficient A; rhizomes elongation	Shoot density; meadow cover; percentage of plagiotropic rhizomes; depth of the lower limit	Epiphytes biomass		
Valencian CS		Leaves surface; percentage of foliar necrosis	Shoot density; meadow and dead matte cover; percentage of plagiotropic rhizomes; rhizome baring/burial	Herbivore pressure; leaf epiphytes biomass		
PREI		Leaves surface; leaves biomass	Shoot density; lower limit depth and type	Leaf epiphytes biomass		
BiPo		Leaves surface	Shoot density; lower limit depth and type			
CI			Meadow and dead matte cover			Relative proportion between *Posidonia*

						oceanica and dead matte
SI			Meadow cover	Substitutes cover		Relative proportion between *P. oceanica* and substitutes
PSI			Meadow and dead matte cover	Substitutes cover		Relative proportion of *P. oceanica*, dead matte and substitutes
PI						Number of seagrass patches
EBQI		Growth rate of vertical rhizomes	Shoot density; meadow cover		Biomass, density and species diversity in all the compartments; grazing index	

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Annex 1 – Classification scales of the ecological status available in literature for some descriptors of *Posidonia oceanica* meadow

Meadow (population level)

Type of the lower limit (UNEP/MAP-RAC/SPA, 2009)

	High	**Good**	**Moderate**	**Poor**	**Bad**
Lower limit	Progressive	Sharp HC	Sharp LC	Sparse	Regressive

Type of the limit	**Main characteristics**
Progressive	Plagiotropic rhizome beyond the limit
Sharp – High cover (HC)	Sharp limit with cover higher than 25%
Sharp – Low cover (LC)	Sharp limit with cover lower than 25%
Sparse	Shoot density lower than 100 shoots · m^{-2}, cover lower than 15%
Regressive	Dead matte beyond the limit

Depth of the lower limit (in m) (UNEP/MAP-RAC/SPA, 2009)

	High	**Good**	**Moderate**	**Poor**	**Bad**
Lower limit	> 34.2	34.2 to 30.4	30.4 to 26.6	26.6 to 22.8	< 22.8

Meadow cover at the lower limit (in percentage) (UNEP/MAP-RAC/SPA, 2009)

	High	**Good**	**Moderate**	**Poor**	**Bad**
Lower limit	> 35%	35% to 25%	25% to 15%	15% to 5%8	< 5%

Shoot density (number of shoots · m^2) (Pergent-Martini et al., 2005)

Depth (m)	**High**		**Good**			**Moderate**			**Poor**			**Bad**	
1	>	1133	1133	to	930	930	to	727	727	to	524	<	524
2	>	1067	1067	to	863	863	to	659	659	to	456	<	456
3	>	1005	1005	to	808	808	to	612	612	to	415	<	415
4	>	947	947	to	757	757	to	567	567	to	377	<	377
5	>	892	892	to	709	709	to	526	526	to	343	<	343
6	>	841	841	to	665	665	to	489	489	to	312	<	312
7	>	792	792	to	623	623	to	454	454	to	284	<	284
8	>	746	746	to	584	584	to	421	421	to	259	<	259
9	>	703	703	to	547	547	to	391	391	to	235	<	235
10	>	662	662	to	513	513	to	364	364	to	214	<	214
11	>	624	624	to	481	481	to	338	338	to	195	<	195
12	>	588	588	to	451	451	to	314	314	to	177	<	177
13	>	554	554	to	423	423	to	292	292	to	161	<	161
14	>	522	522	to	397	397	to	272	272	to	147	<	147
15	>	492	492	to	372	372	to	253	253	to	134	<	134
16	>	463	463	to	349	349	to	236	236	to	122	<	122
17	>	436	436	to	328	328	to	219	219	to	111	<	111
18	>	411	411	to	308	308	to	204	204	to	101	<	101
19	>	387	387	to	289	289	to	190	190	to	92	<	92
20	>	365	365	to	271	271	to	177	177	to	83	<	83
21	>	344	344	to	255	255	to	165	165	to	76	<	76
22	>	324	324	to	239	239	to	154	154	to	69	<	69
23	>	305	305	to	224	224	to	144	144	to	63	<	63
24	>	288	288	to	211	211	to	134	134	to	57	<	57
25	>	271	271	to	198	198	to	125	125	to	52	<	52
26	>	255	255	to	186	186	to	117	117	to	47	<	47
27	>	240	240	to	175	175	to	109	109	to	43	<	43

28	> 227	227 to 164	164 to 102	102 to 39	< 39
29	> 213	213 to 154	154 to 95	95 to 36	< 36
30	> 201	201 to 145	145 to 89	89 to 32	< 32
31	> 189	189 to 136	136 to 83	83 to 30	< 30
32	> 179	179 to 128	128 to 77	77 to 27	< 27
33	> 168	168 to 120	120 to 72	72 to 24	< 24
34	> 158	158 to 113	113 to 68	68 to 22	< 22
35	> 149	149 to 106	106 to 63	< 63	
36	> 141	141 to 100	100 to 59	< 59	
37	> 133	133 to 94	94 to 55	< 55	
38	> 125	125 to 88	88 to 52	< 52	
39	> 118	118 to 83	83 to 48	< 48	
40	> 111	111 to 78	78 to 45	< 45	

Plagiotropic rhizome at the lower limit (in percentage) (UNEP/MAP-RAC/SPA, 2009)

	High	**Good**	**Moderate**	**Poor**	**Bad**
Lower limit	> 70%	70% to 30%	< 30%		

Plant (individual level)

Foliar surface (in cm² per shoot), between June and July (UNEP/MAP-RAC/SPA, 2009)

Depth (m)	**High**	**Good**	**Moderate**	**Poor**	**Bad**
15 m	> 362	362 to 292	292 to 221	221 to 150	< 150

Number of leaves produced per year (UNEP/MAP-RAC/SPA, 2009)

Depth (m)	**High**	**Good**	**Moderate**	**Poor**	**Bad**
15 m	> 8.0	8.0 to 7.5	7.5 to 7.0	7.0 to 6.5	< 6.5

Rhizome elongation (in mm per year) (UNEP/MAP-RAC/SPA, 2009)

Depth (m)	**High**	**Good**	**Moderate**	**Poor**	**Bad**
15 m	> 11	11 to 8	8 to 5	5 to 2	< 2

Physiological or cellular: environment eutrophication

Nitrogen concentration in adult leaves (in percentage), between June and July (UNEP/MAP-RAC/SPA, 2009)

Depth (m)	**High**	**Good**	**Moderate**	**Poor**	**Bad**
15 m	< 1.9%	1.9% to 2.4%	2.4% to 3.0%	3.0% to 3.5%	> 3.5%

Organic matter in the sediment (in percentage, fraction 0.063 mm) (UNEP/MAP-RAC/SPA, 2009)

Depth (m)	**High**	**Good**	**Moderate**	**Poor**	**Bad**
15 m	< 2.5%	2.5% to 3.5%	3.5% to 4.6%	4.6% to 5.6%	> 5.6%

Physiological or cellular: environment contamination

Argent Concentration (mg per g DW), blade of adult leaves, between June and July (Salivas-Decaux, 2009)

Depth (m)	High	Good	Moderate	Poor	Bad
15 m	< 0.08	0.08 to 0.22	0.23 to 0.36	0.37 to 0.45	> 0.45

Cadmium Concentration (mg per g DW), blade of adult leaves, between June and July (Salivas-Decaux, 2009)

Depth (m)	High	Good	Moderate	Poor	Bad
15 m	< 1.88	1.88 to 2.01	2.02 to 2.44	2.45 to 2.84	> 2.84

Mercury Concentration (mg per g DW), blade of adult leaves, between June and July (Salivas-Decaux, 2009)

Depth (m)	High	Good	Moderate	Poor	Bad
15 m	< 0.051	0.051 to 0.064	0.065 to 0.075	0.075 to 0.088	> 0.088

Plumb Concentration (mg per g DW), blade of adult leaves, between June and July (Salivas-Decaux, 2009)

Depth (m)	High	Good	Moderate	Poor	Bad
15 m	< 1.17	1.17 to 1.43	1.44 to 1.80	1.81 to 3.23	> 3.23

2. Guidelines for monitoring coralligenous and other calcareous bioconstructions in Mediterranean

Table of contents

Introduction

The calcareous formations of biogenic origin in the Mediterranean Sea are represented by coralligenous reefs, vermetid reefs, cold water corals, *Lithophyllum byssoides* concrations/trottoirs, banks formed by the corals *Cladocora caespitosa* or *Astroides calycularis*, sabellariid and serpulid worm reefs, and rhodoliths seabeds. Among all, coralligenous reefs (Fig. 1) and rhodoliths seabeds (Fig. 2) are the two most typical and important bioconstructed habitats that develop in the Mediterranean circalittoral zone, built-up by coralline algal frameworks that grow in dim light conditions, for which inventorying and mapping methods, as well as monitoring protocols, still lack of homogeneity and standardisation.

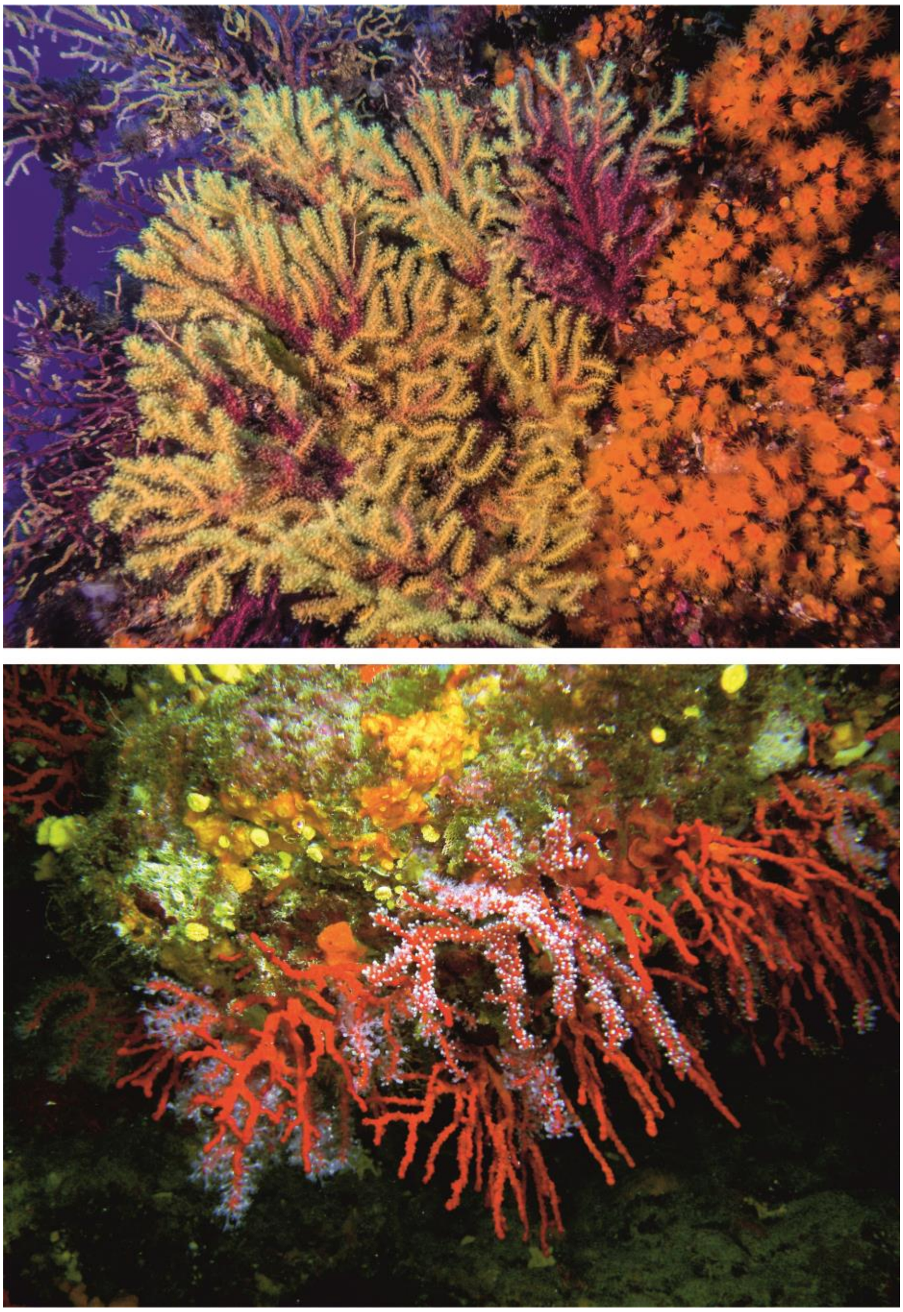

Figure 1: Coralligenous habitat (pictures by Simone Musumeci, Monica Montefalcone).

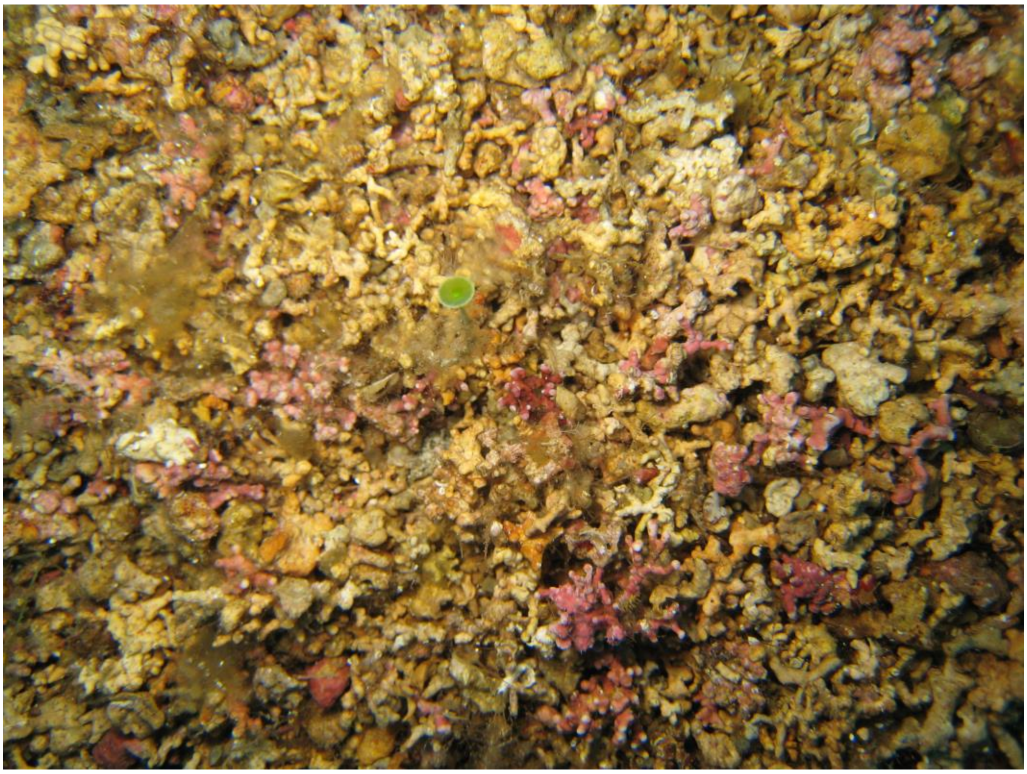

Figure 2: Rhodoliths habitat (picture from UNEP/MAP-RAC/SPA, 2015).

The most important and widespread bioconstructions in the Mediterranean Sea is represented by coralligenous reefs (UNEP/MAP-RAC/SPA, 2008), an endemic and characteristic habitat considered as the climax biocenosis of the circalittoral zone (Pérès and Picard, 1964). Coralligenous is characterised by high species richness, biomass and carbonate deposition values comparable to tropical coral reefs (Bianchi, 2001), and economic values higher than seagrass meadows (Cánovas Molina et al., 2014). Construction of coralligenous reefs started during the post-Würm transgression, about 15000 years ago, and develops on rocky and biodetritic bottoms in relatively constant conditions of temperature, currents and salinity.

Two main coralligenous typologies can be defined, coralligenous growing on the circalittoral rocks (cliffs or outcrops), and coralligenous developing over circalittoral soft/detritic bottoms creating biogenic platforms (Piazzi et al., 2019b). Coralligenous structure results from the dynamic equilibrium between bioconstruction, mainly made by encrusting calcified Rhodophyta belonging to Corallinales and Peyssonneliales (such as the genera *Lithophyllum*, *Lithothamnion*, *Mesophyllum*, *Neogoniolithon*, and *Peyssonnelia*), with an accessory contribution by serpulid polychaetes, bryozoans and scleractinian corals), and destruction processes (by borers and physical abrasion), which create a morphologically complex habitat where highly diverse benthic assemblages develop (Ballesteros, 2006). Light represents the main factor limiting bioconstruction, and coralligenous reefs are able to develop in dim light conditions (<3% of the surface irradiance), from about 20 m down to 120 m depth. Also the upper mesophotic zone (where the light is still present, from 40 m to 120 m depth), embracing the continental shelf, is shaped by extremely rich and diverse coralligenous assemblages dominated by animal forests that grow over biogenic rocky reefs.

Rhodoliths beds are composed by a variable thickness of free-living aggregations of live and dead thalli of calcareous red algae (mostly Corallinales but also Peyssonneliales) and their fragments, creating a biogenic, unstable, three-dimensional habitat typically exposed to bottom currents, which harbours greater biodiversity in comparison to surrounding habitats, and thus viewed as indicators of biodiversity hotspot. They mostly occur on coastal detritic bottoms in the upper mesophotic zone, between 40-60 m depth (Basso et al., 2016). Rhodoliths are made by slow growing organisms and can be long-lived (>100 years) (Riosmena-Rodríguez and Nelson, 2017). These algae can display a branching or a laminar appearance, can sometimes grow as nodules that cover all the seafloor, or accumulate within ripple marks. In the literature, the terms rhodoliths and maërl are often used as synonyms (UNEP/MAP-RAC/SPA, 2009). Maërl is the original Atlantic term to identify deposits of calcified non-nucleated algae mostly composed of *Phymatolithon calcareum* and *Lithothamnion corallioides*. Rhodoliths are intended as unattached nodules formed by calcareous red algae and their growths, showing a continuous spectrum of forms with size spanning from 2 to 250 mm of mean diameter. Thus, rhodoliths beds also includes maërl and calcareous *Peyssonnelia* beds, but the opposite is not true (Basso et al., 2016). Rhodoliths bed is recommended as a generic name to indicate those sedimentary bottoms characterised by any morphology and species of unattached non-geniculate calcareous red algae with >10% of live cover (Basso et al., 206). The name maërl should be restricted to those rhodoliths bed that are composed of non-nucleated, unattached growths of branching, twig-like coralline algae.

Coralligenous reefs provide different ecosystem services to humans (Paoli et al., 2017), but are vulnerable to either global or local impacts. Coralligenous is threatened by direct human activities, such as trawling, pleasure diving and illegal exploitation of protected species, and is also vulnerable to the indirect effects of climate change (e.g., positive thermal anomalies and ocean acidification) (UNEP/MAP-RAC/SPA, 2008). Some invasive algal species (e.g., *Womersleyella setacea*, *Acrothamnion preissii*, *Caulerpa cylindracea*) can also pose a severe threat to these communities, either by forming dense carpets or by increasing sedimentation rate.

Despite the occurrence of many species with high ecological value (some of which are also legally protected, e.g. *Savalia savaglia*, *Spongia officinalis*.), coralligenous reefs were not listed among the priority habitats defined by the EU Habitat Directive (92/43/ EEC), even if they can be included under the habitat “1170 Reefs” of the Directive, and appear also in the Bern Convention. This implies that the most important Mediterranean bio-construction still remained without formal protection as it is not included within the list of Sites of Community Interest (SCIs). Few years after the adoption of the Habitat Directive, coralligenous reefs were listed among the “special habitats types” needing rigorous protection by the Protocol for special protected areas (SPA/BIO) of the Barcelona Convention for the conservation of Mediterranean biodiversity (1995). Only recently, in the frame of the ‘‘Action Plan for the Conservation of Coralligenous and other Mediterranean bio-constructions’’ (UNEP/MAP-RAC/SPA, 2008) adopted by Contracting Parties to Barcelona Convention Barcelona in 2008, encouraged the legal conservation of coralligenous assemblages by the establishment of marine protected areas and emphasized the need for standardised programs for its monitoring. Coralligenous has also been included in the European Red List of marine habitats, where it is classified as “data deficient” (Gubbay et al., 2016), thus demonstrating the urgent need for thorough investigations and accurate monitoring plans. In the same year, the Marine Strategy Framework Directive (MSFD, 2008/56/EC) included “seafloor integrity” as one of the descriptors to be evaluated for assessing the Good Environmental State of the marine environment. Biogenic structures, such as the coralligenous reefs, have thus been recognized as important biological indicators of environmental quality.

Similarly, rhodoliths seabeds are expected to be damaged by dredging, heavy anchors and mooring chains and adversely affected by rising temperatures and ocean acidification. Two maërl forming species, *Phymatolithon calcareum* and *Lithothamnion corallioides* are protected under the EU Habitats Directive (92/43/ EEC) in the Annex V and, in some locations, maërl is also a key habitat within the Annex I list of habitats of the Directive and therefore is given protection through the designation of Special Areas of Conservation. Moreover, a special plan for the legal protection of Mediterranean rhodoliths has been adopted within the framework of the ‘‘Action Plan for the Conservation of Coralligenous and other Mediterranean bio-constructions’’ (UNEP/MAP-RAC/SPA, 2008). Rhodoliths seabeds have also been included in the Natura 2000 sites and in the Red List of Mediterranean threatened habitats.

The Action Plan (UNEP/MAP-RAC/SPA, 2008) identified many priority actions for these two benthic habitats, which mainly concern:

(i) Increase the knowledge on the distribution (compiling existing information, carrying out field activities in new sites or in sites of particular interest) and the composition (list of species) of these habitats

(ii) Set up a standardised spatio-temporal monitoring protocol for coralligenous and rhodoliths habitats.

Detailed information on habitat geographical distribution and bathymetrical ranges is a prerequisite knowledge for a sustainable use of marine coastal areas. Coralligenous and rhodoliths distribution maps are thus a fundamental prerequisite to any conservation action on these habitats. The scientific knowledge concerning several aspects of biogenic concretions (e.g., taxonomy, processes, functioning, biotic relationships, and dynamics) has been currently increasing, but it is still far away from the knowledge we have from other coastal ecosystems, such as seagrass meadows, shallow coastal rocky reefs, etc. One of the major gaps concerning the current state of knowledge on coralligenous and rhodoliths habitats is the limited spatio-temporal studies on their geographical and depth distribution at regional level and basin-wide scale. This information is essential in order to know the real extent of these habitats in the Mediterranean Sea and to implement appropriate management measures to guarantee their conservation (UNEP/MAP-RAC/SPA, 2008). Inventory and monitoring of coralligenous and rhodoliths raise several problems, due to their large bathymetric distribution and the consequent sampling constraints and often limited accessibility, their heterogeneity and the lack of standardised protocols used by different teams working in this field. The operational restrictions imposed by scuba diving (Gatti et al., 2012 and references therein) reduce the amount of collected data during each dive and increase the sampling effort. If some protocols for the inventory and monitoring of coralligenous habitat do exist, common methods for monitoring rhodoliths are comparatively less documented.

Responding to the need of practical guides aimed at harmonising existing methods for bioconstructed habitats monitoring and for subsequent comparison of results obtained by different countries, the Contracting Parties asked the Regional Activity Centre for Specially Protected Areas (RAC/SPA) to improve the existing inventory tools and to propose a standardisation of the mapping and monitoring techniques for coralligenous and rhodoliths. Thus, the main methods used in the Mediterranean for inventory and monitoring of coralligenous and other bioconstructions have been summarised in the "Standard Methods for Inventorying and Monitoring Coralligenous and Rhodoliths Assemblages" (UNEP/MAP-RAC/SPA, 2015). These monitoring guidelines have been the base for the updating and harmonisation process undertaken in this document.

For mapping coralligenous and other bioconstructed habitats, the previous Guidelines (UNEP/MAP-RAC/SPA, 2015) highlighted the following main findings:

- If scuba diving is often used for mapping small areas, it becomes unsuitable when the study area and/or the depth increase (depths >40 m)
- The use of acoustic survey methods (side scan sonar or multibeam) or underwater observation systems (ROV, towed camera) becomes then necessary. However, acoustic techniques must be always integrated and verified by a large number of "field" underwater data.

For monitoring the condition of coralligenous and other bioconstructed habitats, the previous Guidelines (UNEP/MAP-RAC/SPA, 2015) highlighted the following main findings:

- Assessment of the condition of the populations is heavily dependent on the working scale and the resolution requested. Monitoring activities relies mainly on scuba diving but given the above listed constraints, using other tools of investigation (e.g., ROV, towed camera) should be also considered because it allows monitoring with less precision but on larger areas
- Although the use of underwater photograph or video may be relevant, the use of specialists in taxonomy with a good experience in scuba diving is often essential given the complexity of this habitat. If it is possible to estimate the abundance or coverage by standardised indices, detailed characterisations often require the use of square frames (quadrates), transects, or even the removal of all organisms on a given surface. The presences of broken individuals, of necrosis are all factors to be considered as the precise description of the site
- Monitoring of coralligenous habitat starts with the realisation of micro-mapping and then the application of descriptors and/or ecological indices. However, these descriptors vary widely from one team to another, as well as their measurement protocol

- Monitoring of rhodoliths habitats can be done by scuba diving, but the observation using ROV or towed cameras and the collection of samples using dredges, grabs or box corers are privileged because of the greater homogeneity of these populations. However, there is not yet any standardised method widely accepted to date for monitoring rhodoliths, because the action of hydrodynamics may cause a shift of these habitats on the seabed.

Based on recommendations from the previous CPs group meeting, SPA/RAC has been requested to develop an updated version of the Guidelines for monitoring coralligenous and other bioconstructed habitats in Mediterranean (UNEP/MAP-RAC/SPA, 2015), in the context of the IMAP common indicators and in order to ease the task of the MPAs managers when implementing their monitoring programs. A reviewing process on the scientific literature, taking into account the latest techniques and the recent works carried out by the scientific community at the international level, has also been carried out. If standardised protocols for seagrass mapping and monitoring exist and are well-implemented, and a number of ecological indices have already been validated and inter-calibrated among different regions, this is not the case for coralligenous and rhodoliths habitats. In this document a number of “minimal” descriptors to be taken into account for inventorying and monitoring the coralligenous and rhodoliths populations in the Mediterranean are described. The main methods adopted for their monitoring, with the relative advantages, restrictions and conditions of use, are presented. Some of the existing monitoring methods for coralligenous have been already compared or cross-calibrated and are here briefly introduced and, finally, a standardised method recently proposed for coralligenous monitoring is described.

Monitoring methods

a) COMMON INDICATOR 1: Habitat distributional range and extent

Approach

The CI1 is aimed at providing information about the geographical area in which the coralligenous and rhodoliths habitats occur in the Mediterranean and the total extent of surfaces covered. Following the overall procedure suggested for mapping seagrass meadows in the Mediterranean, three main steps can be identified also for mapping bioconstructions (refer to the “Guidelines for monitoring marine vegetation in Mediterranean” in this document for major details):

1) Initial planning, which includes the definition of the objectives in order to select the minimum surface to be mapped and the necessary resolution, tools and equipments
2) Ground survey is the practical phase for data collection, the costliest phase as it generally requires field activities
3) Processing and data interpretation require knowledge and experience to ensure that data collected are usable and reliable.

Resolution

Measures of the total habitat extent may be subjected to high variability, as the final value is influenced by the methods used to obtain maps and by the resolution during both data acquisition and final cartographic restitution. Selecting an appropriate scale is a critical stage in the initial planning phase (Mc Kenzie et al., 2001). When large surface areas have to be mapped and global investigations carried out, an average precision and a lower detail level can be accepted, which means that the habitat distribution and the definition of its extension limits are often only indicative. When smaller areas have to be mapped, a much higher precision and resolution level is required and is easily achievable, thanks to the high-resolution mapping techniques available to date. However, obtaining detailed maps is time consuming and costly, thus practically impossible when time or resources are limited (Giakoumi et al., 2013). These detailed maps provide an accurate localisation of the habitat distribution and a precise definition of its extension limits and total habitat extent, all features necessary for future control and monitoring purposes over a period of time. These high-resolution scales are also used to select remarkable sites where monitoring actions must be concentrated.

A scale of 1:10000 is the best choice for mapping rhodoliths beds at regional level. On this scale, it is possible to delimit areas down to about 500 m^2, which is a good compromise between precise rhodoliths beds delimitation and study effort on a regional basis. Conversely, a scale equal to 1:1000 (or larger) is suggested for detailed monitoring studies of selected rhodoliths beds, where the areal definition and the rhodoliths boundaries should be more accurately located and monitored through time. Two adjacent rhodoliths beds are considered separate if, at any point along their limits, a minimum distance of 200 m occurs (Basso et al., 2016).

Although we have an overall knowledge about the composition and distribution of coralligenous and rhodoliths habitats in the Mediterranean (Ballesteros, 2006; UNEP-MAP-RAC/SPA, 2009; Relini, 2009; Relini and Giaccone, 2009), the scarceness of fine-scale cartographic data on the overall distribution of these habitats is one of the greatest lacunae from the conservation point of view. A first summary by Agnesi et al. (2008) highlighted the scarcity of available cartographic data, with less than 50 cartographies listed for the Mediterranean basin in that period. Most of the available maps are recent (less than ten years old) and are geographically disparate, mostly concerning the north-western basin. Another recent review (Martin et al., 2014) evidenced the occurrence of few datasets on coralligenous reefs and rhodoliths seabeds distribution, coming from 17 Mediterranean countries, and most of them being heterogeneous and with un-standardised legends, even within the same country.

Two global maps showing the distribution of coralligenous (Giakoumi et al., 2013) (Fig. 3) and maërl habitats (Martin et al., 2014) (Fig. 4) in the Mediterranean have been produced based on the review of available information. Coralligenous habitats cover a surface area of about 2763 km^2 in 16 Mediterranean countries, i.e. Albania, Algeria, Croatia, Cyprus, France, Greece, Italy, Israel, Lebanon, Libya, Malta, Monaco, Morocco, Spain, Tunisia, and Turkey. All other ecoregions presented lower coverage, with the Alboran Sea having the lowest. Very limited data were found for the presence of coralligenous formations in the southern and eastern coasts of the Levantine Sea. Information was substantially greater for the northern than the southern part of the Mediterranean. The Adriatic and Aegean Seas presented the highest coverage in terms of presence of coralligenous formations, followed by the Tyrrhenian Sea and the Algero-Provencal Basin. This uneven distribution of data on coralligenous distribution in the Mediterranean is not only a matter of invested research effort or data availability, but also depends on the geomorphologic heterogeneity of the Mediterranean coastline and seafloor: the northern basin encompasses 92.3% of the Mediterranean rocky coastline, while south and extreme south-eastern areas are dominated by sandy coasts (Giakoumi et al., 2013 and references therein). Hence, the extensive distribution of coralligenous in the Adriatic, Aegean, and Tyrrhenian Seas is highly related to the presence of extensive rocky coasts in these areas, with Italy, Greece, and Croatia covering 74% of the Mediterranean's rocky coasts.

Knowledge on maërl seabeds was somewhat limited compared to what is available for coralligenous. Maërl habitats cover a surface area of about 1654 km^2. Only sporadic and punctual information are available, mainly from the North Adriatic, the Aegean Seas and the Tyrrhenian Sea. Datasets are available for Greece, France (Corsica), Cyprus, Turkey, Spain and Italy. Malta and Corsica, in particular, have significant datasets for this habitat as highlighted by fine-scale surveys in targeted areas (Martin et al., 2014).

These low-resolution global maps are still incomplete being the available information highly heterogeneous due to the high variability in the mapping and monitoring efforts across the Mediterranean basin; further mapping is thus required to determine the full extent of these highly variable habitats at the Mediterranean spatial scale. However, they can be very useful for an overall knowledge of the bottom areas covered by coralligenous and rhodoliths, and to evaluate where surveys must be enforced in the future to collect missing data.

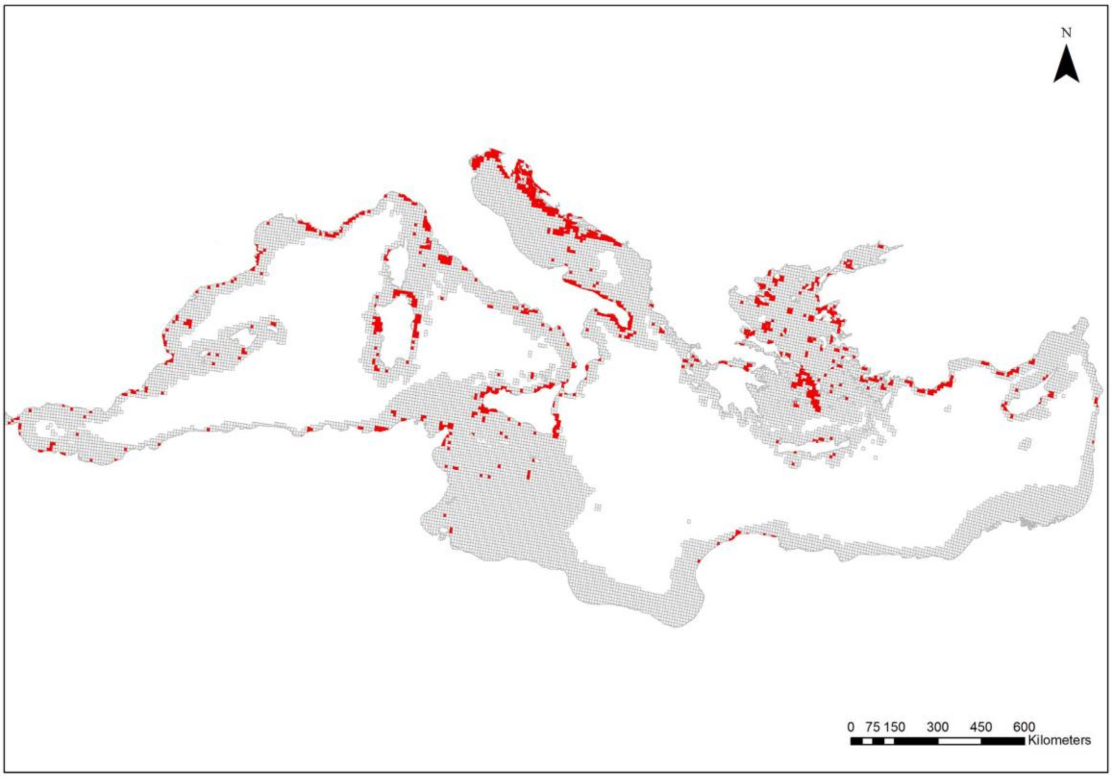

Figure 3: Distribution of coralligenous habitats in the Mediterranean Sea (red areas) (from Giakoumi et al., 2013).

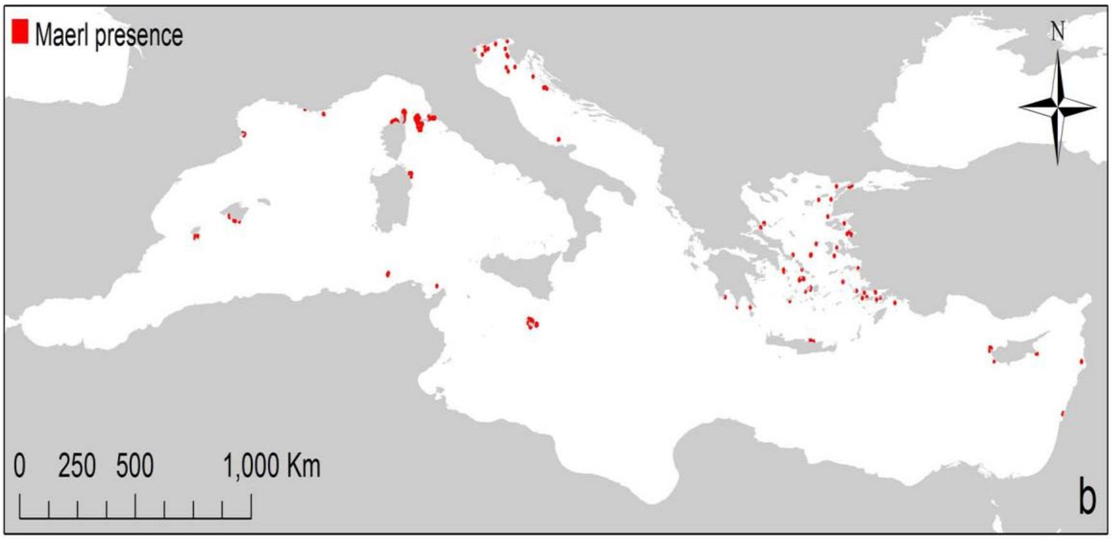

Figure 4: Distribution of maërl habitats in the Mediterranean Sea (red areas) (from Martin et al., 2014).

Methods

Definition of distributional range and extent of coralligenous and rhodoliths habitats requires "traditional" habitat mapping techniques, similar to those used for seagrass meadows in deep waters (Tab. 1). Indirect instrumental mapping techniques and/or direct field visual surveys can be used and are often integrated. The simultaneous use of two or more methods makes it possible to optimize the results being the information obtained complementary. The strategy to be adopted will thus depend on the aim of the study and the area concerned, means and time available.

Underwater observations and sampling methods

Although underwater direct observation by scuba diving (e.g., using transects, permanent square frames) is often used for mapping small areas, this method of investigation quickly shows its limits when the area of study and the depth increase significantly, even if the technique can be optimised for a general description of the site through a towed diver or video transects (Cinelli, 2009). Direct observations provide discrete punctual data that are vital for ground-truthing the instrumental surveys, and for the validation of modelled continuous information (complete coverage of surface areas) obtained from data on limited portions of the study area or along the pathway. Field surveys must be sufficiently numerous and distributed appropriately to obtain the necessary precision, and especially in view of the high heterogeneity of the coralligenous habitat.

In situ underwater observations represent the most reliable, although time-consuming, mapping technique of coralligenous habitat. Surveys can be done along lines (transect), or over small surface areas (permanent square frames) positioned on the seafloor and located to follow the limits of the habitat. The transect consists of marked lines wrapped on a rib and laid on the bottom from fixed points and in a precise direction, typically perpendicular or parallel with respect to the coastline (Bianchi et al., 2004a). Any changes in the habitat and in the substrate typology, within a belt at both sides of the line (considering a surface area of about 1-2 m per side), are recorded on underwater slates. The information registered allows precise and detailed mapping of the sector studied (Tab. 1).

Scuba diving is also suggested for a safe and cost-effective tool to obtain a visual description and sampling of shallow rhodoliths beds (Tab. 1). Underwater observations are effective for a first characterisation of the aboveground facies of this habitat, whilst to describe the belowground community samples on the bottom become necessary. The surface of a living rhodoliths bed is naturally composed of a variable amount of live thalli and their fragments, lying on a variable thickness of dead material and finer sediment. There are no literature data about the required minimum spatial extent for a portion of the seafloor to be defined as a rhodoliths bed. A rhodoliths bed is defined as a habitat that is distinguished from the surrounding seafloor by having >10% of the mobile substratum covered by live calcareous coralline algae as unattached branches and/or nodules (Basso et al., 2016). Live rhodoliths beds are naturally accompanied by a variable quantity of dead rhodoliths and their fragments; thus, a threshold of >50% of the surface cover by dead rhodoliths and their fragments is defined as the condition to identify a dead rhodoliths bed. A seafloor covered by incomplete algal coatings of lithic pebbles and shell remains should not be considered as a rhodoliths bed. The mandatory information needed for a first description of rhodoliths beds includes depth range, areal extent, occurrence of sedimentary structures of the seafloor (such as ripples, mega-ripples, and underwater dunes), thickness of live layer, the mean percentage cover of live thalli, live/dead rhodoliths ratio, dominant morphologies of rhodoliths (see Fig. 5), and identification of the most common and volumetrically important species of calcareous algae. In this first description, the need for specialized taxonomists and time-consuming laboratory analyses is kept to a minimum.

Recently an innovative tool, namely the BioCube, which is a 1 m high device that enables the acquisition of 80 cm x 80 cm frame photo-quadrates, has been implemented for the characterisation of the aboveground detritic and rhodoliths seabottom without scuba diving (Astruch et al., 2019). Photo-quadrates were made with a digital video camera with 30 second-time lapse triggering. Another camera linked to a screen at the surface is fixed to the BioCube to control the workflow and the position of the frame in real time. During the data acquisition, a third camera is filming the surrounding landscape for complementary information on demersal fish and extent of assemblages.

Sampling methods from vessels involving blind grabs, dredges and box corers in a number of randomly selected points within a study area can be used to check for the occurrence of deep rhodoliths beds (ground-truth of acoustic data) and for a complete description of the habitat (Tab. 1). The thickness of the live cover could be measured through the transparent or removable side of a box-corer. Alternatively, a sub-sample could be taken from the recovered box-core using a Plexiglas core of about 10 cm in diameter and at least 20 cm long. Box-coring with a cross-section ≥ 0.16 m^2 is recommended because it has the advantage of preserving the original substratum stratification. The use of dredges for sampling rhodoliths should be discouraged, in order to minimize the impact of the investigation.

<u>Remote sensing surveys</u>

Being the bioconstructed habitats distributed in deep waters (down to 20 m depth), the acoustic techniques (e.g., side scan sonar, multi-beam echosounder) or underwater video recordings (ROVs, towed cameras) are usually recommended, although they require a very long acquisition time given their limited speed and range (Georgiadis et al., 2009). The use of remote sensing allows characterising extensive coastal areas for assessment of the overall spatial patterns of coralligenous and rhodoliths habitats. From maps obtained through remote sensing surveys, the presence/absence of the habitat, its distributional range and the total habitat extent can be easily obtained. Acoustic methods are presently the most convenient technique for mapping rhodoliths beds, associated with ground-truthing by ROV and box-coring. The percentage cover of live thalli over a wide area can also be assessed from a ROV survey. Using acoustic techniques associated with a good geo-location system allow monitoring change in the extent of rhodoliths habitat over time (Bonacorsi et al., 2010).

Observations from the surface can be made by using imagery techniques such as photography and video. Photographic equipment and cameras can be mounted on a vertical structure (sleigh) or within remotely operated vehicles (ROVs). The camera on a vertical structure is submerged at the back of the vessel and is towed by the vessel that advances very slowly (under 1 knot), whilst the ROVs have their own propulsion system and are remotely controlled from the surface. The use of towed video cameras (or ROVs) during surveys makes it possible to see the images on the screen in real time, to identify specific features of the habitat and to evaluate any changes in the habitat or any other characteristic element of the seafloor, and this preliminary video survey may be also useful to locate monitoring stations. Recorded images are then reviewed to obtain a cartographical restitution on a GIS platform for each of the areas surveyed. To facilitate and to improve the results obtained with the camera, joint acquisition modules integrating the depth, images of the seafloor and geographical positioning have been developed (UNEP/MAP-RAC/SPA, 2015).

Sonar provides images of the seafloor through the emission and reception of ultrasounds. Amongst the main acoustic mapping techniques available (Kenny et al., 2003) wide acoustic beam systems like the side scan sonar (SSS) and multi-beam echosounder are usually employed in mapping coralligenous and rhodoliths habitats. All the acoustic mapping techniques are intrinsically affected by uncertainties due to manual classification of the different acoustic signatures of substrate types on sonograms. Errors in sonograms interpretation may arise when two substrate types are not easily distinguished by the observer. Interpretation of remote sensing data requires extensive field calibration and the ground-truthing process remains essential. As the interpretation of sonograms is also a time-consuming and tedious task, several processing techniques were proposed in order to rapidly automate the interpretation of sonograms and make this interpretation more reliable (Montefalcone et al., 2013 and references therein). These methods allow a good discrimination between soft sediments and rocky reefs. Human eye, however, always remains the final judge.

<u>Modelling</u>

Modelling techniques can be used to fill the gaps in the knowledge of the spatial distribution of habitats by predicting the areas that are likely to be suitable for a community to live. Models are usually based on physical and environmental variables (e.g., water temperature, salinity, depth, nutrient concentrations, seabed types), which are typically easier to record and map at the regional and global scales, in contrast to species and habitat data. Despite inherent limitations and associated uncertainties, predictive modelling is a cost-effective alternative to field surveys as it can help identifying and mapping where sensitive marine ecosystems may occur. Based on the spatial datasets available for coralligenous and rhodoliths populations, a predictive modelling was carried out to produce two continuous maps of these two habitats across the Mediterranean Sea (Martin et al., 2014). For coralligenous, bathymetry, slope of the seafloor and nutrient input were the three main contributors to the model. Predicted areas with suitable conditions for the occurrence of coralligenous habitat showed in the North African coast suitable areas for which there were no occurrence data. For rhodoliths, phosphate concentration, geostrophic velocity of sea surface current, silicate concentration and bathymetry were the four main contributors to the model. Given the paucity of occurrence data for this habitat across the Mediterranean, and especially in the North African coast, the model output is relatively informative in highlighting several suitable areas where no occurrence data were available to date.

A recent application of predictive spatial modelling was done starting from a complete acoustic coverage of the seafloor together with a comparatively low number of sea-truths made by scuba diving (Vassallo et al., 2018). This approach was applied to the coralligenous reefs of the Marine Protected Area of Tavolara - Punta Coda Cavallo (NE Sardinia, Italy), through a fuzzy clustering on a set of *in situ* observation. The model allowed recognising and mapping coralligenous habitats within the MPA and showed that the distribution of habitats was mainly driven by distance from coast, depth, and lithotypes. Another example of habitat prediction can be found in Zapata-Ramírez et al. (2016).

Table 1: Synthesis of the main survey tools used for defining the Common Indicator 1_Habitat distributional range and extent for coralligenous and rhodoliths habitats. When available, the depth range, the surface area mapped, the spatial resolution, the efficiency (expressed as area mapped in km^2 per hour), the main advantages or the limits of each tool are indicated, with some bibliographical references.

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Underwater diving	0 m to 40 m	Small areas, less than 250 m^2	From 0.1 m	0.0001 to 0.001 km^2/hour	• Very great precision for the identification (taxonomy) and distribution of species (micro-mapping) • Non-destructive • Low cost, easy to implement	• Small area inventoried • Very time-consuming • Limited operational depth • Highly qualified divers required (safety constraints) • Variable geo-referencing of the dive site	Piazzi et al. (2019a and references therein)
Transects by towed divers	0 m to 50 m	Intermediate areas (less than 1 km^2)	From 1 to 10 m	0.025 to 0.01 km^2/hour	• Easy to implement and possibility of taking pictures • Good identification of populations • Non-destructive and low cost	• Time-consuming • Limited operational depth • Highly qualified divers required (safety constraints) • Variable geo-referencing of the diver route • Water transparency	Cinelli (2009)
Sampling from vessels with blind grabs, dredges or box corers	0 m to about 50 m (until the lower limit of the rhodoliths habitat)	Intermediate areas (a few km^2)	From 1 to 10 m	0.025 to 0.01 km^2/hour	• Very great precision for the identification (taxonomy) and distribution of species (micro-mapping) • All species taken into account • Possibility of *a posteriori* identification • Low cost, easy to implement	• Destructive method • Small area inventoried • Sampling material needed • Work takes a lot of time • Limited operational depth	UNEP/MAP-RAC/SPA (2015)

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Side scan sonar	8 m to over 120 m (until the lower limit of the coralligenous habitat)	From intermediate to large areas (50-100 km²)	From 1 m	1 to 4 km²/hour	• Wide bathymetric range • Realistic representation of the seafloor • Good identification of the nature of the bottom and of assemblages (rhodoliths) with location of edges • Quick execution • Non-destructive	• Flat (2-D) picture to represent 3-D complex habitat • Possible errors in sonograms interpretation • Acquisition of field data necessary to validate sonograms • High cost • Very big mass of data • Not very used for mapping vertical slopes	Cánovas Molina et al. (2016b)
Multi-beam echosounder	2 m to over 120 m (until the lower limit of the coralligenous habitat)	From intermediate to large areas (50-100 km²)	From 1 m (linear) and lower than 1 m (depth)	0.5 to 6 km²/hour	• Possibility of obtaining 3-D picture • Double information collected (bathymetry and seafloor image) • Very precise and wide bathymetric range • Quick execution • Non-destructive	• Very big mass of data • Complex processing of information • Less precise imaging (nature of bed) than side scan sonar • Acquisition of field data necessary to validate sonograms • High cost	Cánovas Molina et al. (2016b)
Remote Operating Vehicle (ROV)	2 m to over 120 m (until the lower limit of the coralligenous habitat)	Small-intermediate areas (a few km²)	From 1 m to 10 m	0.025 to 0.01 km²/hour	• Non-destructive • Possibility of taking pictures • Good identification of habitat and species • Wide bathymetric range	• Small area surveyed • High cost • Slow recording and processing of information • Variable positioning (geo-referencing) • Difficult to handle with currents	Cánovas Molina et al. (2016a); Enrichetti et al. (2019)

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Towed camera	2 m to over 120 m (until the lower limit of the coralligenous habitat)	Intermediate areas (a few km^2)	From 1 m to 10 m	0.025 to 1 km^2/hour	• Easy to implement and possibility of taking pictures • Good identification of habitat and species • Non-destructive • Large area covered	• Limited to homogeneous and horizontal bottom • Slow recording and processing of information • Variable positioning (geo-referencing) • Water transparency • Hard to handle in heavy surface traffic	UNEP/MAP-RAC/SPA (2015)

Data interpretation

Once the surveying is completed, data collected needs to be organized so that it can be used in the future by everyone and can be appropriately archived and easily consulted. A clear definition of all metadata must be provided with the dataset in order to ensure future integration with similar data from other sources. Four important steps for the production of a habitat map must be followed:

1. Processing, analysis and classification of the biological data, through a process of interpretation of acoustic images when available
2. Selecting the most appropriate physical layers (e.g., substrate, bathymetry, hydrodynamics)
3. Integration of biological data and physical layers, and use of statistical modelling to predict habitat distribution and interpolate information
4. The map produced must then be evaluated for its accuracy, i.e. its capacity to represent reality, and therefore its reliability.

During the processing analysis and classification step, the updated list of benthic marine habitat types for the Mediterranean region should be consulted (UNEP/MAP-SPA/RAC, 2019) to recognize any specific habitat type (i.e., coralligenous or rhodoliths) and its main characteristic associations and facies. A complete description of these habitats and the criteria for their identification are also available in Bellan-Santini et al. (2002). Habitats that must be reported on maps are the following (UNEP/MAP-SPA/RAC, 2019):

INFRALITTORAL

MB1.5 Infralittoral rock

MB1.55 Coralligenous (enclave of circalittoral, see MC1.51)

CIRCALITTORAL

MC1.5 Circalittoral rock

MC1.51 Coralligenous

MC1.51a Algal-dominated coralligenous

MC1.511a Association with encrusting Corallinales

MC1.512a Association with Fucales or Laminariales

MC1.513a Association with algae, except Fucales, Laminariales, Corallinales and Caulerpales

MC1.514a Association with non-indigenous Mediterranean *Caulerpa* spp.

MC1.51b Invertebrate-dominated coralligenous

MC1.511b Facies with small sponges (sponge ground, e.g. *Ircinia* spp.)

MC1.512b Facies with large and erect sponges (e.g. *Spongia lamella*, *Sarcotragus foetidus*, *Axinella* spp.)

MC1.513b Facies with Hydrozoa

MC1.514b Facies with Alcyonacea (e.g. *Eunicella* spp., *Leptogorgia* spp., *Paramuricea* spp., *Corallium rubrum*)

MC1.515b Facies with Ceriantharia (e.g. *Cerianthus* spp.)

MC1.516b Facies with Zoantharia (e.g. *Parazoanthus axinellae*, *Savalia savaglia*)

MC1.517b Facies with Scleractinia (e.g. *Dendrophyllia* spp., *Leptopsammia pruvoti*, *Madracis pharensis*)

MC1.518b Facies with Vermetidae and/or Serpulidae

MC1.519b Facies with Bryozoa (e.g. *Reteporella grimaldii*, *Pentapora fascialis*)

MC1.51Ab Facies with Ascidiacea

MC1.51c Invertebrate-dominated coralligenous covered by sediment

See MC1.51b for examples of facies

MC1.52 Shelf edge rock

MC1.52a Coralligenous outcrops

MC1.521a Facies with small sponges (sponge ground)

MC1.522a Facies with Hydrozoa

MC1.523a Facies with Alcyonacea (e.g. *Alcyonium* spp., *Eunicella* spp., *Leptogorgia* spp., *Paramuricea* spp., *Corallium rubrum*)

MC1.524a Facies with Antipatharia (e.g. *Antipathella subpinnata*)

MC1.525a Facies with Scleractinia (e.g. *Dendrophyllia* spp., *Madracis pharensis*)

MC1.526a Facies with Bryozoa (e.g. *Reteporella grimaldii*, *Pentapora fascialis*)

MC1.527a Facies with Polychaeta

MC1.528a Facies with Bivalvia

MC1.529a Facies with Brachiopoda

MC1.52b Coralligenous outcrops covered by sediment

See MC1.52a for examples of facies

MC1.52c Deep banks

MC1.521c Facies with Antipatharia (e.g. *Antipathella subpinnata*)

MC1.522c Facies with Alcyonacea (e.g. *Nidalia studeri*)

MC1.523c Facies with Scleractinia (e.g. *Dendrophyllia* spp.)

MC1.531d Facies with *Lithistida* spp. sponges

MC2.5 Circalittoral biogenic habitat

MC2.51 Coralligenous platforms

MC2.511 Association with encrusting Corallinales

MC2.512 Association with Fucales

MC2.513 Association with non-indigenous Mediterranean *Caulerpa* spp.

MC2.514 Facies with small sponges (sponge ground, e.g. *Ircinia* spp.)

MC2.515 Facies with large and erect sponges (e.g. *Spongia lamella*, *Sarcotragus foetidus*, *Axinella* spp.)

MC2.516 Facies with Hydrozoa

MC2.517 Facies with Alcyonacea (e.g. *Alcyonium* spp., *Eunicella* spp., *Leptogorgia* spp., *Paramuricea* spp., *Corallium rubrum*)

MC2.518 Facies with Zoantharia (e.g. *Parazoanthus axinellae*, *Savalia savaglia*)

MC2.519 Facies with Scleractinia (e.g. *Dendrophyllia* spp., *Madracis pharensis*, *Phyllangia mouchezii*)

MC2.51A Facies with Vermetidae and/or Serpulidae

MC2.51B Facies with Bryozoa (e.g. *Reteporella grimaldii*, *Pentapora fascialis*)

MC2.51C Facies with Ascidiacea

MC3.5 Circalittoral coarse sediment

MC3.52 Coastal detritic bottoms with rhodoliths

MC3.521 Association with maërl (e.g. *Lithothamnion* spp., *Neogoniolithon* spp., *Lithophyllum* spp., *Spongites fruticulosa*)

MC3.522 Association with *Peyssonnelia* spp.

MC3.523 Association with Laminariales

MC3.524 Facies with large and erect sponges (e.g. *Spongia lamella*, *Sarcotragus foetidus*, *Axinella* spp.)

MC3.525 Facies with Hydrozoa

MC3.526 Facies with Alcyonacea (e.g. *Alcyonium* spp., *Paralcyonium spinulosum*)

MC3.527 Facies with Pennatulacea (e.g. *Veretillum cynomorium*)

MC3.528 Facies with Zoantharia (e.g. *Epizoanthus* spp.)

MC3.529 Facies with Ascidiacea

The selection of physical layers to be shown on maps and to be used for following predictive statistical analyses may be an interesting approach within the general framework of mapping coralligenous and rhodoliths habitats, as it would reduce the processing time. However, it is still of little use as only few of the physical parameters are able to clearly predict the distribution of these two habitats, i.e. bathymetry, slope of the seafloor and nutrient input for coralligenous and phosphate concentration, geostrophic velocity of sea surface current, silicate concentration and bathymetry for rhodoliths (Martin et al., 2014).

The data integration and modelling are often a necessary step because indirect visual or remote sensing surveys from vessels are often limited due to time and costs involved, and only rarely allow to obtain a complete coverage of the study area. Coverage under 100% automatically means that it is impossible to obtain high resolution maps and therefore interpolation procedures have to be used, so that from partial surveys a lower resolution map can be obtained. Spatial interpolation is a statistical procedure for estimating data values at unsampled sites between actual data collection locations. For elaborating the final distribution map of benthic habitats on a GIS platform, different spatial interpolation tools (e.g., Inverse Distance Weighted, Kriging) can be used and are provided by the GIS software. Even though this is rarely mentioned, it is important to provide information on the number and the percentage of data acquired on field and the percentage of interpolations run.

The processing and digital analysis of acoustic data on GIS allows to creating charts where each tonality of grey is associated to a specific texture representing a type of habitat or substrate, also on the basis of the situ observations. Although remote sensing data must be always integrated by a great amount of field visual inspections for ground-truthing, especially given the 3-D distribution and complexity of the coralligenous seascape developing over hard substrates, high quality bathymetric data often constitutes an indispensable and appreciation element.

To facilitate the comparison among maps, the standardised red colour is generally used for the graphic representation of coralligenous and rhodoliths habitats. On the resulting maps the habitat distributional range and its total extent (expressed in square meters or hectares) can be defined. These maps could be also compared with previous historical available data from literature to evaluate any changes experienced by benthic habitats over a period of time (Giakoumi et al., 2013). Using the overlay vector methods on GIS, a diachronic analysis can be done, where temporal changes are measurements in term of percentage gain or loss of the habitat extension, through the creation of concordance and discordance maps (Canessa et al., 2017).

Finally, reliability of the map produced should be evaluated. No evaluation scales of reliability have been proposed for coralligenous and rhodoliths habitats mapping; however, scales of reliability evaluation available for seagrass meadows can be adapted also for these habitats (see the "Guidelines on marine vegetation in this document for further details). These scales usually take into account the processing of sonograms, the scale of data acquisition and restitution, the methods adopted, and the positioning system.

b) COMMON INDICATOR 2: Condition of the habitat's typical species and communities

Approach

Monitoring are necessary for conservation purposes, which require efficient management measures to ensure that marine benthic habitats, their constituent species and their associated communities are and remain in a satisfactory ecological status. The good state of health of both coralligenous and rhodoliths habitats will then reflect the Good Environmental Status (GES) pursued by the Contracting Parties to the Barcelona Convention under the Ecosystem Approach (EcAp) and under the Marine Strategy Framework Directive (MSFD).

Monitoring the condition (i.e., the ecological status) of coralligenous and rhodoliths habitats is today mandatory also because:

- Two maërl forming species, *Phymatolithon calcareum* and *Lithothamnion corallioides* are protected under the EU Habitats Directive (92/43/ EEC) in the Annex V
- Coralligenous reefs and rhodoliths seabeds are listed among the "special habitats types" needing rigorous protection by the Protocol for special protected areas (SPA/BIO) of the Barcelona Convention for the conservation of Mediterranean biodiversity (UNEP/MAP-RAC/SPA, 2008).

According to the EcAp, the CI2 fixed by IMAP guidelines and related to "biodiversity" (EO1) is aimed at providing information about the condition (i.e., ecological status) of coralligenous and rhodoliths habitats, being two of the main hotspots of biodiversity in the Mediterranean (UNEP/MAP, 2008). The MSFD (2008/56/EC) included both "biological diversity" (D1) and "seafloor integrity" (D6) as descriptors to be evaluated for assessing the GES of the marine environment. In this regard, biogenic structures, such as the coralligenous reefs and rhodoliths seabeds, have been recognized as important biological indicators of environmental quality.

A defined and standardised procedure for monitoring the status of coralligenous and rhodoliths habitats, comparable to that provided for their mapping, should follow these three main steps:

1. Initial planning, to define objective(s), duration, sites to be monitored, descriptors to be evaluated, sampling strategy, human, technical and financial needs
2. Setting-up the monitoring system and realisation of the monitoring program. This phase includes costs for going out to sea during field activities, equipment for sampling, and human resources. To ensure effectiveness of the program, field activities should be planned during a favourable season, and it would be preferred to monitor during the same season
3. Monitoring over time and analysis is a step where clear scientific competences are needed because the acquired data must be interpreted. Duration of the monitoring, in order to be useful, must be medium-time at least.

The objectives of the monitoring are primarily linked with the conservation of bio-constructed habitats, but they also answer to the necessity of using them as ecological indicators of the marine environment quality. The main aims of the monitoring programs are generally:

- Preserve and conserve the heritage of bioconstructions, with the aim of ensuring that coralligenous and rhodoliths habitats are in a satisfactory ecological status (GES) and also identify as early as possible any degradation of these habitats or any changes in their distributional range and extent. Assessment of the ecological status of these habitats allows to measure the effectiveness of local or regional policies in terms of management of the coastal environment

- Build and implement a regional integrated monitoring system of the quality of the environment, as requested by the Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP) during the implementation of the EcAp in the framework of the Mediterranean Action Plan (UNEP/MAP, 2008). The main goal of IMAP is to gather reliable quantitative and updated data on the status of marine and coastal Mediterranean environment
- Evaluate effects of any coastal activity likely to impact coralligenous and rhodoliths habitats during environmental impact assessment procedures. This type of monitoring aims to establish the condition of the habitat at the time "zero" before the beginning of activities, then monitor the state of health of the habitat during the development works phase or at the end of the phase, to check for any impacts.

The objective(s) chosen will influence the choices of the monitoring criteria in the following steps (e.g., duration, sites to be monitored, descriptors, and sampling methods). The duration of the monitoring should be at least medium-long term (minimum 5-10 years long) for heritage conservation and monitoring environmental quality objectives. The interval of data acquisition could be annually, as most of the typical species belonging to coralligenous assemblages and to rhodoliths beds display slow grow rates and long generation times. In general, and irrespective of the objective advocated, it is judicious to focus initially on a small number of sites that are easily accessible and that can be regularly monitored after short intervals of time. The sites chosen must be: i) representative of the portion of the coastal area investigated, ii) cover most of the possible range of environmental situations (e.g., depth range, slope, substrate type), and iii) include sensitive zones, stable zones or reference zones with low anthropogenic pressures (i.e., MPAs). Then, with the experience gained by the surveyors and the means (funds) available, this network could be extended to a larger number of sites. For environmental impact assessment, short term monitoring (generally 1-2 years) is recommended and should be initiated before the interventions ("zero" time), and possibly continued during, or just after the conclusion of the works. A further control can be made one year after the conclusion. The ecological status of the site subjected to coastal interventions (i.e. the impact site) must be contrasted with the status of at least 2 reference/control sites.

To ensure the sustainability of the monitoring system, the following final remarks must be taken into account:

- Identify the partners, competences and means available
- Planning the partnership modalities (who is doing what? when? and how?)
- Ensure training for the stakeholders so that they can set up standardised procedures to guarantee the validity of the results, and so that comparisons can be made for a given site and among sites
- Individuate a regional or national coordinator depending on the number of sites concerned for monitoring and their geographical distribution
- Evaluate the minimum budget necessary for running the monitoring network (e.g., costs for permanent operators, temporary contracts, equipment, data acquisition, processing and analysis).

Methods

Following the preliminary definition of the distributional range and extent of coralligenous and rhodoliths habitats (the previous CI1), the assessment of the condition of the two habitats starts with an overall characterisation of the typical species and communities occurring within each habitat. Monitoring of these two habitats basically relies on underwater diving, although this technique gives rise to many constraints due to the conditions of the environment in which these habitats develop (great depths, weak luminosity, low temperatures, presence of currents, etc.): it can only be done by confirmed and expert scientific divers (for safety) and over a limited underwater time (Bianchi et al., 2004b; Tetzaff and Thorsen, 2005). Adoption of new investigation tools (e.g., ROVs) allows for a less precise assessment but over larger spatial scale. A first characterisation of the habitat (species present, abundance, vitality, etc.) can be done by direct visual underwater inspections, indirect ROVs or towed camera video recordings, or sampling procedure with dredges, grabs or box corers in the case of rhodoliths seabeds. The acoustic methods that were described above are totally inoperative for detailed characterisations of the habitats, especially for coralligenous. The surveys method depends greatly on the scale of the work and the spatial resolution requested (Tab. 2). The complementarities of these techniques must be taken into account when planning an operational strategy (Cánovas Molina et al., 2016b).

The use of ROVs or towed camera can be useful to optimise information obtained and sampling effort (in term of working time) and become essential for monitoring deep coralligenous assemblages and rhodoliths seabeds developing in the upper mesophotic zone (down to 40 m depth), where scuba diving procedures are usually not recommended. High quality photographs recorded will be analysed in laboratory (also with the help of taxonomists) to list the main conspicuous species/taxa or morphological groups recognisable on images and to evaluate their abundance (coverage or surface area in cm^2). Photographs can be then archived to create temporal datasets.

At shallower depths (up to about 40 m), direct underwater visual surveys by scuba diving are mandatory and strongly suggested. Good experience in underwater diving is requested to operate an effective work at these depths. Scientific divers annotate on their slides the list of the main conspicuous species/taxa characterising the assemblages. Given the complexity of the coralligenous habitat (3-D distribution of species and high biodiversity), divers must be specialists in taxonomy of the main coralligenous species to ensure the validity of the information recorded underwater. Photographs or video collected with underwater cameras can be usefully integrated to visual survey to speed the work (Gatti et al., 2015a). The use of operational taxonomical units (OTUs), or taxonomic surrogates such as morphological groups (lumping species, genera or higher taxa displaying similar morphological features; Parravicini et al., 2010), may represent a useful compromise when a consistent species distinction is not possible (either underwater or on photographs) or to reduce the surveying/analysis time.

For a rough and rapid characterisation of the coralligenous assemblages, semi-quantitative evaluations often give sufficient information (Bianchi et al., 2004b); thus, it is possible to estimate the abundance (usually expressed as % cover) by standardised indices directly in situ or using photographs (UNEP/MAP-RAC/SPA, 2008). However, a quality and fine characterisation of the assemblages often requires the use of square frames (quadrates) or transects (with or without photographs; Piazzi et al., 2018) to collect quantitative data, or even the sampling by scraping of all the organisms present over a given area for further laboratory analyses (Bianchi et al., 2004b). Destructive procedures by scraping are not usually recommended on coralligenous being a time-consuming technique and due to the limited available time underwater. In situ observation and samples must be done over defined and, possibly, standardised surface areas (Piazzi et al., 2018), and the number of replicates must be adequate and high enough to catch the heterogeneity of the habitat.

As well as the presence or abundance of a given species, assessing its vitality seems a particularly interesting parameter. The presence of broken individuals (especially of the branching colonies occurring in the intermediate and upper layers, such as bryozoans, gorgonians) and signs of necrosis are important elements to be taken into consideration (Garrabou et al., 1998, 2001; Gatti et al., 2012). Finally, the nature of the substratum (silted up, roughness, interstices, exposure, slope), the temperature of the water, the vagile fauna associated, the coverage by epibionta and the presence of invasive species must also be considered to give a clear characterisation of the habitat (Harmelin, 1990; Gatti et al., 2012).

Table 2: Synthesis of the main methods used to characterise coralligenous and rhodoliths habitats in the Mediterranean, as the first necessary step for defining the Common Indicator 2_Condition of the habitat's typical species and communities for. When available, the depth range, the surface area surveyed, the spatial resolution, the efficiency (expressed as area surveyed in km^2 per hour), the main advantages or the limits of each tool are indicated, with some bibliographical references.

Methods	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Remote Operating Vehicle (ROV)	From 2 m to over 120 m	Small-Intermediate areas of about 1 km^2	From 1 m to 10 m	0.025 to 0.01 km²/hour	• Non-destructive method • Possibility of taking pictures • Wide bathymetric range • Good identification of facies and associations • Possibility of semi-quantitative/quantitative evaluation	• Need of specialists in taxonomy • High cost, major means out at sea • Slow recording and processing of information • Positioning difficult in the presence of currents • Difficulty of observation and access according to the complexity of the habitat (multilayer assemblages) • Quantitative assessments only on conspicuous species/taxa	Cánovas Molina et al. (2016a); Enrichetti et a. (2019); Piazzi et al. (2019b)
Underwater diving observation	0 m to 40 m	Small areas (less than 250 m^2)	From 1 m	0.0001 to 0.001 km²/hour	• Non-destructive • Very good precision for the identification (taxonomy) and characterisation of the habitat (also its 3-D) • Low cost, easy to implement • Possibility to collect samples • Data already available after dive	• Need of specialists in taxonomy • Small area inventoried • Very time-consuming underwater • Limited operational depth • Highly qualified divers required • Subjectivity of the observer • Quantitative assessments only on conspicuous species/taxa	Gatti et al. (2012, 2015a) Piazzi et al. (2019a)
Methods	**Depth range**	**Surface area**	**Resolution**	**Efficiency**	**Advantages**	**Limits**	**References**
Underwater diving photography or video recording	0 m to 40 m	Small areas (less than 250 m^2)	From 0.1 m	0.0001 to 0.001 km²/hour	• Non-destructive • Good precision for the identification (taxonomy)	• Need of specialists in taxonomy • Small area inventoried • Photographs or video analysis very time-consuming	Gatti et al. (2015b); Montefalcone et al. (2017); Piazzi et al.

					and characterisation of the habitat • *A posteriori* identification possible • Quantitative assessments only on conspicuous species/taxa • Low cost, easy to implement • Possibility to collect samples • Possibility to create archives	• Limited operational depth • Highly qualified divers required • Tools to collect photos/video necessary • Limited number of species/taxa observed • Only 2-D observation allowed	(2017a, 2019a)
Underwater diving sampling by scraping or collection	0 m to 40 m	Small areas (less than 10 m^2)	From 1 m	0.0001 to 0.001 km²/hour	• Very good precision for the identification (taxonomy) and characterisation of the habitat • All species taken into account • *A posteriori* identification • Low cost, easy to implement	• Destructive method • Very small area inventoried • Sampling material needed • Limited operational depth • Highly qualified divers required • Very time-consuming underwater • Analysis of samples in laboratory very time-consuming	Bianchi et al. (2004b)
Methods	**Depth range**	**Surface area**	**Resolution**	**Efficiency**	**Advantages**	**Limits**	**References**
Sampling from vessels with blind grabs, dredges or box corers	0 m to about 120 m (until the lower limit of the rhodoliths habitat)	Intermediate areas (a few km^2)	From 1 to 10 m	0.025 to 0.01 km²/hour	• Very good precision for the identification (taxonomy) and characterisation of the habitat • All species taken into account • *A posteriori* identification • Low cost, easy to implement	• Destructive method • Small area inventoried • Sampling material needed • Samples analysis in laboratory very time-consuming	UNEP/MAP-RAC/SPA (2015)

An effective monitoring should be done at defined intervals over a period of time, even if it could mean a reduced number of sites being monitored. The reference "zero-state" will be then contrasted with data coming from subsequent monitoring periods, always assuring reproducibility of data over time. Thus, the experimental protocol has capital importance. Geographical position of surveys and sampling stations must be located with precision (using buoys on the surface and recording their coordinates with a GPS), and it often requires the use of marking underwater (with fixed pickets into the rock) for positioning the square frames or transects in the exact original position. Finally, even if it cannot be denied that there are logistical constraints linked to the observation of coralligenous and rhodoliths habitats, their long generation time enables sampling to be done at long intervals of time (> 1 year) to monitor them in the long term (Garrabou et al., 2002).

Although destructive methods (total scraping of the substrate and of all organisms present over a given area) have long been used and recognized as the most suitable approach to describe the structure of assemblages and an irreplaceable method for exhaustive species lists, they are not desirable for long-term regular monitorings (UNEP/MAP-RAC/SPA, 2008) and especially within MPAs. Moreover, identification of organisms needs great taxonomic expertise and a long time to analyse samples, making it difficult to process the large number of replicates required for ecological studies and monitoring surveys. It is more suitable to favour non-destructive methods, like photographic sampling or direct underwater observation in given areas (using square frames or transects) to collect quantitative data. These methods do not require sampling of organisms and are therefore absolutely appropriate for long-term monitoring. These different methods can be used separately or together according to the aims of the study, the area inventoried and means available (Tab. 3). Non-destructive methods are increasingly used and – mainly for photographic sampling – enjoy significant technological advances.

Table 3: Comparison between three traditional methods used to monitor coralligenous and other bioconstructions (Bianchi et al., 2004b).

In situ sampling	
Advantages	Taxonomical precision, objective evaluation, reference samples
Limits	High cost, slow laborious work, intervention of specialists, limited area inventoried, destructive method
Use	Studies integrating a strong taxonomical element
Video or photography	
Advantages	Objective evaluation, can be reproduced, reference samples, can be automated, speedy diving work, big area inventoried, non-destructive method
Limits	Low taxonomical precision, problem of *a posteriori* interpretation of pictures
Use	Studies on the biological cycle or over-time monitoring, large depth-range investigated
Underwater visual observation	
Advantages	Low cost, results immediately available, large area inventoried, can be reproduced, non-destructive method
Limits	Risk of taxonomic subjectivity, slow diving work
Use	Exploratory studies, monitoring of populations, bionomic studies

Differently from seagrass, the descriptors used to monitor coralligenous assemblages vary greatly from one team to another and from one region to another, as well as their measuring protocol (Piazzi et al., 2019a and references therein). A first standardised sheet for coralligenous monitoring was created in the context of the Natura 2000 programmes, which solved only partially the issues about comparability among data (Fig. 5). However, methods and descriptors taken into account must be the subject of a standardised protocol. Although many disparities among data acquisition methods still occur, an integrated and standardised procedure named STAR (STAndaRdized coralligenous evaluation procedure) for monitoring the condition of coralligenous reefs has recently been proposed (Piazzi et al., 2019a).

Natura 2000 - Fiche Coralligène – ANTONIOLI 2010 – GIS Posidonie

- Date : …………………. - Observateur :…………………… - N° de plongée & site :………..………..

- **Type de faciès :** *Cystoseira zosteroides* ☐ *Eunicella singularis* ☐ *Eunicella cavolinii* ☐ *Lophogorgia sarmentosa* ☐ *Paramuricea clavata* ☐ Autre : ………………… ☐

- **Gorgone :**

Non ⟶ Oui

	- -	-	+	+ +
Toutes les classes de taille				
Nécrose				
Gorgone arrachée				
Epibiontes				
Recrutement (<3cm)				

Gorgonaire	Espèce :……………
…………….cm	..…………….cm
…………….cm	……………..cm
……………..cm	……………..cm
……………..cm	……………..cm
…………..….cm	…..……..…..cm
……………..cm	…………..….cm

- **Aspect général :**

Non ⟶ Oui

	- -	-	+	+ +
Sédimentation / vase				
Voiles algaux				
Impression de diversité (très coloré)				
Faune cryptique riche				

Filet ☐
Ancrage ☐
Fil ☐
Déchet ☐

Profondeur d'observation des gorgonaires :
- Max :
- Min :

- **Inventaire :**

Macrophytes	
Lithophyllum & Mesophyllum en 3D	
Couverture de *Lithophyllum incrusans* sans relief	
Taches blanches sur Lithophyllum ou Mesophyllum	
Présence d'espèces dressées *Halimeda*, *Udotea* ; *Cystoseira*…	

Spongiaire & Bryozoaire	
Eponges perforantes (Clione)	
Espèces dressées (*Axinella sp.*, *Spongia agaricina*,…)	
Grands bryozoaires branchus	

Ichtyofaune	
Présence d'espèces-cibles avec grands individus	
Poissons benthiques ou nectobenthiques	

- **Observation :**

Photos quadrats et paysagères à réaliser

Figure 5: Example of a standardised sheet for coralligenous monitoring created in the context of the Natura 2000 programmes by GIS Posidonie (Antonioli, 2010).

A standardized protocol for monitoring shallow water (up to 40 m depth) coralligenous habitat

The protocol STAR (STAndaRdized coralligenous evaluation procedure) (Piazzi et al., 2019a) has been proposed for monitoring the condition of coralligenous reefs to obtain information about most of the descriptors used by the different ecological indices adopted to date on coralligenous reefs, through a single sampling effort and data analysis.

Monitoring plans should first distinguish between the two major bathymetrical ranges where coralligenous reefs develop, i.e. the shallow and the deep reefs, within and deeper than about 40 m depth respectively (UNEP/MAP-RAC/SPA, 2008). In fact, shallow and deep coralligenous habitats can show different structure of assemblages, and they are usually subject to different types of anthropogenic pressures. Shallow reefs can be effectively surveyed by scuba diving, allowing obtaining information about descriptors that cannot be evaluated or measured through any other instrumental methods (Gatti et al., 2012, 2015a).

Season: coralligenous assemblages comprise mostly organisms with long life cycles that are subjected to less evident seasonal changes (mainly in water temperature) than shallower assemblages. In contrast, several temporal changes throughout the year have been observed for macroalgal assemblages, and some seasonal erect algae and filamentous species constituting turfs decrease in cover during the cold season. In addition, coralligenous assemblages are often subjected to the invasion of alien macroalgae and most of the invasive macroalgae display seasonal dynamics, thus contributing to modifying the structure of coralligenous assemblages. The most widespread invasive species on coralligenous reefs are the turf-forming Rhodophyta *Womersleyella setacea* and the Chlorophyta *Caulerpa cylindracea*. These two species reach their highest abundance between the end of summer and autumn. The seasonal dynamics of native and invasive macroalgae thus suggest planning monitoring activities between April and June, and no more that once per year.

Depth and slope: the depth range where coralligenous reefs can develop changes with latitude and characteristics of the water. Moreover, different kind of assemblages may develop within the depth range of shallow coralligenous reefs. The slope of the rocky substrate is also important to determine the structure of coralligenous assemblages. In order to define a standardised sampling procedure suitable to collect comparable data, the range of sampling depth and substrate inclination must be fixed. In this context, a depth of around 35 m on a vertical substrate (i.e., slope 85–90°) can be considered as optimal to ensure the presence of coralligenous assemblages in most of the Mediterranean Sea, including the southern areas in oligotrophic waters. Vertical rocky substrates at about 35 m depth can also be easily found near the coast, which is in the zone mostly subjected to anthropogenic impacts.

Sampling design, sampling surface and number of replicates: Coralligenous assemblages show a homogeneous structure when subjected to similar environmental conditions, at least within the same geographic area. They are thus characterised by low variability at spatial scales between hundreds of metres to kilometres, while variability at smaller spatial scales (from metres to tens of metres) is usually high (Abbiati et al., 2009; Ferdeghini et al., 2000; Piazzi et al., 2016). These findings suggest planning sampling designs focusing on high replication at small scales (i.e., tens of metres), whereas intermediate or large scales (i.e. hundreds of metres to kilometres respectively) will require fewer replicates.

The sampling surface is related to the number of replicates and represents an important factor to be considered. A minimum surface suitable to sample coralligenous assemblages has never been established unambiguously, so different replicated sampling surfaces have been proposed depending on the methods adopted (Piazzi et al., 2018 and references therein). Researchers agree that the replicated sampling surface has to be larger than that utilized for shallow Mediterranean rocky habitats (i.e., ≥400 cm^2; Boudouresque, 1971), since the abundance of large colonial animals that characterise coralligenous assemblages could be underestimated when using small sampling areas (Bianchi et al., 2004b). Independent of the number of replicates, most of the proposed approaches suggest a total sampling area ranging between 5.6 and 9 m^2. Parravicini et al. (2009) reported that a sufficiently large sampling surface is more important than the specific method (e.g., visual quadrates or photography) to measure human impacts on Mediterranean rocky reef communities. Larger sampling areas with a lower number of replicates are used for seascape approaches (Gatti et al., 2012). On the contrary, most of the proposed sampling techniques for biocenotic approaches consider a greater number of replicates with a comparatively smaller sampling area, usually disposed along horizontal transects (Cecchi et al., 2014; Deter et al., 2012; Kipson et al., 2011, 2014; Piazzi et al., 2015; Sartoretto et al., 2017; Teixidó et al., 2013). A comparison between the two sampling designs tested in the field showed no significant differences (Piazzi et al., 2019a), suggesting that both approaches can be usefully employed. Thus, three areas of 4 m^2 located tens of metres apart should be sampled, and a minimum of 10 replicated photographic samples of 0.2 m^2 each should be collected in each area by scientific divers, for a total sampling surface area of 6 m^2. This design can be repeated depending on the size of the study site and allows analysis of the data through both seascape and biocenotic approaches (see the *Ecological Indices* paragraph below).

<u>Sampling techniques</u>: coralligenous assemblages have been usually studied by destructive methods employing the total scraping of the substrate, by photographic methods associated with determination of taxa and/or morphological groups and by visual census techniques. The best results can be obtained integrating photographic sampling and in situ visual observations. The former is the most cost-effective method that requires less time spent underwater and allows collecting the large number of samples required for community analysis in a habitat with high spatial variability at small spatial scales. The latter method, using square frames enclosing a standard area of the substrate, has been shown equally effective, but requires longer working time underwater (Parravicini et al., 2010), which may represent a limiting factor at the depths where coralligenous assemblages thrive. A rapid visual assessment (RVA) method has been proposed for a seascape approach (Gatti et al., 2012, 2015a). RVA allows capturing additional information compared with the photographic technique, such as the size of colonies of erect species and the thickness and consistency of the calcareous accretion (see *Descriptors* below). A combination of photographic and visual approaches, using photographic sampling to assess the structure of assemblages and integrating information by collecting a reduced amount of data with the RVA method (i.e., the size of colonies of erect species and the thickness and consistency of the calcareous accretion) is thus suggested.

<u>Photographic samples analysis</u>: the analysis of photographic samples can be performed by different methods (Piazzi et al., 2019a and reference therein); the use of a very dense grid (e.g., 400 cells) or manual contouring techniques through appropriate software may be useful in order to reduce the subjectivity of the operator's estimate.

<u>Descriptors</u>:

- *Sediment load.* Coralligenous reefs are particularly exposed to sediment deposition, especially of fine sediments. Both correlative and experimental studies have demonstrated that the increase of sedimentation rate can lead to changes in the structure of coralligenous assemblages, facilitating the spread of more tolerant and opportunistic species and causing the reduction of both α- and β-diversity. Increased sedimentation may affect coralligenous assemblages by covering sessile organisms, clogging filtering apparatus and inhibiting the rate of recruitment, growth and metabolic processes. Moreover, sediment re-suspension can increase water turbidity, limiting algal production, and can cause death and removal of sessile organisms through burial and scouring. Thus, the amount of sediment deposited on coralligenous reefs has been considered by several researchers (Deter et al., 2012; Gatti et al., 2012, 2015a) and represents valuable information, together with biotic descriptors, to assess the ecological quality of a study area. The amount of sediment may be indirectly evaluated as percentage cover in photographic samples, as this method showed consistent results with those obtained through techniques directly estimating sediment deposition (i.e., by a suction pump).
- *Calcareous accretion*. The calcareous accretion of coralligenous reefs may be impaired by human-induced impacts. The growth of the calcareous organisms that deposit calcium carbonate on coralligenous reefs is a slow process that can be easily disrupted by environmental alterations. Thus, the thickness and consistency of the calcareous deposit can be considered an effective indicator of the occurrence of a positive balance in the bioconstruction process (Gatti et al., 2012, 2015a). The thickness and consistency of the calcareous deposit can be measured underwater through a hand-held penetrometer, with six replicated measures in each of the three areas of about 4 m^2 and tens of metres apart. For each measure, the handheld penetrometer marked with a millimetric scale must be pushed

into the carbonate layer, allowing the direct measurement of the calcareous thickness. By definition, a penetrometer measures the penetration of a device (a thin blade in this case) into a substrate, and the penetration depth will depend on the force exerted and on the strength of the material. In the case of a hand-held penetrometer, the force is that of the diver, and thus cannot be measured properly and provides a semi-quantitative estimate only. Supposing that the diver always exerts approximately the same force, the depth of the penetration will provide a rough estimate of the thickness of the material penetrated. A null penetration is indicative of a hard rock and suggests that the biogenic substrate is absent or the bioconstructional process is no longer active; a millimetric penetration indicates the presence of active bioconstruction resulting in a calcareous biogenic substrate; and a centimetric penetration reveals a still unconsolidated bioconstruction.

- *Erect anthozoans*. The long-living erect anthozoans, such as gorgonians, are considered key species in coralligenous reefs, as they contribute to the typical three-dimensional structure of coralligenous assemblages, providing biomass and biogenic substrata and contributing greatly to the aesthetic value of the Mediterranean sublittoral seascape. However, presence and abundance of these organisms may not necessarily be related to environmental quality, but rather to specific natural factors acting at the local scale (Piazzi et al., 2017a). Accordingly, coralligenous reefs without erect anthozoans may anyway possess a good ecological quality status. Most erect species are, however, affected by regional or global physical and climatic factors, such as global warming, ocean acidification and increased water turbidity, independent of local measures of protection. Several human activities acting locally, such as fishing, anchoring or scuba diving, may also damage erect. Thus, where erect anthozoans are structuring elements of coralligenous assemblages, they can be usefully adopted as ecological indicators through the measure of different variables. The size (mean height) and the percentage of necrosis and epibiosis of erect anthozoans should be assessed through the RVA visual approach, measuring the height of the tallest colony for each erect species and estimating the percentage cover of the colonies showing necrosis and epibiosis signs in each of the three areas of about 4 m^2 and tens of metres apart.

Structure of assemblages. Coralligenous assemblages are considered very sensitive to human induced pressures (Piazzi et al., 2019a and references therein). Correlative and experimental studies highlighted severe shifts in the structure of coralligenous assemblages subjected to several kinds of stressors. The most effective bioindicators used to assess the ecological quality of coralligenous reefs are erect bryozoans, erect anthozoans, and sensitive macroalgae, such as Udoteaceae, Fucales and erect Rhodophyta. On the other hand, the dominance of algal turfs, hydroids and encrusting sponges seems to indicate degraded conditions. Thus, the presence and abundance of some taxa/morphological groups may be considered as an effective indicator of the ecological status of coralligenous assemblages. A value of sensitivity level (SL) has been assigned to each taxon/morphological group on the basis of its abundance in areas subjected to different levels of anthropogenic stress, with SL values varying within a numerical scale from 1 to 10, where low values correspond to the most tolerant organisms and high values to the most sensitive ones (Piazzi et al., 2017a; Fig. 6). Recently, a method has been proposed to distinguish and measure sensitivity to disturbance (DSL) and sensitivity to stress (SSL), the former causing mortality or physical damage and the latter physiological alteration, of the sessile organisms thriving in coralligenous assemblages (Montefalcone et al., 2017). Discriminate effects of stress from effects of disturbance may allow a better understanding of the impacts of human and natural pressures on coralligenous reefs.

The percentage cover of the conspicuous taxa/morphological groups can be evaluated for each photographic sample. The cover values (in %) of each taxon/morphological group are then classified in eight classes of abundance (Boudouresque, 1971): (1) 0 to ≤0.01%; (2) 0.01 to ≤0.1%; (3) 0.1 to ≤1%; (4) 1 to ≤5%; (5) 5 to ≤25%; (6) 25 to ≤50%; (7) 50 to ≤75%; (8) 75 to ≤100%). The overall SL of a sample is then calculated by multiplying the value of the SL of each taxon/group (Fig. 6) for its class of abundance and then summing up all the final values. Coralligenous assemblages are characterised by high biodiversity that is mostly related to the heterogeneity of the biogenic substrate, which increases the occurrence of microhabitats and exhibits distinct patterns at various temporal and spatial scales. A decrease in species richness (i.e., α-diversity) in stressed conditions has been widely described for coralligenous reefs (Balata et al., 2007), but also the number of taxa/morphological groups per sample can be considered a further effective indicator of ecological quality. Thus, the richness (α-diversity, i.e. the mean number of the taxa/groups per photographic sample) should be computed.

Taxon/group	SL
Algal turf	1
Hydrozoans (e.g. *Eudendrium* spp.)	2
Pseudochlorodesmis furcellata	2
Perforating sponges (e.g. *Cliona* spp.)	2
Dyctiotales	3
Encrusting sponges	3
Encrusting bryozoans	3
Encrusting ascidians (also epibiontic)	3
Encrusting Corallinales, articulated Corallinales	4
Peyssonnelia spp.	4
Valonia spp., *Codium* spp.	4
Sponges prostrate (e.g. *Chondrosia reniformis, Petrosia ficiformis*)	5
Large serpulids (e.g. *Protula tubularia, Serpula vermicularis*)	5
Parazoanthus axinellae	5
Leptogorgia sarmentosa	5
Flabellia petiolata	6
Erect corticated terete Ochrophyta (e.g. *Sporochnus pedunculatus*)	6
Encrusting Ochrophyta (e.g. *Zanardinia typus*)	6
Azooxantellate individual scleractinians (e.g. *Leptopsammia pruvoti*)	6
Ramified bryozoans (e.g. *Caberea boryi, Cellaria fistulosa*)	6
Palmophyllum crassum	7
Arborescent and massive sponges (e.g. *Axinella polypoides*)	7
Salmacina-Filograna complex	7
Myriapora truncata	7
Erect corticated terete Rodophyta (e.g. *Osmundea pelagosae*)	8
Bushy sponges (e.g. *Axinella damicornis, Acanthella acuta*)	8
Eunicella verrucosa, Alcyonium acaule	8
Erect ascidians	8
Corallium rubrum, Paramuricea clavata, Alcyonium coralloides	9
Zooxantellate scleractinians (e.g. *Cladocora caespitosa*)	9
Pentapora fascialis	9
Flattened Rhodophyta with cortication (e.g. *Kallymenia* spp.)	10
Halimeda tuna	10
Fucales (e.g. *Cystoseira* spp., *Sargassum* spp.), *Phyllariopsis brevipes*	10
Eunicella singularis, Eunicella cavolini, Savalia savaglia	10
Aedonella calveti, Reteporella grimaldii, Smittina cervicornis	10

Figure 6: Values of the sensitivity level (SL) assigned to each of the main taxon/morphological group in the coralligenous assemblages (Piazzi et al., 2017a).

- *Spatial heterogeneity*. Coralligenous assemblages are also characterised by a high variability at small spatial scale, and consequently by high values of β-diversity, which is linked to the patchy distribution of the organisms. Under stressed conditions, the importance of biotic factors in regulating an organism's distribution decreases, and occurrence and abundance mostly follow the gradient of stress intensity (Balata et al., 2005). The loss of structuring perennial species and the proliferation of ephemeral algae lead to widespread biotic homogenization (Balata et al., 2007; Gatti et al., 2015b, 2017), and to a consequential reduction of β-diversity (Piazzi et al., 2016). Thus, the β-diversity of assemblages may be considered a valuable indicator of human pressure on coralligenous reefs. β-diversity, in general, can be calculated through different methods; in the case of coralligenous assemblages, variability of species composition among sampling units (heterogeneity of assemblages) has been measured in terms of multivariate dispersion calculated on the basis of distance from centroids (Piazzi et al., 2017a) through permutational analysis of multivariate dispersion (PERMDISP). Thus, any changes in compositional variability displayed by PERMDISP may be directly interpretable as changes of β-diversity.

Protocol for monitoring mesophotic (down to 40 m depth) coralligenous habitat

The use of unmanned vehicles, such as ROVs, may be considered suitable to survey deep coralligenous reefs in mesophotic environments, down to 40 m depth (UNEP/MAP-RAC/SPA, 2008; Cánovas-Molina et al., 2016a; Ferrigno et al., 2017). The Italian MSFD protocol (MATTM/ISPRA, 2016) for monitoring mesophotic coralligenous and rocky reefs includes a standard sampling design conceived to gather various quantitative components, such as the occurrence and extent of the habitat (either biogenic or rocky reefs), the siltation level, and the abundance, condition and population structure of habitat-forming megabenthic species (i.e., animal forests), as well as presence and typology of marine litter.

Three replicated video-transects, each at least 200 m long, should be collected in each area investigated (Enrichetti et al., 2019). Footages can be obtained by means of a ROV, equipped with a high definition digital camera, a strobe, a high definition video camera, lights, and a 3-jaw grabber. The ROV should also host an underwater acoustic positioning system, a depth sensor, and a compass to obtain georeferenced tracks to be overlapped to multi-beam maps when available. Two parallel laser beams (90° angle) can provide a scale for size reference. In order to guarantee the best quality of video footages, ROV are expected to move along linear tracks, in continuous recording mode, at constant slow speed ($< 0.3\ ms^{-1}$) and at a constant height from the bottom (< 1.5 m), thus allowing for adequate illumination and facilitating the taxonomic identification of the megafauna. Transects are then positioned along dive tracks by means of a GIS software editing. Each video transect is analysed through any of the ROV-imaging techniques, using starting and end time of the transect track as reference. Visual census of megabenthic species is carried out along the complete extent of each 200 m-long transect and within a 50 cm-wide visual field, for a total of 100 m^2 of bottom surface covered per transect.

From each transect the following parameters are measured on videos:

- Extent of hard bottom, calculated as percentage of total video time showing this type of substratum (rocky reefs and biogenic reefs) and subsequently expressed in m^2
- Species richness, considering only the conspicuous megabenthic sessile and sedentary species of hard bottom in the intermediate and canopy layers (*sensu* Gatti et al., 2015a). Organisms are identified to the lowest taxonomic level and counted. Fishes and encrusting organisms are not considered, as well as typical soft bottoms species. Some hard-bottom species, especially cnidarians, can occasionally invade soft bottoms by settling on small hard debris dispersed in the sedimentary environment. For this reason, typical hard bottom species (e.g., *Eunicella verrucosa*) encountered on highly silted environments have to be considered in the analysis
- Structuring species are counted, measured (height expressed in cm) and the density of each structuring species is computed and referred to the hard-bottom surface (as n° of colonies or individuals m^{-2})
- The percentage of colonies with signs of epibiosis, necrosis and directly entangled in lost fishing gears are calculated individually for all structuring anthozoans
- Marine litter is identified and counted. The final density (as n° of items m^{-2}) is computed considering the entire transect (100 m^2).

Within each transect, 20 random high definition photographs targeting hard bottom must be obtained, and for each of them four parameters are estimated, following an ordinal scale. Modal values for each transect are calculated. Evaluated parameters on photos include:

- Slope of the substratum: 0°,<30° (low), 30°-80° (medium),>80°(high)

- Basal living cover, estimated considering the percentage of hard bottom covered by organisms of the basal (encrusting species) and intermediate (erect species but smaller than 10 cm in height) layers: 0, 1 (<30%), 2 (30-60%), 3 (>60%)
- Coralline algae cover (indirect indicator of biogenic reef), estimated considering the percentage of basal living cover represented by encrusting coralline algae: 0, 1 (sparse), 2 (abundant), 3 (very abundant)
- Sedimentation level, estimated considering the percentage of hard bottom covered by sediments: 0%,<30% (low), 30-60% (medium),>60% (high).

Protocol for monitoring rhodoliths habitat

A standardised and common sampling method for monitoring rhodoliths seabeds is not available to date (UNEP/MAP-RAC/SPA, 2008). Mediterranean rhodoliths seabeds appear to possess more diverse species assemblages of coralline and peyssonneliacean algae than their Atlantic counterparts, and to be structured by a suite of combinations of rhodolith shapes and coralline compositions: from monospecific branched growth-forms, to multispecific rhodoliths (Basso et al., 2016). Therefore, the monitoring protocols available for sampling and monitoring rhodoliths in shallow subtidal waters cannot be applied as such and require calibrating to the Mediterranean specificities.

A recent proposal for monitoring rhodoliths beds can be found in Basso et al. (2016). Monitoring the rhodoliths habitat can be done by underwater diving and direct visual observation, with sampling and following taxa identification in laboratory. However, surveys using ROVs, towed cameras, or more usually sampling from vessels using blind grabs, dredges or box corers are often favoured because of the greater homogeneity of these populations (Tab. 4). Monitoring should address all the variables already described for the first descriptive characterisation of the habitat, with the addition of the full quantitative description of the rhodoliths community, through periodical surveys. A decrease in rhodoliths beds extent, live/dead rhodoliths ratio, live rhodoliths percentage cover, associated with change in the composition of the macrobenthic community (calcareous algal engineers and associated taxa) may reveal potential negative impacts acting on rhodoliths beds. All possible variations in growth form, shape, and internal structure of rhodoliths have been simplified in a scheme with three major categories as focal points along a continuum: compact and nodular pralines, larger and vacuolar boxwork rhodoliths, and unattached branches (Fig. 5). Each of the three end-members within rhodoliths morphological variability corresponds to a typical (but not exclusive) group of composing coralline species and associated biota and is possibly correlated with environmental variables, among which substratum instability (mainly due to hydrodynamics) and sedimentation rate are the most obvious. Thus, the indication of the percentage cover by the three live rhodoliths categories at the surface of each rhodoliths beds is a proxy of rhodoliths habitat structural and ecological complexity. The high species diversity hosted by rhodoliths beds requires time-consuming and expensive laboratory analysis for species identification. Videos and photos provide no information on rhodoliths composition owing to the absence of conspicuous, easy-to-detect species. Moreover, since most coralline species belong to a few genera only, the use of taxonomic ranks higher than species is not useful.

Table 4: Comparison between four traditional methods used to monitor rhodoliths habitat.

Underwater visual observation	
Advantages	Low cost, results immediately available, non-destructive method, reference samples, taxonomical precision, information on the distribution of species
Limits	Work limited as regards to depth, small area inventoried
Use	Exploratory studies, monitoring of assemblages, bionomic studies

Blind sampling (dredges, grabs or box corers)	
Advantages	Low cost, easy to implement, taxonomical precision, reference samples, analysis of substratum (granulometry, calcimetry, % of organic matter), large depth-range investigated
Limits	Low precision of observation, several replicates needed, limited area inventoried, destructive method
Use	Localised studies integrating a taxonomical element, validation of acoustic methods
ROV and towed cameras	
Advantages	Objective evaluation, reference samples (images), large area inventoried, non-destructive method, information on the distribution of species, large depth-range investigated
Limits	High cost, low taxonomical precision, problem of *a posteriori* interpretation of images, observation only of the superficial layers, little information on the substratum and basal layer
Use	Studies on distribution and temporal monitoring, validation of acoustic methods
Acoustic methods	
Advantages	Very large areas inventoried, information on hydrodynamics (sedimentary figures), can be reproduced, non-destructive method, large depth-range investigated
Limits	High cost, interpreting of sonograms, additional validation (inter-calibration), observation only of the superficial layers, no taxonomical information
Use	Studies over large spatial scales, monitoring of populations, bionomic studies

A minimum of three box-cores with opening ≥0.16 m^2 should be collected in each rhodoliths bed at the same depth, and to a depth of about 20 cm of sediment. One box-corer must be collected within the rhodoliths area with the highest percentage of live cover (on the basis of preliminary ROV dives), and the others as far as possible from it, following the depth gradient in opposite directions of the maximum rhodoliths bed extension. In many instances grab samples could be useful, but attention must be paid to seafloor surface disruption and mixing, and the possible loss of material during recovery. In those extreme cases of very coarse material preventing box-core penetration and closure, a grab could be used instead, although it cannot preserve stratification. Once the box-core is recovered a colour photograph of the whole surface of the box-core, at a high enough resolution to recognise the morphology of single live rhodoliths and other conspicuous organisms, must be collected. In addition, the possible occurrence of heavy overgrowths of fleshy algae that may affect rhodoliths growth rate must be reported. The following descriptors must then be assessed: 1) visual estimation of the percentage cover of live red calcareous algae; 2) visual estimation of the live/dead rhodoliths ratio calculated for the surface of the box-core; 3) visual assessment of the rhodoliths morphologies characterising the sample (Fig. 5); 4) measurement of the thickness of the live rhodoliths layer. The sediment sample is then washed through a sieve (e.g., 0.5 mm mesh) and the sample treated with Rose Bengal to stain living material before being preserved for sorting under a microscope for taxa identification. All live calcareous algae and accompanying phytobenthos and zoobenthos should be identified and quantified, in order to allow for detection of variability in space and time, and any changes after possible impacts. Algal species must be evaluated using a semi-quantitative approach (classes of abundance of algal coverage: absent, 1-20%, 21-40%, 41-60%, 61-80%, >81%). For molecular investigations, samples from voucher rhodoliths morphotypes should be air-dried, and preserved in silica gel. The sediment sample should be analysed for grain-size (mandatory), and carbonate content.

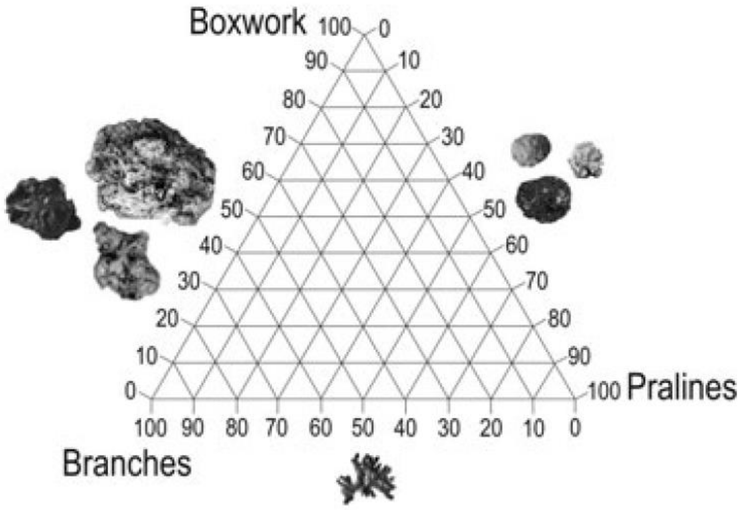

Figure 5: ternary diagram for the description of the rhodoliths bed tridimensionality. The percentage cover of each rhodoliths morphotype, relative to the total rhodoliths cover, can be plotted on the correspondent axis. The three main rhodoliths morphotypes (boxwork rhodoliths, pralines and unattached branches) are intended as focal points of a continuum, to which any possible rhodoliths morphology can be approximately assigned. From Basso et al. (2016).

Ecological Indices

To assess the ecological status of coralligenous reefs several ecological indices have been developed based on different approaches (Kipson et al., 2011, 2014; Teixidó et al., 2013; Zapata-Ramírez et al., 2013; David et al., 2014; Féral et al., 2014; Piazzi et al., 2019), which are summarised in Tab. 5. Most of the ecological indices available for monitoring shallow coralligenous reefs require underwater surveys by scuba diving. These indices have been developed following different approaches and adopt distinct descriptors and sampling techniques, thus hampering the comparison of data and results, and requiring inter-calibration procedures. Detailed descriptions of the sampling tools and the methodologies adopted for each index listed in Table 5 can be found in the relative bibliographic references.

For instance, ESCA (Ecological Status of Coralligenous Assemblages; Cecchi et al., 2014; Piazzi et al., 2015, 2017a), ISLA (Integrated Sensitivity Level of coralligenous Assemblages; Montefalcone et al., 2017), and CAI (Coralligenous Assessment Index; Deter et al., 2012) indices are based on a biocenotic approach where coralligenous assemblages are investigated in terms of composition and abundance of all species for ESCA and ISLA, and percentage cover of mud, bryozoans, and builder organisms (i.e., Corallinales, bryozoans, scleractinians) for CAI.

EBQI (Ecosystem-Based Quality Index; Ruitton et al., 2014) adopts a trophic web approach at the ecosystem level, in which the different functional components are identified, and an ecological status index is measured for each of them.

COARSE (COralligenous Assessment by ReefScape Estimate; Gatti et al., 2012, 2015a) uses a seascape approach to provide information about the structure of coralligenous reefs in order to assess the seafloor integrity. Since the coralligenous is characterised by high heterogeneity, extreme patchiness and coexistence of several biotic assemblages, a seascape approach seems to be the most reasonable solution for its characterisation.

OCI (Overall Complexity Index; Paoli et al., 2016) combines measures of structural and functional complexity, while the INDEX-COR (Sartoretto et al., 2017) integrates three descriptors (the sensitivity of taxa to organic matter and sediment deposition, the observable taxonomic richness, and the structural complexity of assemblages) to assess the health status of coralligenous assemblages.

Inter-calibrations among some of the above listed ecological indices have already been carried out. Comparison between ESCA and COARSE (Montefalcone et al., 2014; Piazzi et al., 2014, 2017a, 2017b), which are the two indices with the greatest number of successful applications to date (Piazzi et al., 2017b) in 24 sites of the NW Mediterranean Sea showed that the two indices provided different but complementary information to determine the intrinsic quality of coralligenous reefs and to detect the effects of human pressures on the associated assemblages. The concurrent use of ESCA and COARSE can thus be effective in providing information about the alteration of ecological quality of coralligenous reefs. A recent comparison among ESCA, ISLA, and COARSE has also been carried out (Piazzi et al., 2018), which proved that main differences among indices are linked to the different approaches used, and that ESCA and ISLA showed highly consistent results being based on a biocenotic approach. Finally, CAI, ESCA, COARSE, and INDEX-COR have been compared in 21 sites along the southern coasts of France (Gatti et al., 2016). Results showed that the four indices are not always concordant in indicating the ecological quality of coralligenous habitats, some metrics being more sensitive than others to the increasing pressure levels.

Few efforts have been made to define indices for mesophotic environments based on ROV footages, resulting in three seascape indices (Tab. 6), namely MAES (Mesophotic Assemblages Ecological Status; Cánovas-Molina et al., 2016a), CBQI (Coralligenous Bioconstructions Quality Index; Ferrigno et al., 2017), and MACS (Mesophotic Assemblages Conservation Status; Enrichetti et al., 2019). MACS is a new multi-parametric index that is composed by two independent units, the Index of Status (Is) and the Index of Impact (Ii) following a DPSIR (Driving forces – Pressures – Status – Impacts – Response) approach. The index integrates three descriptors included in the MSFD and listed by the Barcelona Convention to define the environmental status of seas, namely biological diversity, seafloor integrity, and marine litter. The Is depicts the biocoenotic complexity of the investigated ecosystem, whereas the Ii describes the impacts affecting it. Environmental status is the outcome of the status of benthic communities plus the amount of impacts upon them: the integrated MACS index measures the resulting environmental status of deep coralligenous habitats reflecting the combination of the two units and their ecological significance. The MACS index has been effectively calibrated on 14 temperate mesophotic reefs of the Ligurian and Tyrrhenian seas, all characterised by the occurrence of temperate reefs but subjected to different environmental conditions and levels of human pressures.

Final remarks

Inventorying and monitoring the condition of coralligenous reefs and rhodoliths seabeds in the Mediterranean constitutes a unique challenge given the ecological and economic importance of these habitats and the threats that hang over their continued existence. Long ignored due to their difficult accessibility and the limited means of investigation, today these habitats are widely included in monitoring programs to assess environmental quality.

A standardised approach must be encouraged for monitoring the condition of coralligenous reefs and rhodoliths seabeds, and in particular:

- Knowledge on coralligenous reefs and rhodoliths seabeds distribution should be continuously enhanced at the Mediterranean scale and reference areas/sites should be individuated
- Long chronological dataset must be envisaged, and a network of Mediterranean experts settled up
- Monitoring networks, locally managed and coordinated on a regional scale, should be started, and the standardised protocols here proposed should be applied to the entire Mediterranean both on coralligenous reefs and rhodoliths seabeds.

Table 5: Descriptors used in the ecological indices mostly adopted in the regional/national monitoring programs to evaluate environmental quality of shallow (up to 40 m depth) coralligenous habitat and based on different approaches.

Index	Method	Image analysis	Descriptors
Biocenotic			
ESCA	Photographic samples: 30 photographic quadrates (50 cm×37.5 cm) in two areas hundreds of metres apart	Software Image J' for the estimation of the % cover of the main taxa and/or morphological groups of sessile macro-invertebrates and macroalgae	3 descriptors: Sensitivity Level of all species (SL); α diversity (diversity of assemblages); β diversity (heterogeneity of assemblages)
ISLA	Photographic samples: 30 photographic quadrates (50 cm×37.5 cm) in two areas hundreds of metres apart	Software Image J' for the estimation of the % cover of the main taxa and/or morphological groups of sessile macro-invertebrates and macroalgae	2 descriptors: Integrated Sensitivity Level of all species (ISL) i.e., SL to stress and SL to disturbance
CAI	Photographic samples: 30 photographic quadrates (50 cm×50 cm) along a 40 m long transect	Software CPCe 3.6 for the estimation of the % cover by each species	3 descriptors: % cover of mud; % cover of builders; % cover of bryozoans
Ecosystem			
EBQI	Direct in situ observation and samples. A simplified conceptual model of the functioning of the ecosystem with 10 functional compartments		11 descriptors: % cover of builders; % cover of non-calcareous species; abundance of filter and suspension feeders; occurrence of bioeroders and density of sea urchins; abundance of browsers and grazers; biomass of planktivorous fish; biomass of predatory fish; biomass of piscivorous fish; Specific Relative Diversity Index for fish; % cover of benthic detritus matter; density of detritus feeders
Seascape			
COARSE	Direct in situ observations with Rapid Visual Assessment (RVA): 3 replicated		9 descriptors, 3 per each layer: Basal layer: % cover of encrusting calcified rhodophyta, non-calcified encrusting algae, encrusting animals, turf-

	visual estimation over an area of about 2 m^2 each		forming algae and sediment; a semi-quantitative assessment of boring species marks; thickness and consistency of calcareous layer with a hand held penetrometer (5 replicates) Intermediate layer: specific richness; n° of erect calcified organisms; sensitivity of bryozoans Upper layer: total % cover of species; % of necrosis of each population; maximum height of the tallest specimen
Integrated			
INDEX-COR	Photographic samples and direct observations: 30 photographic quadrates (60 cm×40 cm) along two 15 m long transects (15 photos per transect); visual census of marine litter, conspicuous benthic sessile and mobile species (echinoderms, crustacean decapods and nudibranchs), estimation of the % cover of gorgonians and sponges, % of necrotic gorgonian colonies	Free software photoQuad, using the uniform point count technique	3 descriptors: Taxa Sensitivity level (TS) to organic matter and sediment input; taxonomic richness of conspicuous taxa that were recognizable visually on photo-quadrates and in situ; structural complexity of the habitat, defined from the % cover of the taxa belonging to basal and intermediate layers estimated from the photo-quadrates and the % cover of gorgonians and large sponges observed in situ along the transects for the upper layer
OCI	Available detailed maps of benthic habitats		Surface area covered by coralligenous obtained from maps; list of the main taxonomic groups found in the habitat; biomass per unit area of each taxonomic group obtained from the literature. These descriptors are used to compute exergy and specific exergy as a measure of structural complexity, whilst throughput and information as a measure of functional complexity

Table 6: Descriptors used in the ecological indices mostly adopted in the regional/national monitoring programs to evaluate environmental quality of deep (from 40 m to about 120 m depth) coralligenous habitat occurring in the shallow mesophotic zone.

Index	Method	Image analysis	Descriptors
Seascape			
MAES	ROV survey: 500 m long video transects per area and 20 random high-resolution photographs frontally on the seafloor	VLC program for video and Image J' software for photos	6 descriptors: n° of megabenthic taxa, % biotic cover in the basal layer; density of erect species; average height and % cover of the dominant erect species; % of colonies with epibiosis/necrosis; density of marine litter
CBQI	ROV survey and photographs	VisualSoft software for video and DVDVideoSoft software to obtain random frames every 10 s for quantitative analysis	9 descriptors: % cover of coralligenous on the bottom; n° of morphological groups; density of fan corals; % of colonies with epibiosis/necrosis; % of colonies with covered/entangled signs; % of fishing gear; depth; slope; substarte type
MACS	ROV survey: three replicated video transects, each at least 200 m long, and 20 random high-resolution photographs frontally on the seafloor	VLC program for video and Image J' software for photos	12 descriptors: species richness of the conspicuous megabenthic sessile and sedentary species in the intermediate and canopy layers; % cover of basal encrusting species; % cover of coralline algae; dominance of structuring species; density of structuring species; height of structuring species; % cover of sediment; % of colonies with signs of epibiosis; % of colonies with signs of necrosis; % of colonies directly entangled in lost fishing gears; density of marine litter; typology of marine litter

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Annex 1

List of the main species to be considered in the inventorying and monitoring coralligenous and rhodoliths habitats (from UNEP/MAP-RAC/SPA, 2015)

Coralligenous

Builders

Algal builders

Lithophyllum cabiochae (Boudouresque & Verlaque) Athanasiadis, 1999

Lithophyllum stictaeforme (J.E. Areschoug) Hauck, 1877

Lithothamnion sonderi Hauck, 1883

Lithothamnion philippii Foslie, 1897

Mesophyllum alternans (Foslie) Cabioch & M.L. Mendoza, 1998

Mesophyllum expansum (Philippi) Cabioch & M.L. Mendoza, 2003

Mesophyllum macedonis Athanasiadis, 1999

Mesophyllum macroblastum (Foslie) W.H. Adey, 1970

Neogoniolithon mamillosum (Hauck) Setchell & L.R. Mason, 1943

Peyssonnelia rosa-marina Boudouresque & Denizot, 1973

Peyssonnelia polymorpha (Zanardini) F. Schmitz, 1879

Sporolithon ptychoides Heydrich, 1897

Animal builders

Foraminifera

Miniacina miniacea Pallas, 1766

Bryozoans

Myriapora truncata Pallas, 1766

Schizomavella spp.

Turbicellepora spp.

Adeonella calveti Canu & Bassler, 1930

Smittina cervicornis Pallas, 1766

Pentapora fascialis Pallas, 1766

Schizoretepora serratimargo (Hincks, 1886)

Rhynchozoon neapolitanum Gautier, 1962

Polychaeta

Serpula spp.

Spirorbis sp.

Spirobranchus polytrema Philippi, 1844

Cnidaria

Caryophyllia (Caryophyllia) inornata (Duncan, 1878)

Caryophyllia (Caryophyllia) smithii Stokes & Broderip, 1828

Leptopsammia pruvoti Lacaze-Duthiers, 1897

Hoplangia durotrix Gosse, 1860

Polycyathus muellerae Abel, 1959

Cladocora caespitosa Linnaeus, 1767

Phyllangia americana mouchezii Lacaze-Duthiers, 1897

Dendrophyllia ramea Linnaeus, 1758

Dendrophyllia cornigera Lamarck, 1816

Bioeroders

Sponges

Clionidae (Cliona, Pione)

Echinoids

Echinus melo Lamarck, 1816

Sphaerechinus granularis (Lamarck, 1816)

Molluscs

Rocellaria dubia (Pennant, 1777)

Hiatella arctica Linnaeus, 1767

Lithophaga lithophaga Linnaeus, 1758

Petricola lithophaga (Retzius, 1788)

Polychaetes

Polydora spp.

Dipolydora spp.

Dodecaceria concharum Örsted, 1843

Sipunculids

Aspidosiphon (Aspidosiphon) muelleri muelleri Diesing, 1851

Phascolosoma (Phascolosoma) stephensoni Stephen, 1942

OTHER RELEVANT SPECIES (*invasive; **disturbed or stressed environments-usually, when abundant)

Algae

Green algae

Flabellia petiolata (Turra) Nizamuddin, 1987

Halimeda tuna (J. Ellis & Solander) J.V. Lamouroux, 1816

Palmophyllum crassum (Naccari) Rabenhorst, 1868

Caulerpa cylindracea Sonder, 1845

Caulerpa taxifolia (M. Vahl) C. Agardh, 1817*

Codium bursa (Olivi) C. Agardh, 1817**

Codium fragile (Suringar) Hariot, 1889*

Codium vermilara (Olivi) Chiaje, 1829**

Brown algae

Cystoseira zosteroides (Turner) C. Agardh, 1821

Cystoseiramontagnei var. compressa (Ercegovic) M. Verlaque, A. Blanfuné, C.F. Boudouresque, T. Thibaut & L.N. Sellam, 2017

Laminaria rodriguezii Bornet, 1888

Halopteris filicina (Grateloup) Kützing, 1843

Phyllariopsis brevipes (C. Agardh) E.C. Henry & G.R. South, 1987

Dictyopteris lucida M.A. Ribera Siguán, A. Gómez

Garreta, Pérez Ruzafa, Barceló Martí & Rull Lluch, 2005**

Dictyota spp.**

Stypopodium schimperi (Kützing) M. Verlaque & Boudouresque, 1991*

Acinetospora crinita (Carmichael) Sauvageau, 1899**

Stilophora tenella (Esper) P.C. Silva in P.C. Silva, Basson & Moe, 1996**

Stictyosiphon adriaticus Kützing, 1843**

“Yellow” algae (Pelagophyceae)

Nematochrysopsis marina (J.Feldmann) C. Billard, 2000**

Red algae

Osmundaria volubilis (Linnaeus) R.E. Norris, 1991

Rodriguezella spp.

Ptilophora mediterranea (H.Huvé) R.E. Norris, 1987

Kallymenia spp.

Halymenia spp.

Sebdenia spp.

Peyssonnelia spp. (non calcareous)

Phyllophora crispa (Hudson) P.S. Dixon, 1964

Gloiocladia spp.

Leptofauchea coralligena Rodríguez-Prieto & De Clerck, 2009

Acrothamnion preissii (Sonder) E.M. Wollaston, 1968*

Lophocladia lallemandii (Montagne) F. Schmitz, 1893*

Asparagopsis taxiformis (Delile) Trevisan de Saint-Léon, 1845*

Womersleyella setacea (Hollenberg) R.E. Norris, 1992*

Animals

Sponges

Acanthella acuta Schmidt, 1862

Agelas oroides Schmidt, 1864

Aplysina aerophoba Nardo, 1843

Aplysina cavernicola Vacelet, 1959

Axinella spp.

Chondrosia reniformis Nardo, 1847

Clathrina clathrus Schmidt, 1864

Cliona viridis (Schmidt, 1862)

Dysidea spp.

Haliclona (Reniera) mediterranea Griessinger, 1971

Haliclona (Soestella) mucosa Griessinger, 1971

Hemimycale columella Bowerbank, 1874

Ircinia oros Schmidt, 1864

Ircinia variabilis Schmidt, 1862

Oscarella sp.

Petrosia (Petrosia) ficiformis (Poiret, 1789)

Phorbas tenacior Topsent, 1925

Sarcotragus fasciculatus (Pallas, 1766)

Spirastrella cunctatrix Schmidt, 1868

Spongia (Spongia) officinalis Linnaeus, 1759

Spongia (Spongia) lamella Schulze, 1879

Cnidaria

Alcyonium acaule Marion, 1878

Alcyonium palmatum Pallas, 1766

Corallium rubrum Linnaeus, 1758

Paramuricea clavata Risso, 1826

Eunicella spp.

Leptogorgia sarmentosa Esper, 1789

Ellisella paraplexauroides Stiasny, 1936

Antipathes spp.

Parazoanthus axinellae Schmidt ,1862

Savalia savaglia Bertoloni, 1819

Callogorgia verticillata Pallas, 1766

Polychaeta

Sabella spallanzanii Gmelin, 1791

Filograna implexa Berkeley, 1835

Salmacina dysteri Huxley, 1855

Protula spp.

Bryozoans

Chartella tenella Hincks, 1887

Margaretta cereoides Ellis & Solander, 1786

Hornera frondiculata (Lamarck, 1816)

Tunicates

Pseudodistoma cyrnusense Pérès, 1952

Aplidium spp.

Microcosmus sabatieri Roule, 1885

Halocynthia papillosa Linnaeus, 1767

Molluscs

Charonia lampas Linnaeus, 1758

Charonia variegata Lamarck, 1816

Pinna rudis Linnaeus, 1758

Naria spurca (Linnaeus, 1758)

Luria lurida Linnaeus, 1758

Decapoda

Palinurus elephas Fabricius, 1787

Scyllarides latus Latreille, 1803

Maja squinado Herbst, 1788

Echinodermata

Antedon mediterranea Lamarck, 1816

Hacelia attenuata Gray, 1840

Centrostephanus longispinus Philippi, 1845

Holothuria (Panningothuria) forskali Delle Chiaje, 1823

Holothuria (Platyperona) sanctori Delle Chiaje, 1823

Pisces

Epinephelus spp.

Mycteroperca rubra Bloch, 1793

Sciaena umbra Linnaeus, 1758

Scorpaena scrofa Linnaeus, 1758

Raja spp.

Torpedo spp.

Mustelus spp.

Phycis phycis Linnaeus, 1766

Serranus cabrilla Linnaeus, 1758

Scyliorhinus canicula Linnaeus, 1758

Rhodoliths

(*invasive; **disturbed or stressed environments-usually, when abundant). Species that can be dominant or abundant are preceded by #

Algae

Red algae (calcareous)

Lithophyllum racemus (Lamarck) Foslie, 1901

Lithothamnion corallioides (P.L. Crouan & H.M. Crouan) P.L. Crouan & H.M. Crouan, 1867

Lithothamnion valens Foslie, 1909

Peyssonnelia crispata Boudouresque & Denizot, 1975

Peyssonnelia rosa-marina Boudouresque & Denizot, 1973

Phymatolithon calcareum (Pallas) W.H. Adey & D.L. McKibbin ex Woelkering & L.M. Irvine, 1986

Spongites fruticulosa Kützing, 1841

Tricleocarpa cylindrica (J. Ellis & Solander) Huisman & Borowitzka, 1990

Lithophyllum cabiochae (Boudouresque et Verlaque) Athanasiadis

Lithophyllum stictiforme (J.E. Areschoug) Hauck, 1877

Lithothamnion minervae Basso, 1995

Mesophyllum alternans (Foslie) Cabioch & Mendoza, 1998

Mesophyllum expansum (Philippi) Cabioch & Mendoza, 2003

Mesophyllum philippii (Foslie) W.H. Adey, 1970

Neogoniolithon brassica-florida (Harvey) Setchell & L.R. Mason, 1943

Neogoniolithon mamillosum (Hauck) Setchell & L.R. Mason, 1943

Peyssonnelia heteromorpha (Zanardini) Athanasiadis, 2016

Sporolithon ptychoides Heydrich, 1897

<u>Red algae (non builders)</u>

Osmundaria volubilis (Linnaeus) R.E. Norris, 1991

Phyllophora crispa (Hudson) P.S. Dixon, 1964

Peyssonnelia spp. (non calcareous)

Acrothamnion preissii (Sonder) E.M. Wollaston,1968*

Alsidium corallinum C. Agardh, 1827

Cryptonemia spp.

Felicinia marginata (Roussel) Manghisi, Le Gall, Ribera, Gargiulo & M. Morabito, 2014

Gloiocladia microspora (Bornet ex Bornet ex Rodríguez y Femenías) N. Sánchez & C.Rodríguez-Prieto ex Berecibar, M.J. Wynne, Barbara & R. Santos, 2009

Gloiocladia repens (C.Agardh) Sánchez & Rodríguez-Prieto, 2007

Gracilaria spp.

Halymenia spp.

Kallymenia spp.

Leptofauchea coralligena Rodríguez-Prieto & De Clerck, 2009

Nitophyllum tristromaticum J.J. Rodríguez y Femenías ex Mazza, 1903

Osmundea pelagosae (Schiffner) K.W. Nam, 1994

Phyllophora heredia (Clemente) J. Agardh, 1842

Rhodophyllis divaricata (Stackhouse) Papenfuss, 1950

Rytiphlaea tinctoria (Clemente) C. Agardh, 1824

Sebdenia spp.

Vertebrata byssoides (Goodenough & Woodward) Kuntze, 1891

Vertebrata subulifera (C.Agardh) Kuntze, 1891

Womersleyella setacea (Hollenberg) R.E. Norris, 1992*

<u>Green algae</u>

Flabellia petiolata (Turra) Nizamuddin, 1987

Caulerpa cylindracea Sonder, 1845*

Caulerpa taxifolia (M. Vahl) C. Agardh, 1817*

Codium bursa (Olivi) C. Agardh, 1817

Microdictyon umbilicatum (Velley) Zanardini, 1862

Palmophyllum crassum (Naccari) Rabenhorst, 1868

Umbraulva dangeardii M.J. Wynne & G. Furnari, 2014

Brown algae

Arthrocladia villosa (Hudson) Duby, 1830

Laminaria rodriguezii Bornet, 1888

Sporochnus pedunculatus (Hudson) C. Agardh, 1817

Acinetospora crinita (Carmichael) Sauvageau, 1899**

Carpomitra costata (Stackhouse) Batters, 1902

Cystoseira abies-marina (S.G. Gmelin) C. Agardh, 1820

Cystoseira foeniculacea (Linnaeus) Greville, 1830

Cystoseira foeniculacea f. *latiramosa* (Ercegovic?) A. Gómez Garreta, M.C. Barceló, M.A. Ribera & J.R. Lluch, 2001

Cystoseira montagnei var. compressa (Ercegovic) M. Verlaque, A. Blanfuné, C.F. Boudouresque, T. Thibaut & L.N. Sellam, 2017

Cystoseira zosteroides (Turner) C. Agardh, 1821

Dictyopteris lucida M.A. Ribera Siguán, A. Gómez Garreta, Pérez Ruzafa, Barceló Martí & Rull Lluch, 2005

Dictyota spp.

Halopteris filicina (Grateloup) Kützing, 1843

Nereia filiformis (J. Agardh) Zanardini, 1846

Phyllariopsis brevipes (C. Agardh) E.C. Henry & G.R. South, 1987

Spermatochnus paradoxus (Roth) Kützing, 1843

Stictyosiphon adriaticus Kützing, 1843

Stilophora tenella (Esper) P.C. Silva, 1996

Zanardinia typus (Nardo) P.C. Silva, 2000

Animals

Sponges

Aplysina spp.

Axinella spp.

Cliona viridis Schmidt, 1862

Dysidea spp.

Haliclona spp.

Hemimycale columella Bowerbank, 1874

Oscarella spp.

Phorbas tenacior Topsent, 1925

Spongia (Spongia) officinalis Linnaeus, 1759

Spongia (Spongia) lamella Schulze, 1879

Cnidaria

Alcyonium palmatum Pallas, 1766

Eunicella verrucosa Pallas, 1766

Paramuricea macrospina Koch, 1882

Aglaophenia spp.

Adamsia palliata (Müller, 1776)

Calliactis parasitica Couch, 1838

Cereus pedunculatus Pennant 1777

Cerianthus membranaceus (Gmelin, 1791)

Funiculina quadrangularis Pallas, 1766

Leptogorgia sarmentosa Esper, 1789

Nemertesia antennina Linnaeus, 1758

Pennatula spp.

Veretillum cynomorium Pallas, 1766

Virgularia mirabilis Müller, 1776

Polychaetes

Aphrodita aculeata Linnaeus, 1758

Sabella pavonina Savigny, 1822

Sabella spallanzanii Gmelin, 1791

Bryozoans

Cellaria fistulosa Linnaeus, 1758

Hornera frondiculata (Lamarck, 1816)

Pentapora fascialis Pallas, 1766

Turbicellepora spp.

Tunicates

Aplidium spp.

Ascidia mentula Müller, 1776

Diazona violacea Savigny, 1816

Halocynthia papillosa Linnaeus, 1767

Microcosmus spp.

Phallusia mammillata Cuvier, 1815

Polycarpa spp.

Pseudodistoma crucigaster Gaill, 1972

Pyura dura Heller, 1877

Rhopalaea neapolitana Philippi, 1843

Synoicum blochmanni Heiden, 1894

Echinodermata

Astropecten irregularis Pennant, 1777

Chaetaster longipes (Bruzelius, 1805)

Echinaster (Echinaster) sepositus Retzius, 1783

Hacelia attenuata Gray, 1840

Holothuria (Panningothuria) forskali Delle Chiaje, 1823

Leptometra phalangium Müller, 1841

Luidia ciliaris Philippi, 1837

Ophiocomina nigra Abildgaard in O.F. Müller, 1789

Parastichopus regalis Cuvier, 1817

Spatangus purpureus O.F. Müller 1776

Sphaerechinus granularis Lamarck, 1816

Stylocidaris affinis Philippi, 1845

Pisces

Mustelus spp.

Pagellus acarne (Risso, 1827)

Pagellus erythrinus (Linnaeus, 1758)

Raja undulata Lacepède, 1802

Scyliorhinus canicula (Linnaeus, 1758)

Squatina spp.

Trachinus radiatus Cuvier, 1829

3. Guidelines for monitoring dark habitats in Mediterranean

Table of contents

Introduction

Dark habitats[2] are environments where the luminosity is extremely weak (deep mesophotic zone), or even absent (aphotic zone) distributed throughout the Mediterranean basin from the sea surface (i.e., caves) to the deep-sea realm. The bathymetric extension of this lightless zone depends to a great extent on the turbidity of the water and corresponds to benthic and pelagic habitats starting from the deep circalittoral. Caves, which show peculiar environmental conditions that favour the installation of organisms typical of dark habitats, are also taken into account. Dark habitats are dependent on very diverse geomorphologic structures, e.g. underwater caves, submarine canyons, seamounts, slopes, isolated rocks, abyssal plains, brine anoxic lakes, and chemo-synthetic features such as cold seeps and hydrothermal springs. Dark habitats are considered as sensitive habitats in the Mediterranean Sea requiring protection (Habitat Directive 92/43), supporting peculiar assemblages that constitute veritable reservoirs of biodiversity that, therefore, must be protected and need further attention. Thus, dark habitats were considered under the Action Plan for their conservation adopted in the 18th Ordinary Meeting of the Contracting Parties to the Barcelona Convention (Turkey, December 2013). Among the objectives of the Action Plan (UNEP/MAP-RAC/SPA, 2015) there was the need to improve knowledge about dark populations (e.g., location, specific richness, functioning, and typology) through national and regional programs aimed at establishing a shared knowledge of dark habitats, of their distribution around the Mediterranean in the form of a geo-referenced information system (GIS), and of their condition to implement specific management interventions at the basin scale.

In this context, the need of practical guidelines aimed at harmonising existing methods for dark habitats monitoring and for subsequent comparison of results obtained by different countries has been highlighted. Based on recommendations from the previous CPs group meetings, the Regional Activity Centre for Specially Protected Areas (RAC/SPA) has been asked to improve the existing inventory tools and to propose a standardisation of the mapping and monitoring techniques for dark habitats in the context of the IMAP common indicators and in order to ease the task of the MPAs managers when implementing their monitoring programs. Thus, the main methods used in the Mediterranean for inventory and monitoring of dark habitats have been recently summarised in the "Draft guidelines for inventorying and monitoring of dark habitats (UNEP/MAP-SPA/RAC, 2017)" and the "Guidelines for inventorying and monitoring of dark habitats in the Mediterranean Sea" (SPA/RAC-UN Environment/MAP OCEANA, 2017). These guidelines are the base for the updating and harmonisation process undertaken in this document.

These updated guidelines aim to establish common methods 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[3], inventorying and monitoring initiatives focusing on marine caves should consider the cave habitat as a whole. Therefore, this updated document presents methodologies which cover both semi-dark and dark caves. Notwithstanding the increased scientific knowledge on dark habitats 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 Sea, 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.) the high cost and difficulties for accessing, and (2) the lack of historical data and long-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 remains limited. This issue should be addressed by CPs at the earliest convenience in order to put in

[2] 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 120-200 m).
[3]<0.01% of the light at the sea surface level, according to Harmelin et al. (1985).

place control systems aiming at the implementation of Ecosystem Approach (EcAp) procedures, and particularly an IMAP at the regional level.

A reviewing process on the scientific literature, taking into account the latest techniques and the recent works carried out by the scientific community at the international level, has been carried out to update the former draft guidelines. If some standardised protocols do exist for seagrass and coralligenous mapping and monitoring (and are also well-implemented in the case of seagrass), this is not the case for dark habitats. In this document a number of “minimal” descriptors to be taken into account for inventorying and monitoring dark habitats in the Mediterranean are described. The main methods adopted for their monitoring, with the relative advantages, restrictions and conditions of use, are presented.

Marine caves

Marine caves support well diversified and unique biological communities (Pérès and Picard, 1949; Pérès 1967; Riedl 1966; Harmelin et al., 1985), harbouring a variety of sciaphilic communities, usually distributed according to the following zonation scheme: (a) a (pre-)coralligenous[4] 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 at the end or at the confined areas of the cave, 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) (see Appendix I). The most frequently recorded sponge species are *Agelas oroides*, *Petrosia ficiformis* (often discoloured), *Spirastrella cunctatrix*, *Chondrosia reniformis* (often discoloured), *Phorbas tenacior*, and *Axinella damicornis* (Fig. 1). 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 significantly contribute 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* (in the southern areas of the Central and Western Mediterranean Sea) (Fig. 1); (ii) facies of *Corallium rubrum*, which is more common in the North-Western Mediterranean Sea but can be found only in deep waters (below 50 m depth) in the north-eastern basin (Fig. 1); 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) (Fig. 1). Facies of erect bryozoans (e.g., *Adeonella* spp. and *Reteporella* spp.) often develop in semi-dark caves (Pérès, 1967; Ros et al., 1985) (Fig. 1).

- *Dark cave communities*

The shift from semi-dark to dark cave communities is evidenced through a sharp decrease in biotic coverage, biomass, three-dimensional biotic 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, serpulids, bryozoans and brachiopods (Pérès, 1967) (see Appendix I). 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*, *Jaspis johnstoni*, *Haliclona mucosa*, and

[4] 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).

Lycopodina hypogea. Serpulid polychaetes are among the dominant taxa in these caves, with the typical species being *Serpula cavernicola* and *Spiraserpula massiliensis* (Zibrowius, 1971; Bianchi and Sanfilippo, 2003; Sanfilippo and Mòllica, 2000). In some caves, the species *Protula tubularia* forms 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. *Onychocella marioni*) can also produce nodular constructions in the transitional zone between semi-dark and dark cave communities (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, anthozoans, 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., 2002).

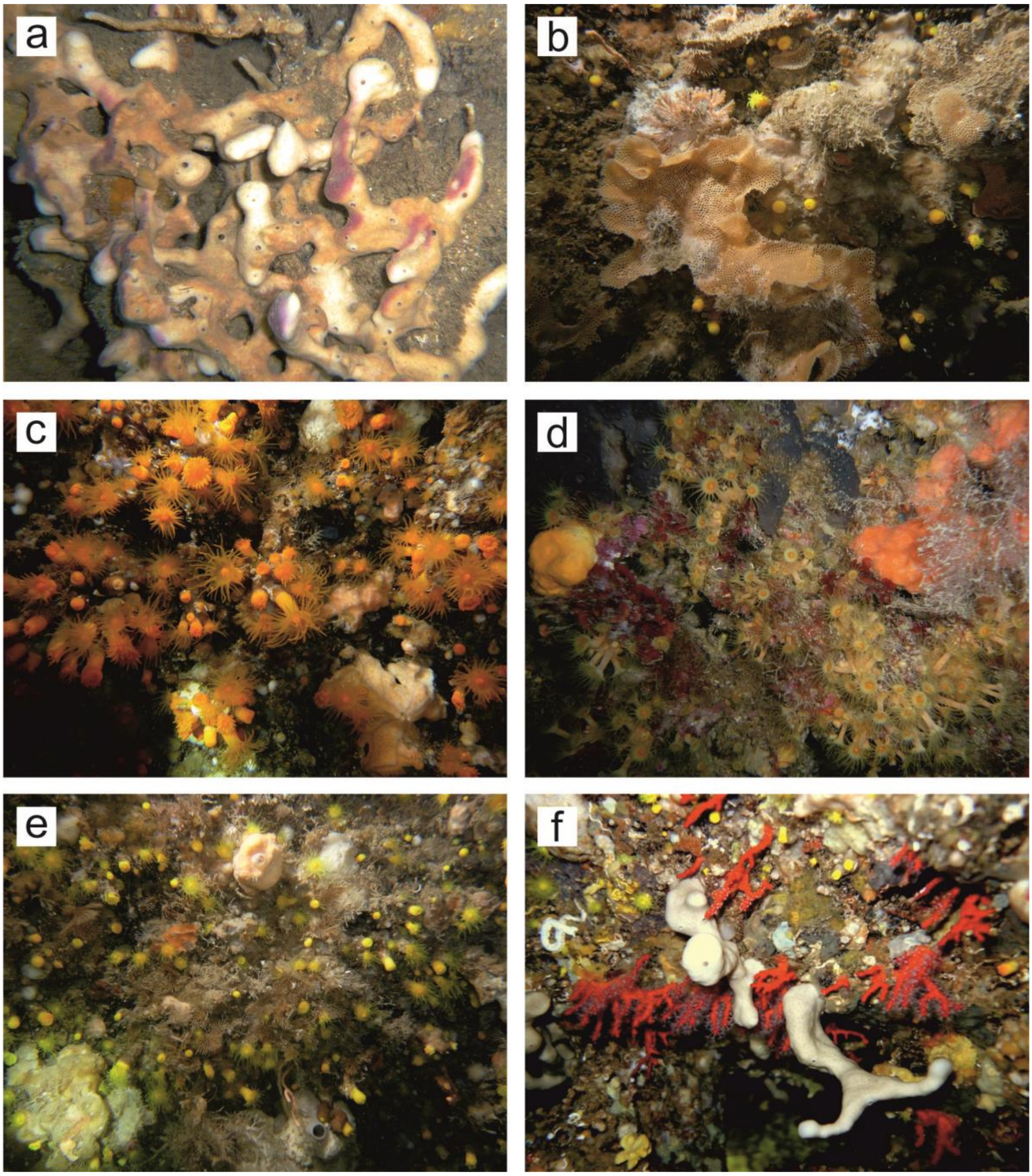

Figure 1: facies with *Petrosia ficiformis* (a), *Reteporella grimaldii* and other bryozoans (b), *Astroides calycularis* (c), *Parazoanthus axinellae* (d), *Leptopsammia pruvoti* (e), and *Corallium rubrum* (f) in semi-dark marine caves. Pictures by Monica Montefalcone (a-e) and Vasilis Gerovasileiou (f).

Knowledge on the marine caves distribution and ecology in the different sectors of the Mediterranean Sea can be summarised as follow:

Western Mediterranean Sea

A total of 1046 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 and 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 and Picard, 1949; Riedl, 1966; Harmelin et al., 1985; Ros et al., 1985; Bianchi and

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 characterised 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 submarine cave of Bergeggi (Ligurian Sea, Italy) provides the longest series of data on the status of benthic communities, being studied regularly since 1986 (Parravicini et al., 2010; Montefalcone et al., 2018).

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 and 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).

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 and 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 and Guidetti, 2009) and in Sicily (e.g., Rosso et al., 2013, 2014; Sanfilippo et al. 2015). Marine caves in this area were recently studied and evaluated for their ecological status.

Adriatic Sea

Up to date 708 marine caves have been recorded in the Adriatic Sea (Giakoumi et al., 2013), supporting approximately 400 taxa (Gerovasileiou and Voultsiadou, 2014). The coasts of Croatia are among the most 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 and 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. 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 *Monachus monachus* (Dendrinos et al., 2007). The coasts of Lebanon host most of the studied Levantine caves (e.g., Bitar and Zibrowius, 1997; Logan et al., 2002; Pérez et al., 2004; Vacelet et al., 2007; Morri et al., 2009). Forty-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.

Deep-sea habitats

Deep-sea habitats are those where either no sunlight arrives (aphotic zone) or where the light that does arrive is insufficient for the development of plant communities (deep mesophotic zone), usually at depths below 120-200 m. Deep-sea habitats display diverse geomorphologic structures: submarine canyons, seamounts, slopes, isolated rocks, abyssal plains, brine anoxic lakes, and chemo-synthetic features such as cold seeps and hydrothermal springs. Given their wide bathymetric range, parts of these geomorphologic formations may start in the upper mesophotic zone (down to 40 m depth). This is the case of the summits of seamounts and the heads of canyons, as well as some off-shore isolated rocks. To maintain their integrity, all of these habitats are included within the classification of dark habitats.

Deep-sea habitats may host complex three-dimensional animal forests over rocky reefs and detritic or muddy bottoms, and are mainly dominated by arborescent, structuring anthozoans, sponges and bryozoans. As agreed, and set out in the Dark Habitats Action Plan (UNEP/MAP-RAC/SPA, 2015), the existing biological communities characterising deep-sea habitats are the following:

- ✓ Assemblages of underwater canyons
- ✓ Assemblages associated with seamounts
- ✓ Engineering benthic invertebrate assemblages
  - Black coral and gorgonian forests on hard substrata
  - Beds with *Isidella elongata* and beds with pennatulaceans on detritic substrata
  - Associations of sponges on both types of substrata
- ✓ Deep-sea chemo-synthetic assemblages

However, thanks to advances in scientific knowledge, other recently discovered types are being added to the lists of deep-sea habitats.

The most characteristic habitat-forming species of the deep mesophotic and aphotic zones are sponges and anthozoans, although other phyla and classes, such as molluscs, polychaete tube-worms, bryozoans, and cirriped crustaceans, may also have a predominant role in some cases or be a fundamental part of mixed habitats, also through the formation of complex bioconstructions that provide three-dimensional structures (Fig. 2).

- *Habitats dominated or formed by stony corals (Scleractinia)*

The best known are Cold-Water coral (CWC) reefs, mainly formed by *Desmophyllum pertusum (*ex *Lophelia pertusa*) and *Madrepora oculata* (Orejas and Jiménez, 2019). 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 1000 m. They have been found both in the western and eastern central Mediterranean Sea, in areas such as the Cabliers, Chella and Avempace seamounts in the Alboran Sea (Pardo et al., 2011; de la Torriente et al., 2014; Lo Iacono et al. 2014), in canyons in the Gulf of Lion and the surrounding area such as Cassidaigne and Creus (Bourcier and Zibrowius, 1973; Orejas et al., 2009; Fourt and Goujard, 2012; Gori et al. 2013), in the estern Ligurian Sea (Fanelli et al., 2017), in the southern Catalan canyons (e.g., La Fonera canyon; Lastras et al., 2016; Taviani et al., 2019), south of Sardinia in the Nora Canyon (Taviani et al., 2017), in the Gulf of Naples (Taviani et al., 2017), offshore Santa Maria di Leuca in the Northern Ionian Sea

(Taviani et al., 2005a, b; Mastrototaro et al., 2010; Savini et al., 2014; Vertino et al., 2010; D'Onghia et al., 2012), south of Malta and other sites in the Strait of Sicily (Schembri et al., 2007; Freiwald et al., 2009; Taviani et al., 2009, 2011a; Evans et al., 2016), next to the Jabuka-Pomo depression (Županović, 1969), in the Bari canyon and off Apulia in the Southwestern Adriatic Sea (Freiwald et al., 2009; Angeletti et al., 2014; D'Onghia et al., 2015), 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., 2011a), 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 Sea it is rare to find places with dense populations (Pardo et al., 2011; Bo et al., 2014a). 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 (Pardo et al., 2011; de la Torriente et al., 2014), in submarine canyons in the Gulf of Lion and Corsica (Orejas et al., 2009; Gori et al. 2013; Fourt et al., 2014a), in the Balearic Archipelago continental shelf and slope (Orejas et al., 2014), on seamounts in the Tyrrhenian Sea (Bo et al., 2011), at mesophotic depths in the Ligurian Sea (Bo et al., 2014a), in some areas of the Central Mediterranean Sea (Würtz and Rovere, 2015), including the banks of the Ionian Sea (Amendolara Bank, Tursi et al., 2004; Bo et al., 2014a), and in the southern Adriatic Sea (Freiwald et al., 2009; Angeletti et al., 2015a). *D. ramea* is more common in shallower waters, especially at mesophotic depths. Recently, however, *D. ramea* communities have been found in deep waters in the eastern Mediterranean Sea, such as the deep seabeds of Cyprus (Orejas et al., 2017) 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 showed a more diffuse abundance and distribution in the past.

Other colonial stony corals that have been found forming dense aggregations in certain areas are *Madracis pharensis*, a typical component of cave assemblages that is particularly abundant in the coralligenous outcrops of the eastern Mediterranean basin, which is also abundant in the heads of canyons and coastal waters of Lebanon, at depths down to nearly 300 m, sometimes in mixed aggregations with brachiopods, molluscs 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 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 *Desmophyllum pertusum* and *Madrepora oculata* (Galil and Zibrowius, 1998; Montagna et al., 2006; Freiwald et al., 2009; Taviani et al., 2011b, 2016a, 2017; de la Torriente et al., 2014; Fourt et al., 2014a).

Species of the genus *Caryophyllia* settle on rocky and detritic bottoms and may become important. For example, *Caryophyllia (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 scleractinians such as *Javania cailleti*, *Stenocyathus vermiformis* and other *Caryophyllia* spp. It has been found in seamounts, escarpments or rocky bottoms (Galil and Zibrowius, 1998; Mastrototaro et al., 2010; Aguilar et al., 2013, 2014). In the case of soft bottoms, mainly in detritic sands, beginning in the deep circalittoral sand and extending to depths down to 400-500 m, *Caryophyllia (Caryophyllia) smithii* can cover significant areas (de la Torriente et al., 2014), similar to *Flabellum* spp. in the Atlantic (Baker et al., 2012; Serrano et al., 2016).

- *Habitats dominated or structured by black corals*

Antipatharians or black corals are represented in the Mediterranean by just a few species, although this number may increase with the new deep-sea explorations. They 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. 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, 2019a, 2019b; Ingrassia et al., 2016). *Antipathes dichotoma* can also occur with high densities, but many times are part of other black coral communities alongside gorgonians. They have a wide bathymetric distribution with some species occurring also in the upper mesophotic zone at relatively shallow depths (about 60 m) (Bo et al., 2009, 2019b), and others extending to the superficial bathyal zone and reaching depths of over 2000 m. It is known that some *Leiopathes* sp. inhabit depths down to 4000 m outside the Mediterranean Sea (Molodtsova, 2011). Dense aggregations have been found on seamounts in the Alboran (de la Torriente et al., 2014), the Balearic Archipelago (Grinyó, 2016), the Ligurian Sea (Bo et al., 2014a, 2019a), and the Tyrrhenian Seas (Bo et al., 2011, 2012; Fourt et al., 2014a; Ingrassia et al., 2016), in South Western Sardinia (Bo et al., 2015; Cau et al., 2016a), on the escarpments in the south of Malta (Deidun et al., 2015; Evans et al., 2016), 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 the Malta Escarpment and offshore Rhodes (Taviani et al., 2011b; Angeletti et al., 2015b).

Antipathella subpinnata, similarly to *Antipathes dichotoma*, normally occupies offshore mesophotic rocky elevations or deep coastal bottoms but may thrive also on seamount summits (Bo et al., 2009, 2014; de la Torriente et al., 2014), and reach greater depths. It has a wide distribution in the Mediterranean Sea, being recorded within white coral regions (Bo and Bavestrello, 2019), mainly in the western and central basins but also in the Aegean Sea (Vafidis and Koukouras, 1998; Bo et al., 2008). *A. wollastoni* has also been recorded near the Strait of Gibraltar (Ocaña et al., 2007).

Recently other black coral species have also been observed forming dense aggregations. Some examples are *Parantipathes larix* found 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., 2014a), and *Phanopathes rigida*, newly reported on seamounts between 180-400 m from the South of the Alboran Sea in the Cabliers Bank (Bo et al., 2019b). *Parantipathes larix* has a wide bathymetric distribution, from 120 m down to over 2000 m (Opresko and Försterra, 2004; Fabri et al., 2011; Bo et al., 2012b).

- *Habitats dominated by gorgonians*

Deep Mediterranean gorgonian assemblages (Alcyonacea excluding Alcyoniina) can be highly diverse and present a wide geographic and bathymetric distribution (Gori et al., 2017, 2019). 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 (Mastrototaro et al., 2017). Some of the assemblages that reach high densities are those formed by the Atlanto-Mediterranean gorgonian *Callogorgia verticillata*. Dense forests have been found that can begin in the deep mesophotic zone and extend to a depth of more than 1000 m (de la Torriente et al., 2014; Angeletti et al., 2015a; Evans et al., 2016; Gori et al., 2017, 2019). 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., *Desmophyllum pertusum*, *Dendrophyllia* spp). A frequent association of this species is with the whip coral (*Viminella flagellum*), especially in the deep circalittoral and upper bathyal zones (Giusti et al., 2012; Lo Iacono et al., 2012; Chimienti et al., 2019), 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 (Aguilar et al., 2013; Fourt et al., 2014b), 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; Enrichetti et al., 2019). 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 on rocky bottoms from littoral to great depths. *E. cavolini* was observed down to 280 m in the Nice canyon (Fourt and Chevaldonné, pers. obs.), however, they are more common on the tops of seamounts, forming monospecific assemblages or mixed with *Paramuricea clavata* (Aguilar et al., 2013; De la Torriente et al., 2014). 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., 2014a). 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 and Goujard, 2012).

There is a wide range of small gorgonians that can form dense thickets (Angiolillo et al., 2014; Grinyó et al., 2016) or co-occur alongside larger species such as *C. verticillata*, antipatharians or alongside cold-water coral reef building species (Evans et al., 2016; Chimienti et al., 2019). Among these species can be found *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 (Bo et al., 2011, 2012b, 2015; Giusti et al., 2012; Aguilar et al., 2013; Angeletti et al., 2014; Grinyó et al., 2015; Evans et al., 2016; Taviani et al., 2017).

Swiftia pallida 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 Sea, having been found on seamounts of the Alboran Sea (de la Torriente et al., 2014) to places as far away as the canyons off Lebanon (R. Aguilar, pers. obs.) and Israel (Zvi Ben Avraham, pers. obs.). 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 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 (Maldonado et al., 2015; Evans et al., 2016). Both can be found in the western and central Mediterranean Sea in zones ranging from a depth of 300 m to over 1000 m (Sartoretto and Zibrowius, 2018; Chimienti et al., 2019).

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 (Sartoretto, 2012; de la Torriente et al., 2014; Evans et al., 2016).

In the case of *Nicella granifera*, so far this has only been found in the western Mediterranean Sea, 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 1000 m in the bathyal zone (Rossi et al., 2008; Taviani et al., 2010; Knittweis et al., 2016), with a peak at mesophotic depths (Cattaneo 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 (Freiwald et al., 2009; Constatini et al., 2010; 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 Sea and which usually appears in muddy bottoms below depths of 400 m. It has been found on seamounts in the Alboran and Balearic Seas (Aguilar et al., 2013; de la Torriente et al., 2014; 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 at 190 m depth (Bo et al., 2015), in the bathyal plain of Malta (R. Aguilar, pers. obs.), and in the Ionian Sea (Mytilineou et al., 2014), among other places.

Other soft-bottom species include *Spinimuricea* spp. (Aguilar et al., 2008; Bo et al., 2012b; Topçu and Öztürk, 2016), 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 bathyal zone. They can therefore appear in all kinds of soft bottoms on seamounts and in canyons, on bathyal plains and shelf edges (Chimienti et al., 2019). Species of the genera *Pennatula* and *Pteroeides* can form mixed communities that become numerous on the shelf edges and the beginning of the slope (e.g., Chella Bank) (Gili and Pagès, 1987; Aguilar et al., 2013; de la Torriente et al., 2014). 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 Sea on seamount slopes, the shelf edges, plains, and in canyons (Gili and Pagès, 1987; Aguilar et al., 2013), 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 Sea, at depths ranging from the circalittoral to the bathyal zone. It forms dense forests in shelf areas, gently sloping areas in canyons, and muddy-sandy interstices on seamounts (Morri et al., 1991; Fabri et al., 2014; de la Torriente et al., 2014). 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 Sea (Balearic Sea, Central Mediterranean and Ionian Sea). This is *Protoptilum carpenteri* (Mastrototaro et al., 2015, 2017; R. 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 and Goujard, 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).

- *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., 2015) and *Nidalia studeri* (López-González et al., 2012), which create dense aggregations in the lower circalittoral and bathyal zones, between 150 m and 400 m. They can be found on hard bottoms, and on gravel and coarse sediments of seamounts, slope edges and submarine canyons. Their known geographical distribution stretches from the western to the central Mediterranean Sea, although a wider distribution has not been ruled out.

Equally important are species such as *Alcyonium palmatum* and *Paralcyonium spinulosum* (Templado et al., 1993; Fava and Ponti, 2007; Bo et al., 2011; Marin et al., 2011b, 2014; UNEP/MAP-RAC/SPA, 2013), since their plasticity in the occupation of both soft and hard bottoms allows them to colonise large areas of the Mediterranean basin, 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 Sea (R. 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 (Aguilar et al., 2008; Lastras et al., 2016), whereas *Arachnanthus* spp. usually appears in groups of hundreds or thousands of individuals slightly separated from each other (Marín et al., 2011a; Aguilar et al., 2014).

- *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 basin and an important role in deep ecosystems (Bo et al., 2012a; Calcinai et al., 2013; Angeletti et al., 2014; Taviani et al., 2016a), while those of the genus *Phakellia* are more common in the western basin (Aguilar et al., 2013; de la Torriente et al., 2014). 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*), which are common in shallow areas developing on the heads of canyons, shelf edges and in the upper bathyal zones (R. 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 (Bo et al., 2011, 2012b; Aguilar et al., 2013).

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 depths of more than 700 m near the Balearic Islands (Maldonado et al., 2015) and on Mejean bank between 380 and 455 m (Fourt and Chevaldonné, pers. obs.). It is not known whether other "stone 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 the same in the Mediterranean Sea.

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 Sea (Pansini and Musso, 1991; de la Torriente et al., 2014; Fourt et al., 2014a; Evans et al., 2016), sometimes with the presence of the carnivorous sponge *Cladorhiza abyssicola*, while *Rhizaxinella pyrifera* is more common in sandy-detritic bottoms (Bo et al., 2012a), but can also be found in cold seeps on mud volcanoes (Olu-Le Roy et al., 2004).

- *Sponge grounds with hexactinellids*

The large glass sponge *Asconema setubalense* is the most important in the formation of these aggregations of sponges in the Aboran Sea, 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 this area.

With a much wider distribution in the Mediterranean, reaching the eastern basin, *Tetrodictyum reiswegi* (Aguilar et al., 2014; Boury-Esnault et al., 2015, 2017) 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, at depths of 200-2500 m.

It is not known whether other species of hexactinellids that inhabit the Mediterranean Sea 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 of scattered individuals, but in this case on muddy bottoms. In the Mediterranean Sea it has been found from the Alboran to the Tyrrhenian Sea at depths between 350 m and more than 2000 m (Boury-Esnault et al., 2015).

All the species of anthozoans and sponges mentioned above which have a similar bathymetric distribution and substrate preference may form mixed habitats.

- *Habitats dominated 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 (Schembri et al., 2007; Angeletti et al., 2011; Deidun et al., 2015), although *Megabalanus* spp. may also create a number of communities of some importance, usually together with molluscs and corals (R. 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 and Bellan-Santini, 1990; Marín et al., 2014; Esposito et al., 2015; R. Aguilar, pers. obs.), at depths that range from the edge of shelf or on the seamount summits to down to more than 700 m.

- *Habitats dominated by 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*. All of them attach to rocky substrates, but also to gravel or coarse sediment, and their distribution covers the entire Mediterranean basin. 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 (Aguilar et al., 2010; de la Torriente et al., 2014). In soft bottoms, down to 350-400 m depths, some stalked species such as *Kinetoskias* sp. (Harmelin and D'Hondt, 1993; Aguilar et al., 2013, Maldonado et al., 2015), or species from the Candidae family (R. Aguilar, pers. obs.), may begin to appear. These bryozoans living on muddy bottoms have been found in the western and central Mediterranean basin (Mastrototaro et al., 2017).

- *Habitats dominated by polychaetes*

Many polychaetes form associations with species such as anthozoans, sponges, bryozoans, and brachiopods on rocky substrates of escarpments and mountains, in canyons and caves, 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); they may create small reefs together with corals, as for *Serpula vermicularis* in the Bari Canyon (Sanfilippo et al., 2013), or they can be found 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 and 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., 2017).

As for the terebellids, the sand mason worm (*Lanice conchilega*) creates patches 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 Sea, but data from the North Sea record densities of several hundreds or thousands of individuals per square meter, forming structures with some 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 chemo-synthetic communities, such as the Amsterdam mud volcano, between the Anaximenes and Anaxagoras marine ranges in the eastern Mediterranean basin (Shank et al., 2011).

- *Habitats dominated by molluscs*

The main aggregations, concretions and mollusc reefs in deep bottoms are those formed by oysters of the Gryphidae 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 (de la Torriente et al., 2014; Fabri et al., 2014). *N. zibrowii* is found only on rocky bottoms, also belonging to escarpments, seamounts and canyons, but its distribution is usually at greater depths, from 350 m down to more than 1 000 m (Beuck et al., 2016; Taviani et al., 2017). 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*, *Desmophyllum pertusum*, *D. dianthus*, and *Javania cailleti* (Taviani et al., 2016b, 2019).

There are also other species of molluscs, 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., 2017). 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.

- *Other habitats*

Brachiopods such as *Megerlia truncata*, *Terebratulina retusa*, *Argyrotheca* spp., *Megathyris detruncata*, *Novocrania anomala*, 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., 2015a; Taviani et al., 2017). 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: *Gryphus vitreus* (EC, 2006; Madurell et al., 2012; Aguilar et al., 2014).

In other cases, the dominant species are the Ascidiacea such as *Diazona violacea* and *Dicopia antirrhinum* (UNEP/MAP-RAC/SPA, 2013; Mechò et al., 2014) 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., 2004). However, *Leptometra phalangium* is not exclusively restricted to soft bottoms, but can also occur in equal numbers on rocky bottoms (Marín et al., 2011a, b) or even on coral reefs (Pardo et al., 2011; R. 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; Mastrototaro et al., 2017; R. Aguilar, pers. obs.), holothurians such as *Mesothuria intestinalis* and *Penilpidia ludwigi* (Pagès et al., 2007; Cartes et al., 2009), ophiuroids such as *Amphiura* spp., and also on some rocky bottoms and reefs, with an abundance of specimens of *Ophiothrix* spp. and *Holothuria forskali* (Templado et al., 2012).

Equally important are the Archaean communities and microbial mats (Pachiadaki et al., 2010; Pachiadaki and Kormas, 2013; Giovannelli et al., 2016) together with their associated chemo-symbiotic molluscs (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 basin, 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 chemo-synthetic communities usually occur at great depths, down to more than 2000 m.

- *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 reefs of coral, molluscs, brachiopods, polychaetes and sponges. These bottoms are found on seamounts, bathyal plateaus, escarpments, and in canyons. They include the compacted seabeds of old aggregations of *Gryphus vitreus* (R. Aguilar, pers. obs.), reefs and rubble of *Madrepora oculata*, *Desmophyllum pertusum*, *D. dianthus*, *Dendrophyllia cornigera*, oysters (*Neopycnodonte zibrowii*) (Županović, 1969; Taviani and Colantoni, 1979; Zibrowius and Taviani, 2005; Taviani et al., 2005b; Rosso et al., 2010; Bo et al., 2014c; Fourt et al., 2014b), beds of *Modiolus modiolus* shells (Aguilar et al., 2013; Gofas et al., 2014), subfossil reefs of polychaetes such as *Pomatoceros triqueter* (Domínguez-Carrió et al., 2014), fossilised structures of old sponge aggregations such as *Leiodermatium* sp. (R. 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.

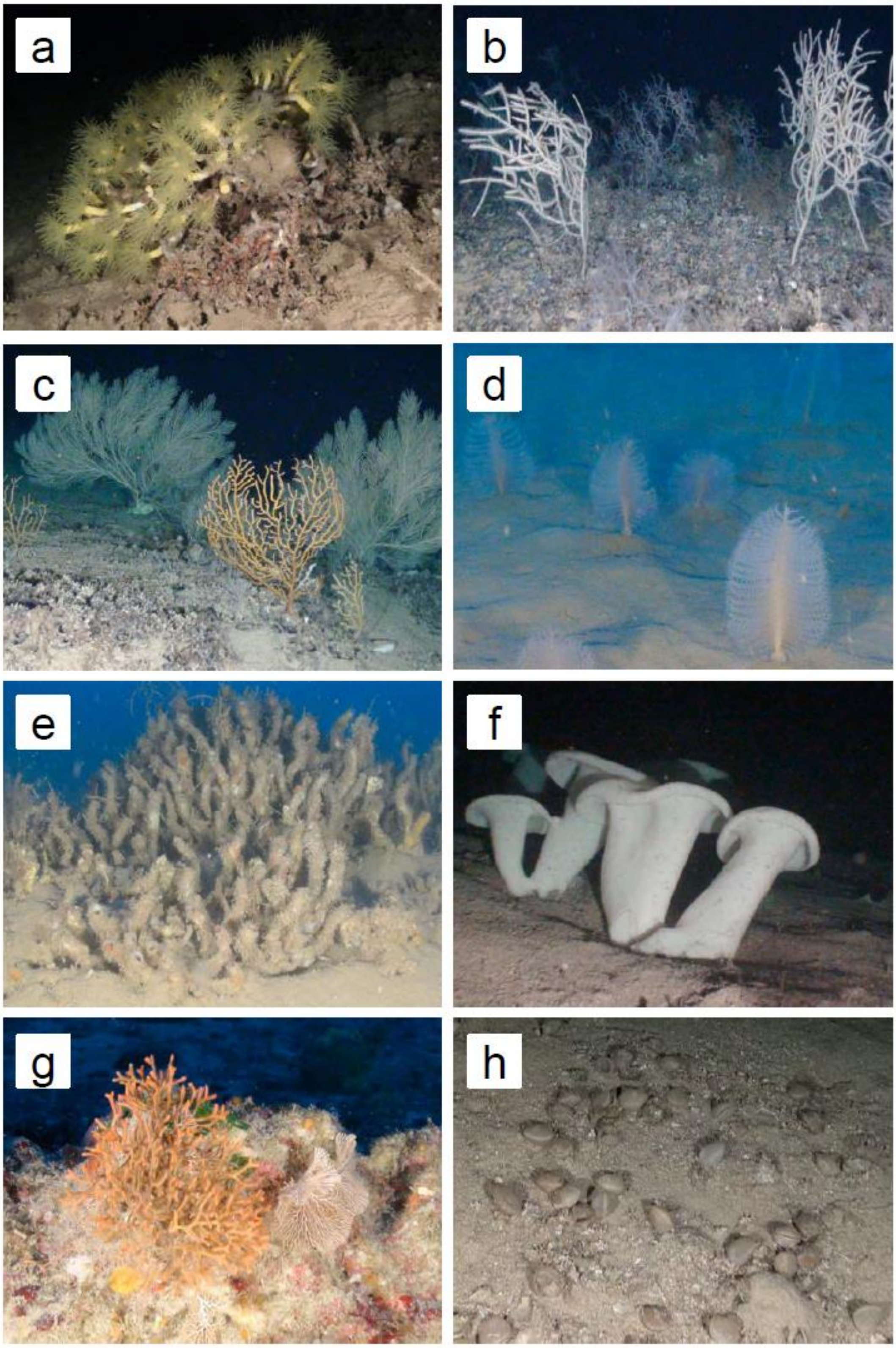

Figure 2: Characteristic species of deep-sea habitats. *Dendrophyllia cornigera*, Catifas Bank (a); *Antipathes dichotoma* and *Leiopathes glaberrima*, Malta (b); *Callogorgia verticillata* and *Placogorgia* sp., Ses Olives Seamount (c); *Pennatula rubra*, Lebanon (d); reef of vermetids, Lebanon (e); *Asconema setubalense*, Chella Bank (f); *Adeonella calveti* and *Hornera frondiculata*, Malta (g); brachiopods *Gryphus vitreus*, Emile Baudot Escarpment (h). Pictures by Oceana (SPA/RAC-UN Environment/MAP OCEANA, 2017).

Monitoring methods

a) COMMON INDICATOR 1: Habitat distributional range and extent

Approach

The CI1 is aimed at providing information about the geographical area in which dark habitats occur in the Mediterranean Sea and the total extent of surfaces covered by these habitats. Mapping dark habitats is particularly challenging because of the operational constrains to manage devices (e.g., SSS or ROV) in very deep waters and within caves, and in this latter case it results often impossible to allow the instrument entering the cave, and the overall high costs associated with oceanographic campaigns.

Three main steps can be identified for mapping dark habitats:

1) Initial planning, which includes the definition of the objectives in order to select the minimum surface to be mapped and the necessary resolution, tools and equipment

2) Ground survey is the practical phase for data collection, the costliest phase as it generally requires field activities

3) Processing and data interpretation require knowledge and experience to ensure that data collected are usable and reliable.

Resolution

Measures of the total habitat extent may be subjected to high variability, as the final value is influenced by the methods used to obtain maps and by the resolution during both data acquisition and final cartographic restitution. Selecting an appropriate scale is a critical stage in the initial planning phase (Mc Kenzie et al., 2001). An average precision and a lower detail can be accepted when large surface areas have to be mapped and global investigations carried out. On the contrary, a much higher precision and resolution is required when smaller areas have to be mapped. Detailed maps provide an accurate localisation of the habitat distribution and a precise definition of its extension limits and total habitat extent, all features necessary for future control and monitoring purposes over a period of time. However, the scarceness of fine-scale cartographic data on the overall distribution of dark habitats is one of the greatest lacunae from the conservation point of view.

<u>Marine caves</u>

To date approximately 3000 marine caves (semi- and entirely submerged) have been recorded in the Mediterranean basin (Fig. 3), according to the latest basin scale census by Giakoumi et al. (2013). Most of these caves (97%) are located in the North Mediterranean Sea, which encompasses a higher percentage of carbonate coasts and has been more extensively studied. Nevertheless, the number of underwater caves penetrating the rocky coasts of the Mediterranean basin remains unknown and comprehensive mapping efforts are still necessary to fill distribution gaps, especially in the eastern and southern regions of our sea.

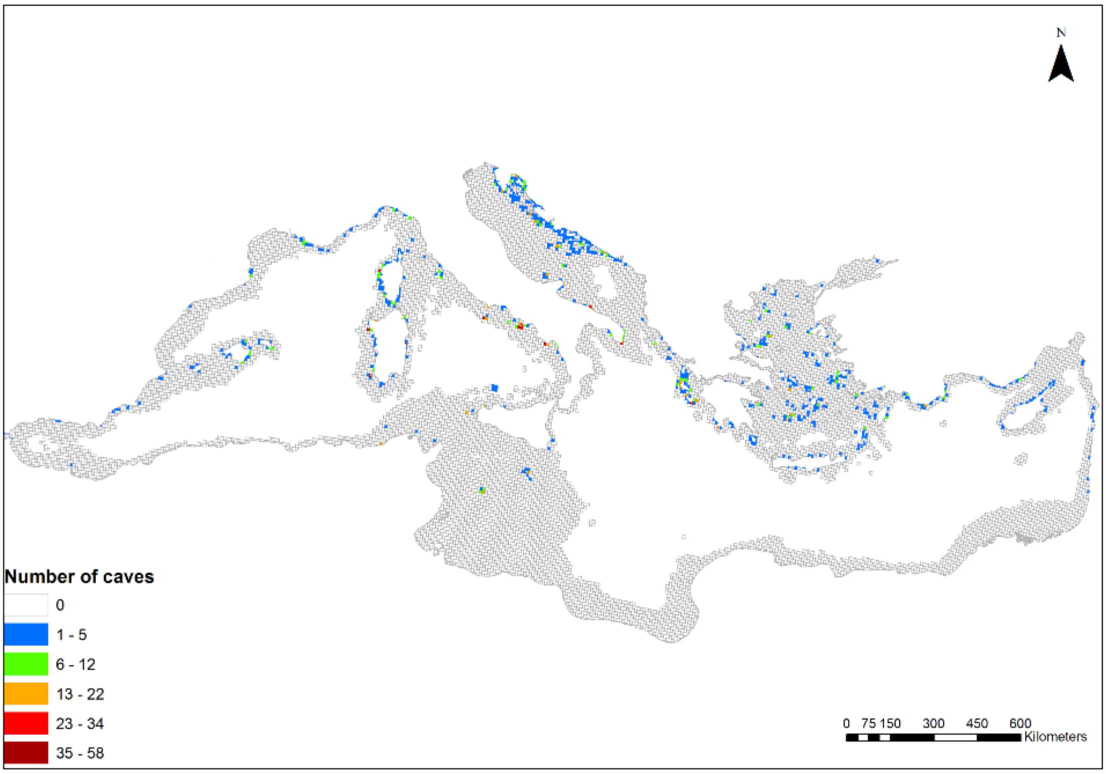

Figure 3: Distribution of marine caves in the Mediterranean Sea; different colours represent the number of caves in 10 km × 10 km cells (from Giakoumi et al., 2013).

<u>Deep-sea habitats</u>

Deep-sea habitats can be found in very diverse and extensive areas of the Mediterranean Sea, given that this sea has an average depth of about 1500 m, with many of its seabeds in aphotic zones (Fig. 4).

In the Mediterranean, 518 large canyons have been identified (Harris and Whiteway, 2011) (Fig. 5), along with around 242 underwater mountains or seamount-like structures (Würtz and Rovere, 2015) (Fig. 6) and there are some twenty sites where deep-water chemo-synthetic assemblages have been confirmed (Taviani, 2014) (Fig. 7). 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.

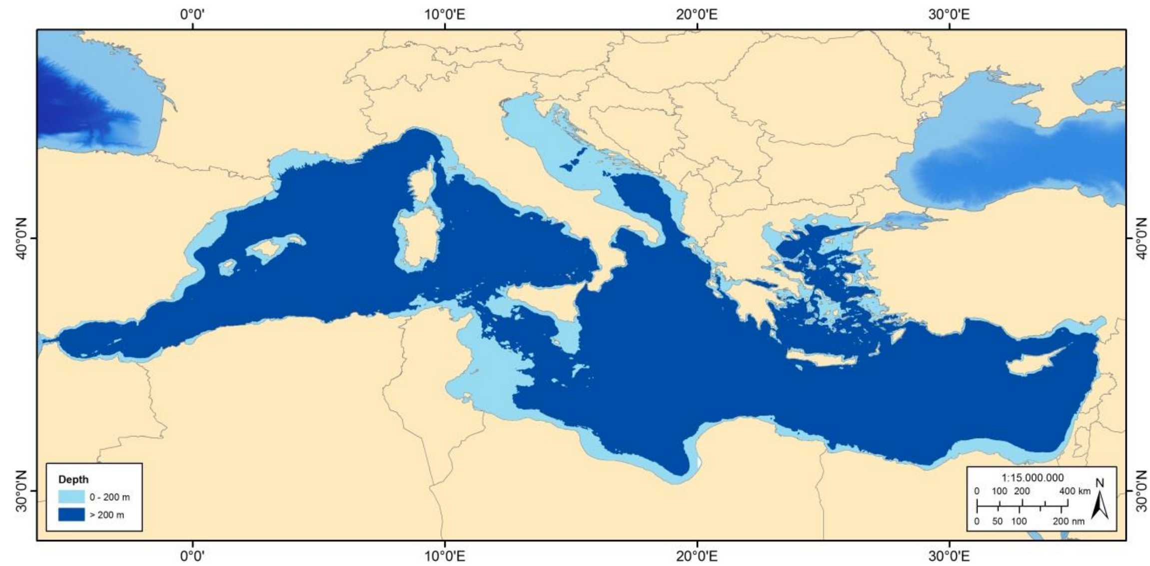

Figure 4: Deep-sea areas in the Mediterranean Sea below 200 m depth.

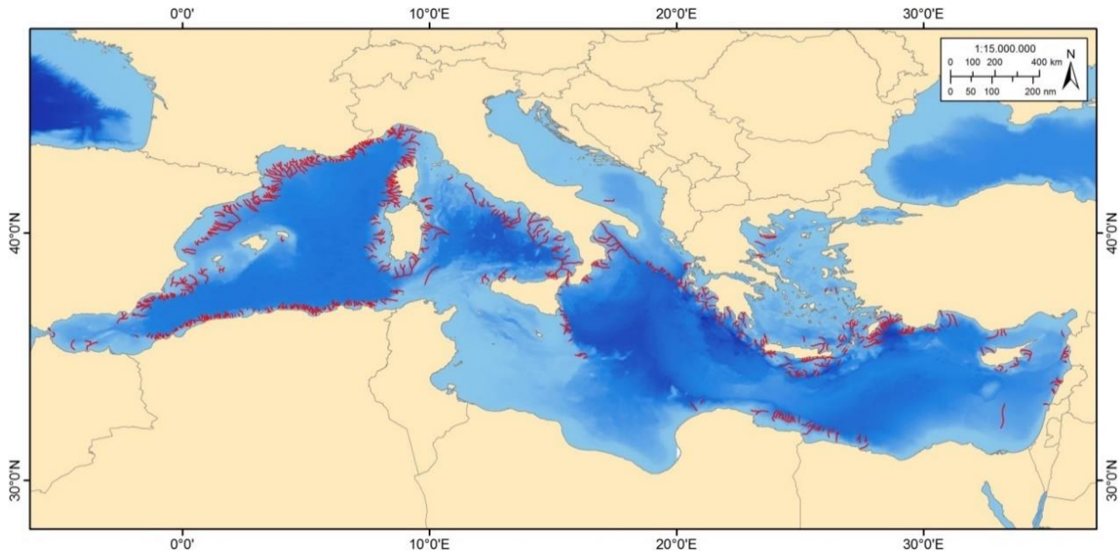

Figure 5: Distribution of Mediterranean submarine canyons.

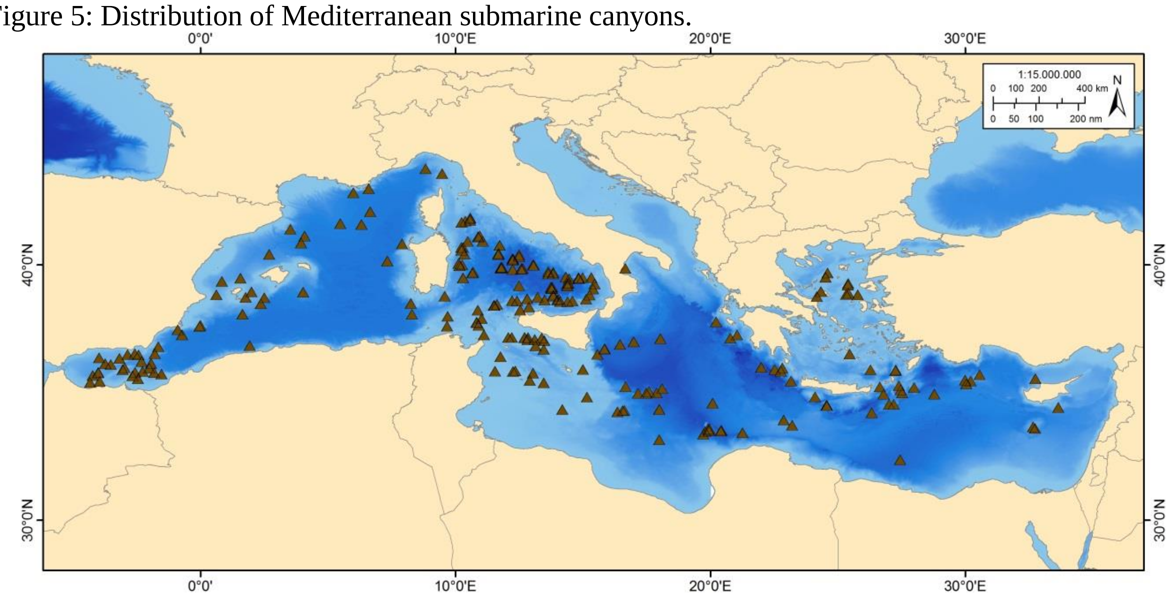

Figure 6: Distribution of Mediterranean seamounts.

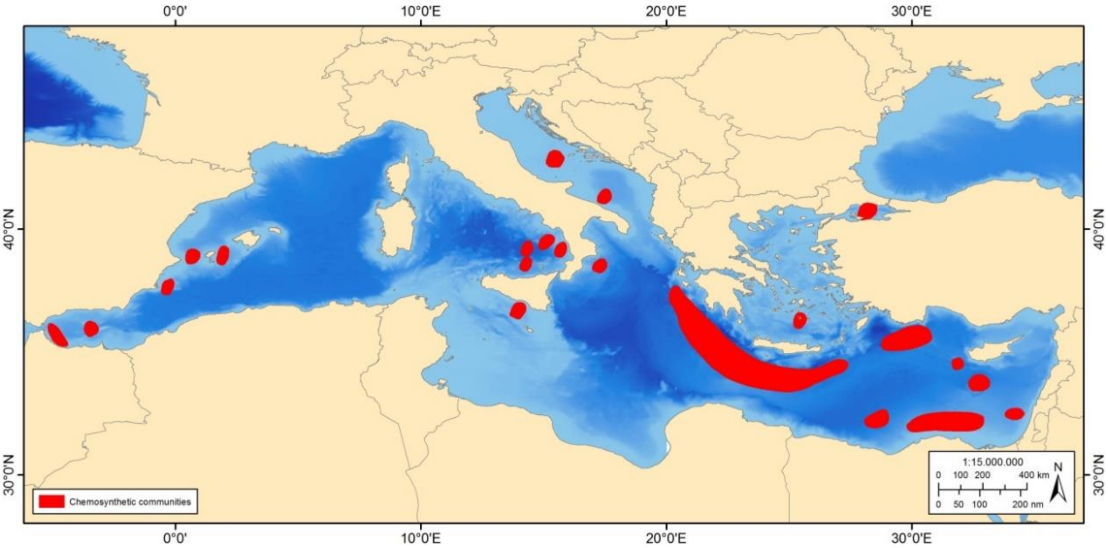

Figure 7: Identified areas with chemo-synthetic assemblages.

Methods

<u>Marine caves</u>

Inventorying of marine cave communities requires two steps:

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

Underwater diving

For marine caves up to 40 m depths diving is necessary for the exploration, mapping and inventorying, except for shallow caves of the semi- submerged type, which can be often spotted and accessed at the sea surface level. 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. 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 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, slate) (Barbieri, 2014). Additional equipment is needed for taking distance measurements (e.g., tape measure, portable echosounder, compass and waterproof range finder for semi-submerged caves).

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 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: i) depth; ii) orientation and dimensions of the cave entrance(s); iii) cave morphology (e.g., blind cave or tunnel); iv) submersion level (e.g., semi-submerged or submerged cave); v) maximum and minimum water depth inside the cave; and vi) 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. A useful protocol for inventorying semi-submerged caves, has been provided by Dendrinos et al. (2007); however, in areas supporting the Mediterranean monk seal (*Monachus monachus*) populations, such initiatives should be undertaken during periods with low in-cave seal activity (e.g., late spring or 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 ROVs, even though several limitations linked with the possibility to penetrate into these confined habitats (Fairfield et al., 2007; Stipanov et al., 2008).

Deep-sea habitats

Acoustic and video surveys

The necessary technology for research and expeditions in deep-sea habitats (e.g., ROV, submarines) has high costs that must be taken into account when planning oceanographic campaigns. Research vessels, suited to work in bathyal zones, are necessary to manage many of the instruments used for deep-sea habitat mapping. High resolution bathymetric maps (e.g., produced by multi-beam echosonar) are very useful tools for location and description of deep-sea habitats; however, they are not usually available. Also, seafloor irregularities make sometimes difficult to explore geomorphologic features, such as seamounts, submarine canyons, and deep caves.

Definition of distributional range and extent of deep-sea habitats requires “traditional” habitat mapping techniques, similar to those used for deep coralligenous reefs (Tab. 1). Being the deep-sea habitats distributed in deep waters (down to 120 m depth), the use of bathyscaphes, submarines, landers, etc., provide visual and georeferenced information on the geological formations and benthic communities on these seabeds. Acoustic techniques (e.g., side scan sonar, multi-beam echosounder) or underwater video recordings (Remote Operating Vehicle, ROV) are usually recommended, athougth they require a very long acquisition time given their limited speed and range. Sonar provides topobathymetric images of the seafloor through the emission and reception of ultrasounds; it creates 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 remote sensing allows characterising extensive areas for the assessment of the overall spatial patterns of deep-sea habitats. From maps obtained through remote sensing surveys, the presence/absence of the habitat, its distributional range and the total habitat extent can be easily obtained. Acoustic methods are presently the most convenient technique for mapping deep-sea habitats, associated with ground-truthing by ROV and, sometimes, box-coring. The simultaneous use of two or more methods makes it possible to optimize the results being the information obtained complementary. The strategy to be adopted will thus depend on the aim of the study and the area concerned, means and time available. Multi-beam 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 specific geomorphologic features such as seamounts, canyons, mud volcanoes, pockmarks, carbonated mounds, reefs, etc.

For all remote sensing techniques, 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. All the acoustic mapping techniques are intrinsically affected by uncertainties due to manual classification of the different acoustic signatures of substrate types on sonograms. Errors in sonograms interpretation may arise when two substrate types are not easily distinguished by the observer. Interpretation of remote sensing data requires extensive field calibration and the ground-truthing process remains essential. As the interpretation of sonograms is also a time-consuming and tedious task, several processing techniques were proposed in order to rapidly automate the interpretation of sonograms and make this interpretation more reliable (Montefalcone et al., 2013 and references therein). These methods allow a good discrimination between soft sediments and rocky reefs. Human eye, however, always remains the final judge.

Observations from the surface can be made by using imagery techniques such as video recordings by ROVs. ROVs have their own propulsion system and are remotely controlled from the surface. The use of ROVs during surveys makes it possible to see the images on the screen in real time, to identify specific features of the habitat and to evaluate any changes in the habitat or any other characteristic element of the seafloor, and this preliminary video survey may be also useful to locate monitoring and sampling stations. Recorded images are then reviewed to obtain a cartographical restitution on a GIS platform for each of the areas surveyed. Seabed inspection by ROV visual methods provides key information for the detection of potential areas where other dark habitats, more difficultly detected using acoustic methods, might occur.

Sampling methods

To obtain a better description of the habitat and for ground-truthing acoustic surveys, sampling methods are sometimes necessary. Special equipments are available for sediment sampling and characterisation from vessels at great depths, varying from grabs, gravity cores, piston cores, box cores, and multiple corers, used in a number of randomly selected points within a study area (Tab. 1) (Danovaro et al., 2010).

Table 1: Synthesis of the main survey tools used for defining the Common Indicator 1_Habitat distributional range and extent for dark habitats. When available, the depth range, the surface area mapped, the spatial resolution, the efficiency (expressed as area mapped in km^2 per hour), the main advantages or the limits of each tool are indicated, with some bibliographical references.

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Underwater diving (only for marine caves)	0 m to 40 m	Small areas, less than 250 m^2	From 0.1 m	0.0001 to 0.001 km^2/hour	• Very great precision for the identification (taxonomy) and distribution of species (micro-mapping) • Non-destructive • Low cost, easy to implement	• Method adapt only for marine caves characterisation • Small area inventoried • Very time-consuming • Limited operational depth • Highly qualified and expert divers required (safety constraints)	Gerovasileiou et al. (2013, 2015); Montefalcone et al. (2018)
Sampling from vessels with grabs, gravity cores, box cores, multiple corers, trawls	Down to 1500 m	Intermediate areas (a few km^2)	From 1 to 10 m	0.025 to 0.01 km^2/hour	• Very great precision for the identification (taxonomy) and distribution of species (micro-mapping) • All species taken into account • Possibility of *a posteriori* identification	• Destructive method • Small area inventoried • Sampling material needed • Difficulty to manage sampling devices at great depths • Laboratory analyses very time consuming • High costs of the research vessels	Danovaro et al. (2010)
Side scan sonar	Down to 4000 m	From intermediate to large areas (50-100 km^2)	From 1 m	1 to 4 km^2/hour	• Wide bathymetric range • High resolution and good identification of the nature of the bottom • Quick execution • Non-destructive	• Flat (2-D) picture to represent 3-D complex habitats • Possible errors in sonograms interpretation • Acquisition of field data necessary to validate sonograms • High cost of instruments and research vessels	Palmiotto and Loreto (2019)

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Multi-beam echosounder	Down to 4000 m	From intermediate to large areas (50-100 km^2)	From 1 m (linear) and lower than 1 m (depth)	0.5 to 6 km^2/hour	• Possibility of obtaining 3-D picture • Double information collected (bathymetry and seafloor image) • Very precise and wide bathymetric range • Realistic representation of the seafloor • Quick execution • Non-destructive	• Very big mass of data • Complex processing of information • Less precise imaging (nature of the bottom) than side scan sonar • Acquisition of field data necessary to validate sonograms • High cost of instruments and research vessels • High resolution maps not usually available	Palmiotto and Loreto (2019)
Remote Operating Vehicle (ROV), bathyscaphes, or submarines	Down to 4000 m	Small-intermediate areas (a few km^2)	From 1 m to 10 m	0.025 to 0.01 km^2/hour	• Non-destructive • Possibility of taking pictures • Good identification of habitat and species • Wide bathymetric range	• Small area surveyed • High cost • Slow recording and processing of information • Variable positioning (geo-referencing) • Difficult to handle at great depths • High cost of instruments and research vessels	Enrichetti et al. (2019); Rogers (2019)

Data interpretation

Once the surveying is completed, data collected needs to be organized so that it can be used in the future by everyone and can be appropriately archived and easily consulted. A clear definition of all metadata must be provided with the dataset in order to ensure future integration with similar data from other sources. Acoustic data must be always integrated by a great amount of samplings or video recordings by ROVs for ground-truthing, especially given the wide distribution and complexity of the deep-sea habitats.

Four important steps for the production of a habitat map must be followed:

1. Processing, analysis, interpretation and classification of field biological data, to be integrated with acoustic data when available
2. Selecting the most appropriate physical layers (e.g., substrate, bathymetry, hydrodynamics)
3. Integration of biological data and physical layers, and use of statistical modelling to predict habitat distribution and interpolate information
4. The map produced must then be evaluated for its accuracy, i.e. its capacity to represent reality, and therefore its reliability.

During <u>the processing analysis and classification step</u>, the updated list of benthic marine habitat types for the Mediterranean region should be consulted (UNEP/MAP-SPA/RAC, 2019) to recognize any specific dark habitat type (i.e., marine cave, circalittoral rock, bathyal sand) and its main characteristic associations and facies. A complete description of these habitats and the criteria for their identification are also available in Bellan-Santini et al. (2002). Dark habitats that must be reported on maps are the following (UNEP/MAP-SPA/RAC, 2019):

LITTORAL

MA1.5 Littoral rock

MA1.52 Mediolittoral caves

MA1.521 Association with encrusting Corallinales or other Rodophyta

INFRALITTORAL

MB1.5 Infralittoral rock

MB1.56 Semi-dark caves and overhangs (see MC1.53)

CIRCALITTORAL

MC1.5 Circalittoral rock

MC1.53 Semi-dark caves and overhangs

MC1.53a Walls and tunnels

MC1.531a Facies with sponges (e.g. *Axinella* spp., *Chondrosia reniformis*, *Petrosia ficiformis*)

MC1.532a Facies with Hydrozoa

MC1.533a Facies with Alcyonacea (e.g. *Eunicella* spp., *Paramuricea* spp., *Corallium rubrum*)

MC1.534a Facies with Scleractinia (e.g. *Leptopsammia pruvoti*, *Phyllangia mouchezii*)

MC1.535a Facies with Zoantharia (e.g. *Parazoanthus axinellae*)

MC1.536a Facies with Bryozoa (e.g. *Reteporella grimaldii*, *Pentapora fascialis*)

MC1.537a Facies with Ascidiacea

MC1.53b Ceilings

See MC1.53a for examples of facies

MC1.53c Detritic bottom

See MC3.51 for examples of associations and facies

MC1.53d Brackish water caves or caves subjected to freshwater runoff

MC1.531d Facies with *Lithistida* spp. sponges

OFFSHORE CIRCALITTORAL

MD1.5 Offshore circalittoral rock

MD1.51 Offshore circalittoral rock invertebrate-dominated

MD1.511 Facies with small sponges (sponge ground, e.g. *Halicona* spp., *Phakellia* spp., *Poecillastra* spp.)

MD1.512 Facies with large and erect sponges (e.g. *Spongia lamella*, *Axinella* spp.)

MD1.513 Facies with Alcyonacea (e.g. *Alcyonium* spp., *Callogorgia verticillata*, *Ellisella paraplexauroides*, *Eunicella* spp., *Leptogorgia* spp., *Paramuricea* spp., *Swiftia pallida*, *Corallium rubrum*)

MD1.514 Facies with Antipatharia (e.g. *Antipathella subpinnata*)

MD1.515 Facies with Scleractinia (e.g. *Dendrophyllia* spp., *Madracis pharensis*)

MD1.516 Facies with Ceriantharia (e.g. *Cerianthus* spp.)

MD1.517 Facies with Zoantharia (e.g. *Savalia savaglia*)

MD1.518 Facies with Polychaeta

MD1.519 Facies with Bivalvia

MD1.51A Facies with Brachiopoda

MD1.51B Facies with Bryozoa (e.g. *Myriapora truncata*, *Pentapora fascialis*)

MD1.52 Offshore circalittoral rock invertebrate-dominated covered by sediments

See MD1.51 for examples of facies

MD1.53 Deep offshore circalittoral banks

MD1.531 Facies with Antipatharia (e.g. *Antipathella subpinnata*)

MD1.532 Facies with Alcyonacea (e.g. *Nidalia* spp.)

MD1.533 Facies with Scleractinia (yellow corals forest, e.g. *Dendrophyllia* spp.)

MD2.5 Offshore circalittoral biogenic habitat

MD2.51 Offshore reefs

MD2.511 Facies with Vermetidae and/or Serpulidae

MD2.52 Thanatocoenosis of corals, or Brachiopoda, or Bivalvia (e.g. *Modiolus modiolus*)

See MD1.51 for examples of facies

MD3.5 Offshore circalittoral coarse sediment

MD3.51 Offshore circalittoral detritic bottoms

MD3.511 Facies with Bivalvia (e.g. *Neopycnodonte* spp.)

ME2.512 Facies with Brachiopoda

MD3.513 Facies with Polychaeta

MD3.514 Facies with Crinoidea (e.g. *Leptometra* spp.)

MD3.515 Facies with Ophiuroidea

MD3.516 Facies with Echinoidea

MD4.5 Offshore circalittoral mixed sediment

MD4.51 Offshore circalittoral detritic bottoms

See MD3.51 for examples of facies

MD5.5 Offshore circalittoral sand

MD5.51 Offshore circalittoral sand

See MD3.51 for examples of facies

MD6.5 Offshore circalittoral mud

MD6.51 Offshore terrigenous sticky muds

MD6.511 Facies with Pennatulacea (e.g. *Pennatula* spp., *Virgularia mirabilis*)

MD6.512 Facies with Polychaeta

MD6.513 Facies with Bivalvia (e.g. *Neopycnodonte* spp.)

MD6.514 Facies with Brachiopoda

MD6.515 Facies with Ceriantharia (e.g. *Cerianthus* spp., *Arachnanthus* spp.)

UPPER BATHYAL

ME1.5 Upper bathyal rock

ME1.51 Upper bathyal rock invertebrate-dominated

ME1.511 Facies with small sponges (sponge ground; e.g. *Farrea bowerbanki*, *Halicona* spp., *Podospongia loveni*, *Tretodictyum* spp.)

ME1.512 Facies with large and erect sponges (e.g. *Spongia lamella*, *Axinella* spp.)

ME1.513 Facies with Antipatharia (e.g. *Antipathes* spp., *Leiopathes glaberrima*, *Parantipathes larix*)

ME1.514 Facies with Alcyonacea (e.g. *Acanthogorgia* spp., *Callogorgia verticillata*, *Placogorgia* spp., *Swiftia pallida*, *Corallium rubrum*)

ME1.515 Facies with Scleractinia (e.g. *Dendrophyllia* spp., *Madrepora oculata*, *Desmophyllum cristagalli*, *Lophelia pertusa*, *Madracis pharensis*)

ME1.516 Facies with Cirripeda (e.g. *Megabalanus* spp., *Pachylasma giganteum*)

ME1.517 Facies with Crinoidea (e.g. *Leptometra* spp.)

ME1.518 Facies with Bivalvia (e.g. *Neopycnodonte* spp.)

ME1.519 Facies with Brachiopoda

ME1.52 Caves and ducts in total darkness

ME2.5Upper bathyal biogenic habitat

ME2.51 Upper bathyal reefs

ME2.511 Facies with small sponges (sponge ground)

ME2.512 Facies with large and erect sponges (e.g. *Leiodermatium* spp.)

ME2.513 Facies with Scleractinia (e.g. *Madrepora oculata*, *Desmophyllum cristagalli*)

ME2.514 Facies with Bivalvia (e.g. *Neopycnodonte* spp.)

ME2.515 Facies with Serpulidae reefs (e.g. *Serpula vermicularis*)

ME2.516 Facies with Brachiopoda

ME2.52 Thanatocoenosis of corals, or Brachiopoda, or Bivalvia, or sponges

See ME1.51 for examples of facies

ME3.5 Upper bathyal coarse sediment

ME3.51 Upper bathyal coarse sediment

ME3.511 Facies with Alcyonacea (e.g. *Alcyonium* spp., *Chironephthya mediterranea*, *Paralcyonium spinulosum*, *Paramuricea* spp., *Villogorgia bebrycoides*)

ME4.5 Upper bathyal mixed sediment

ME4.51 Upper bathyal mixed sediment

ME4.511 Facies with Bivalvia (e.g. *Neopycnodonte* spp.)

ME4.512 Facies with Brachiopoda

ME5.5 Upper bathyal sand

ME5.51Upper bathyal detritic sand

ME5.511 Facies with small sponges (sponge ground, e.g. *Rhizaxinella* spp.)

ME5.512 Facies with Pennatulacea (e.g. *Pennatula* spp., *Pteroeides griseum*)

ME5.513 Facies with Crinoidea (e.g. *Leptometra* spp.)

ME5.514 Facies with Echinoidea

ME5.515 Facies with Bivalvia (e.g. *Neopycnodonte* spp.)

ME5.516 Facies with Brachiopoda

ME5.517 Facies with Bryozoa

ME5.518 Facies with Scleractinia (e.g. *Caryophyllia cyathus*)

ME6.5 Upper bathyal muds

ME6.51 Upper bathyal muds

ME6.511 Facies with small sponges (sponge ground, e.g. *Pheronema* spp., *Thenea* spp.)

ME6.512 Facies with Pennatulacea (e.g. *Pennatula* spp., *Funiculina quadrangularis*)

ME6.513 Facies with Alcyonacea (e.g. *Isidella elongata*)

ME6.514 Facies with Scleractinia (e.g. *Dendrophyllia* spp., *Madrepora oculata*, *Desmophyllum cristagalli*)

ME6.515 Facies with Crustacea Decapoda (e.g. *Aristeus antennatus*, *Nephrops norvegicus*)

ME6.516 Facies with Crinoidea (e.g. *Leptometra* spp.)

ME6.517 Facies with Echinoidea (e.g. *Brissopsis* spp.)

ME6.518 Facies with Bivalvia (e.g. *Neopycnodonte* spp.)

ME6.519 Facies with Brachiopoda

ME6.51A Facies with Ceriantharia (e.g. *Cerianthus* spp., *Arachnanthus* spp.)

ME6.51B Facies with Bryozoa (e.g. *Candidae* spp., *Kinetoskias* spp.)

ME6.51C Facies with giant Foraminifera (e.g. Astrorhizida)

LOWER BATHYAL

MF1.5 Lower bathyal rock

MF1.51 Lower bathyal rock

MF1.511 Facies with small sponges (e.g. *Stylocordyla* spp.)

MF1.512 Facies with Alcyonacea (e.g. *Dendrobrachia* spp.)

MF1.513 Facies with Scleractinia (e.g. *Dendrophyllia* spp., *Madrepora oculata*, *Desmophyllum cristagalli*, *Lophelia pertusa*)

MF1.514 Facies with chemiosynthetic benthic species (e.g. Siboglinidae, *Lucinoma* spp.)

MF2.5 Lower bathyal biogenic habitat

MF2.51 Lower bathyal reefs

MF2.511Facies with Scleractinia (e.g. *Dendrophyllia* spp., *Madrepora oculata*, *Desmophyllum cristagalli*, *Lophelia pertusa*)

MF2.52 Thanatocoenosis of corals, or Brachiopoda, or Bivalvia, or sponges

See MF1.51 for examples of facies

MF6.5 Lower bathyal muds

MF6.51 Sandy muds

MF6.511 Facies with small sponges (e.g. *Thenea* spp.)

MF6.512 Facies with Alcyonacea (e.g. *Isidella elongata*)

MF6.513 Facies with Echinoidea (e.g. *Brissopsis* spp.)

MF6.514 Facies with Pennatulacea (e.g. *Pennatula* spp., *Funiculina quadrangularis*)

MF6.515 Facies with bioturbations

ABYSSAL

MG1.5 Abyssal rock

MG1.51 Abyssal rock

MG1.511 Facies with small sponges

MG1.512 Facies with Alcyonacea

MG1.513 Facies with Polychaeta

MG1.514 Facies with Crustacea (Amphipoda, Isopoda, Tanaidacea)

MG6.5 Abyssal muds

MG6.51 Abyssal muds

MG6.511 Facies with small sponges

MG6.512 Facies with Alcyonacea (e.g. *Isidella elongata*)

MG6.513 Facies with Polychaeta

MG6.514 Facies with Crustacea (Amphipoda, Isopoda, Tanaidacea)

MG6.515 Facies with bioturbations

Although the selection of physical layers to be shown on maps and to be used for following predictive statistical analyses might be a promising approach within the general framework of mapping dark habitats, no examples of prediction of the distribution of dark habitats are reported in literature to date. Inspiring from the examples of habitat predictions performed on coralligenous reefs (see the "Guidelines on coralligenous" in this document for further details), the following physical attributes could be investigated for predicting potential deep-sea habitat types starting from a general geomorphologic data: bathymetry, slope of the seafloor, seafloor types, currents, and nutrient input (Giannoulaki et al., 2013; Martin et al., 2014).

The data integration and spatial interpolation is often a necessary step because indirect visual or remote sensing surveys from vessels are often limited due to time and costs involved, and only rarely allow to obtain a complete coverage of the study area. Spatial interpolation is a statistical procedure for estimating data values at unsampled sites between actual data collection locations. For elaborating the final distribution map of dark habitats on a GIS platform, different spatial interpolation tools (e.g., Inverse Distance Weighted, Kriging) can be used and are provided by the GIS software. Even though this is rarely mentioned, it is important to provide information on the number and the percentage of data acquired on field and the percentage of interpolations run.

On the resulting maps the habitat distributional range and its total extent (expressed in square meters or hectares) can be defined. These maps could be also compared with previous historical available data from literature (very scarce for deep-sea habitats) to evaluate any changes experienced by the habitat over a period of time. Using the overlay vector methods on GIS, a diachronic analysis can be done, where temporal changes are measurements in term of percentage gain or loss of the habitat extension, through the creation of concordance and discordance maps (Canessa et al., 2017). Mapping of protected habitats (e.g., under SPA/BD) is a necessary step to evaluate habitat loss or increase in the total area covered. Conservation targets require that the habitat maintains stable and Member States have generally adopted a 5% tolerance above the baseline to represent a 'stable' situation. However, in some cases a more stringent <1% tolerance has been used for the maintenance of the habitat extent. For protected habitats that have historically been reduced, the target should be that the total area increases towards the size of the baseline. However, for most of the deep-sea habitats no information on their reference state is available.

Various software platforms have been developed for three-dimensional (3D) cave modelling (e.g., Sellers and Chamberlain, 1998; Boggus and Crawfis, 2009; Gallay et al., 2015; Oludare Idrees and Pradhan, 2016). A rapid and cost-effective 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), using handheld echosounder. 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 of the 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).

Finally, reliability of the map produced should be evaluated. No evaluation scales of reliability have been proposed for dark habitats mapping; however, scales of reliability evaluation available for seagrass meadows can be adapted also for these habitats (see the “Guidelines on marine vegetation” in this document for further details). These scales usually take into account the processing of sonograms, the scale of data acquisition and restitution, the methods adopted, and the positioning system.

b) COMMON INDICATOR 2: Condition of the habitat’s typical species and communities

Approach

Monitoring the condition (i.e., the ecological status) of dark habitats is today mandatory for conservation and management purposes, to ensure dark habitats, their constituent species and their associated communities to maintain a satisfactory ecological status in terms of structure and functions. The good state of health of dark habitats will then reflect the Good Environmental Status (GES) pursued by the Contracting Parties to the Barcelona Convention under the Ecosystem Approach (EcAp) and under the Marine Strategy Framework Directive (MSFD).

According to the EcAp and following the IMAP recommendations, it is suggested that future monitoring schemes for marine caves and deep-habitats should mainly consider common indicators related to biodiversity (EO1), and in particular the Common Indicator 2 - Condition of the habitat’s typical species and communities. Being important biodiversity hot-spots in the Mediterranean Sea, dark habitats have been recognized as biological indicators of environmental quality.

Defined and standardised procedures for monitoring the status of marine caves and deep-sea habitats are not available to date. For planning an effective monitoring program, however, the following three main steps must be undertaken:

1. Initial planning, to define objective(s), duration, sites to be monitored, descriptors to be evaluated, sampling strategy, human, technical and financial needs
2. Setting-up the monitoring system and realisation of the monitoring program. This phase includes costs for going out to sea during field activities, equipment for sampling, and human resources. To ensure effectiveness of the program, field activities should be planned during a favourable season, and it would be preferred to monitor during the same season
3. Monitoring over time and analysis, where clear scientific competences are needed because acquired data must be interpreted. Duration of the monitoring, in order to be useful, must be medium-time at least.

The objectives of the monitoring are primarily linked with the conservation of dark habitats, to maintain their ecological status (GES) and also to identify, as early as possible, any degradation or any change in their distributional range and extent. Assessment of the ecological status of these habitats allows to measure the effectiveness of local or regional policies in terms of management of the coastal areas and of fisheries activities. The Integrated Monitoring and Assessment Programme (IMAP) requires a regional integrated monitoring system of the quality of the environment, which can be reached through reliable quantitative and updated data on the status of Mediterranean dark habitats.

The sites chosen must be: i) representative of the portion of the seafloor investigated, ii) cover most of the possible range of environmental situations (e.g., depth range, slope, substrate type), and iii) include sensitive zones, stable zones or reference zones with low anthropogenic pressures and especially low fishing pressure. The selection of sites to be monitored must be done to keep the monitoring effort cost-effective. Special habitats essential for the early developmental stages of mobile fauna (e.g., spawning, feeding grounds) or hosting benthic assemblages considered as key components of the deep-sea assuring ecosystem functioning (e.g., engineer species or species listed in the Red List), must be included among the selected sites. The duration of the monitoring should be at least medium-long term (minimum 5-10 years long). An effective monitoring should be done at defined intervals over a period of time, even if it could mean a reduced number of sites being monitored. The interval of data acquisition could be annually, as most of the typical species belonging to deep-sea habitats (e.g., animal forests) display slow grow rates and long generation times (> 1 year). In general, and irrespective of the objective advocated, it is judicious to focus initially on a small number of sites and that can be regularly monitored after short intervals of time. Then, with the experience gained by the surveyors and the means (funds) available, this network could be extended to a larger number of sites.

The reference “zero-state” will be contrasted with data coming from subsequent monitoring periods, always assuring reproducibility of data over time. Geographical position of surveys and sampling stations must therefore be located with precision.

To ensure the sustainability of the monitoring system, the following final remarks must be taken into account:

- Identify the partners, competences and means available
- Planning the partnership modalities (who is doing what? when? and how?)
- Ensure training for the stakeholders so that they can set up standardised procedures to guarantee the validity of the results, and so that comparisons can be made for a given site and among sites
- Individuate a regional or national coordinator depending on the number of sites concerned for monitoring and their geographical distribution
- Evaluate the minimum budget necessary for running the monitoring network (e.g., costs for permanent operators, temporary contracts, equipment, data acquisition, processing and analysis).

The lack or scarcity of quantitative data and long-time-series from marine caves and deep-sea habitats in most of the Mediterranean areas is a major impediment to evaluate changes in their ecological status. There is evidence of alterations through time in caves of the north-western Mediterranean Sea, 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; Nepote et al., 2017; Montefalcone et al., 2018). 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 and Lejeusne, 2003; Guarnieri et al., 2012; Giakoumi et al., 2013; Gerovasileiou et al., 2016b). Main threats to deep-sea habitats include climate change-related pressures (e.g., ocean warming, changes in primary production, hypoxia, and ocean acidification) and deep-water fishing, including bottom trawling (Rogers, 2019). Increased temperatures can lower oxygen thresholds and reduce the tolerance of species to acidification, while, in turn, hypoxia and acidification can reduce thermal tolerance. Physical disturbances caused by bottom trawling, deep-sea mining, and oil and gas extraction can increase physiological stress due to climate change factors.

Methods

<u>Monitoring marine cave communities</u>

Following the preliminary definition of the localisation and topography of a marine cave (the previous CI1), assessment of its condition starts with an overall characterisation of the typical species and communities occurring within each cave. Monitoring of this habitat basically relies on underwater diving, although this technique gives rise to many constraints due to the peculiar conditions of this habitat (weak luminosity, complex topography, etc.). Good experience in underwater diving is requested to operate an effective work within submerged caves.

The general principles and methods for the characterisation of hard substrate cave communities are similar to those described in the guidelines for coralligenous monitoring (see “Guidelines for monitoring coralligenous” in this document). The use of non-destructive quantitative visual survey methods for studying the structure and the status of cave sessile communities is highly recommended (e.g., Martí et al., 2004; Bussotti et al., 2006; Gerovasileiou and Voultsiadou, 2016; Montefalcone et al., 2018). Direct *in situ* visual census techniques or photographic methods, associated with determination of taxa and/or morphological groups, can be adopted. Scientific divers annotate on their slides the list of the main conspicuous species/taxa characterising the assemblages. Divers must be specialists in the taxonomy of the main species that can be found in these habitats, to ensure the validity of the information recorded underwater. The best results can be obtained integrating photographic sampling and *in situ* visual observations. The former is the most cost-effective method that requires less time spent underwater and allows collecting the large number of samples required for community analysis in such a complex and confined habitat at small spatial scales. The latter method, using square frames enclosing a standard area of the substrate, has been shown equally effective, but requires longer working time underwater (Parravicini et al., 2010), which may represent a limiting factor when working within caves. Both methods minimise human impact on these fragile communities, still providing reference conditions for monitoring at given sites (Bianchi et al., 2004). For the study of sessile communities a minimum of 3 replicated photographic samples (photo-quadrates) of about 0.16 m^2 each should be collected at each sampling station, covering a total surface of about 1-4 m^2. Positioning and number of sampling stations depend on the cave topography and its bathymetric range (Nepote et al., 2017). Being benthic assemblages of marine caves highly variable, even at small scales, and subjected to strong gradient, a systematic sampling method must be adopted, with stations regularly spaced from one another starting from the entrance and moving to the terminal part of the caves. All replicates must be taken on the vertical walls of the caves and at the same depth.

Given the limitations of the visual identification of several benthic taxa, the collection of supplementary qualitative samples is often necessary. The use of operational taxonomical units (OTUs), or taxonomical surrogates such as morphological groups (lumping species, genera or higher taxa displaying similar morphological features; Parravicini et al., 2010), may represent a useful compromise for the study of cave sessile benthos when a consistent species distinction is not possible (either underwater or on photographs), or to reduce the surveying/analysis time (Gerovasileiou and Voultsiadou, 2016; Nepote et al., 2017; Montefalcone et al., 2018). Semi-quantitative evaluations through underwater visual census could also provide valuable information in certain cases.

A list of the main conspicuous species/taxa or morphological groups recognisable underwater, or on images, is then produced. A list of species that are frequently reported in Mediterranean marine caves is presented in Appendix 1. 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 (Gerovasileiou and Voultsiadou, 2012, 2014). 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 and Morri, 2003). Marine caves are characterised by a high degree of natural heterogeneity and their communities present qualitative and quantitative differences in species composition across different Mediterranean eco-regions (Gerovasileiou and Voultsiadou, 2012). 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. Advanced image processing software dedicated to marine biological research integrate methods and tools for the following accurate extraction of species coverage (%) or abundance (cm^2) from photo-quadrates (e.g., Teixidó et al., 2011; Trygonis and Sini, 2012). 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).

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 and Guidetti, 2009), as well as for decapods crustaceans (Denitto et al., 2009). The number of species and individuals observed at 5 min interval must be recorded on the slate.

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).

The disappearance 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 thermo-tolerant congeners are among the most striking examples of negative alterations on cave communities (Chevaldonné and Lejeusne, 2003; Guarnieri et al., 2012; Nepote et al., 2017). Growth forms are used to investigate different strategies of substratum occupation, which are strictly influenced by environmental conditions. For instance, the shift from a flattened morphology to a peduncolated one observed in some sponges of the genus *Petrosia* and *Chondrosia* in two marine caves of the Liguria Sea affected by costal constructions, is a clear strategy to counteract silting in environments with low water exchanges because it allows a greater efficiency in the elimination of catabolites (Nepote et al., 2017). Similarly, the use of trophic guilds can effectively show any change in the functioning of the eosysystem, providing information about trophic organization (which depends on light penetration and particulate matter availability) (Montefalcone et al., 2018).

An ecosystem-based index (CavEBQI) for the evaluation of the ecological quality of marine cave ecosystems has been 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-dimensional form (e.g., *Corallium 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. In the framework of a 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; Montefalcone et al., 2018). 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 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 shown in Figure 8.

Figure 8: 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, photo-quadrates covering a total surface of about 1-4 m^2 should be acquired for the study of sessile communities.

<table>
<tr><td colspan="5">Area: Date: Observer:</td></tr>
<tr><td>Latitude:</td><td colspan="4">Longitude:</td></tr>
<tr><td>Submersion level: Submerged / Semi-submerged</td><td colspan="4">Cave morphology: Blind cave / Tunnel
No. of entrances: …</td></tr>
<tr><td>Total length of cave: …</td><td colspan="2">Maximum water depth: …</td><td colspan="2">Minimum water depth: …</td></tr>
<tr><td colspan="5">Entrance A – Max depth (m): …   Height (m): …   Width (m): …   Orientation: …</td></tr>
<tr><td colspan="5">Entrance B – Max depth (m): …   Height (m): …   Width (m): …   Orientation: …</td></tr>
<tr><td colspan="5">Other topographic features: Internal beach / Air pockets / Speleothems / …</td></tr>
<tr><td colspan="5">Micro-habitats:</td></tr>
<tr><td colspan="5">Detritivorous / omnivorous species (number of species and individuals observed at 5 min interval)</td></tr>
<tr><td>Herbstia condyliata</td><td>1–2</td><td>3–4</td><td>5–10</td><td>>10</td></tr>
<tr><td>Galathea strigosa</td><td>1–2</td><td>3–4</td><td>5–10</td><td>>10</td></tr>
<tr><td>Scyllarus arctus</td><td>1–2</td><td>3–4</td><td>5–10</td><td>>10</td></tr>
<tr><td>...</td><td>1–2</td><td>3–4</td><td>5–10</td><td>>10</td></tr>
<tr><td>…</td><td>1–2</td><td>3–4</td><td>5–10</td><td>>10</td></tr>
<tr><td>…</td><td>1–2</td><td>3–4</td><td>5–10</td><td>>10</td></tr>
<tr><td>…</td><td>1–2</td><td>3–4</td><td>5–10</td><td>>10</td></tr>
<tr><td>…</td><td>1–2</td><td>3–4</td><td>5–10</td><td>>10</td></tr>
<tr><td colspan="5">Mysids   0   few   swarm</td></tr>
<tr><td>Fish species observed/ cave zone
(CE: entrance, SD: semi-dark zone, DZ: dark zone)</td><td colspan="4">Decapods species observed / cave zone
(CE: entrance, SD: semi-dark zone, DZ: dark zone)</td></tr>
<tr><td>… /</td><td colspan="4">… /</td></tr>
<tr><td>… /</td><td colspan="4">… /</td></tr>
<tr><td>… /</td><td colspan="4">… /</td></tr>
</table>

… /		… /	
… /		… /	
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… /		… /	
… /		… /	
***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	

<u>Monitoring deep-sea habitats</u>

Following the preliminary definition of the distributional range and extent of deep-sea habitats (the previous CI1), assessment of the condition of these habitats starts with an overall characterisation of the typical species and communities occurring within each habitat. Methodologies to monitor the condition of deep-sea dark habitats include a wide array of technologies and equipment (see Tab. 1). Selection of the methods for monitoring depends on the habitat type (and selected target species) to be addressed. Large sessile epibenthic species on hard substrates are preferably monitored using optical, non-destructive methods, such as ROVs. Living specimens can be collected by ROV arm. Endobenthic communities are sampled using standardised grabs or corers. The use of ROVs, bathyscaphes, or submarines provide visual and georeferenced information on the benthic communities on these habitats. Data about the presence of species, distribution patterns, estimates of densities, biological associations, etc., can be obtained. In the case of the ROVs and submarines, these allow the completion of video 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. High quality photographs and video recorded will then be analysed in laboratory (also with the help of taxonomists) to list the main conspicuous species/taxa or morphological groups recognisable on images and to evaluate their abundance (coverage or surface area in cm^2). Photographs can be archived to create temporal datasets. A selection of target species should be defined per sub-region (or bioregion) to allow for the consistent assessment of their 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 shortlived species if they characteristically occur in the habitat under natural conditions, as they can also be functionally very important for the community. This list should be updated every six years.

Although destructive methods are not desirable for long-term regular monitoring (UNEP/MAP-RAC/SPA, 2008), they become indispensable for a high-resolution characterisation of deep-sea communities. A variety of sampling gears has been used to collect sediment samples from vessels to identify the type of substrate, the granulometry, the organic matter content, and for the study of deep-sea organisms (Danovaro et al., 2010). Common devices are grabs, gravity cores, piston cores, box cores, and multiple corers, used in a number of randomly selected points within a study area. 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. 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, such as rocky reefs, soft bottom communities dominated by long-lived species (e.g., large sponges, gorgonians, bamboo corals).

Deep-sea macrofauna has been typically sampled using a modified Agassiz benthic trawl (2.3 m wide and 0.9 m high), a 14.76 m Marinovich-type deep-water trawl (codend mesh 6 mm) with a 0.5 mm plankton net secured on top, and different types and sizes of box corers, depending on the depth considered and the research teams. A 0.062 m^2 box corer with an effective penetration of 40 cm (Ocean Instruments model 700 AL) has been used in the Levantine Sea. The samples are typically preserved in 10% buffered formalin aboard the vessel. In the laboratory, samples are washed and sieved through 250 µm mesh (Danovaro et al., 2010).

Deep-sea megafauna has been sampled in the western Mediterranean by different methods, depending on the depth considered (Danovaro et al., 2010 and references therein). Commercial trawls can eb used, having horizontal mouth openings of 20-25 m and 3-5 m of vertical opening, with a 40 mm stretched mesh in the codend liner, which are trawled over the seafloor at about 3 knots. The otter semiballoon trawl gear (OTSB: 8 m horizontal spread and 0.8 vertical mouth opening) has been also used in the Mediterranean Sea. This sampling device was subsequently transformed into the otter trawl Maireta System (OTMS: 12 m horizontal spread and 1.4 m vertical opening approximately). The OTMS is equipped with SCANMAR sensors that provide information on bottom contact time and vertical and horizontal opening of the trawl's mouth down to 1500 m depth, allowing calculation of sampled area. Furthermore, the Agassiz trawl has been commonly used to sample the deep western and eastern Mediterranean benthos since the late 1980s.

The use of AUVs, CTDs, Niskin bottles and other methods to analyse the water column provides complementary information on water masses, currents, and physicochemical data, which combined with all the other information allows a better interpretation of deep ecosystems. Regarding AUVs, those equipped with multi-beam echosounder (or with side scan sonar) and cameras are also 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 advantageous approach with respect to use of large vessels for several days.

New techniques of DNA analysis, besides providing information on populations and species, 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.

Protocol for monitoring rocky reefs habitats down to 120 m depth

Although no standardised protocols exist to date for monitoring deep-sea habitats, the protocol recently proposed for monitoring mesophotic coralligenous reefs (down to 40 m depth) (Enrichetti et al., 2018) can be applied and adapted for monitoring deep-sea rocky habitats in the offshore circalittoral and the bathyal zones. The proposed protocol (all details can be found in Cánovas-Molina et al., 2016; Enrichetti et al., 2018) suggests a standard sampling design conceived to gather various quantitative components, such as the occurrence and extent of the rocky habitat, the siltation level, and the abundance, condition and population structure of habitat-forming megabenthic species (i.e., animal forests), as well as presence and typology of marine litter, through ROVs surveys.

Three replicated video-transects, each at least 200 m long, should be collected in each area investigated. Footages can be obtained by means of a ROV, equipped with a high definition digital camera, a strobe, a high definition video camera, lights, and a 3-jaw grabber. The ROV should also host an underwater acoustic positioning system, a depth sensor, and a compass to obtain georeferenced tracks to be overlapped to multi-beam maps when available. Two parallel laser beams (90° angle) can provide a scale for size reference. In order to guarantee the best quality of video footages, ROV are expected to move along linear tracks, in continuous recording mode, at constant slow speed (< 0.3 ms^{-1}) and at a constant height from the bottom (< 1.5 m), thus allowing for adequate illumination and facilitating the taxonomic identification of the megafauna. Transects are then positioned along dive tracks by means of a GIS software editing. Each video transect is analysed through any of the ROV-imaging techniques, using starting and end time of the transect track as reference. Visual census of megabenthic species is carried out along the complete extent of each 200 m-long transect and within a 50 cm-wide visual field, for a total of 100 m^2 of bottom surface covered per transect.

From each transect the following parameters are measured on videos:

- Extent of hard bottom, calculated as percentage of total video time showing this type of substratum (rocky reefs and biogenic reefs) and subsequently expressed in m^2
- Species richness, considering only the conspicuous megabenthic sessile and sedentary species of hard bottom in the intermediate and canopy layers. Organisms are identified to the lowest taxonomic level and counted. Fishes and encrusting organisms are not considered, as well as typical soft bottoms species. Some hard-bottom species, especially cnidarians, can occasionally invade soft bottoms by settling on small hard debris dispersed in the sedimentary environment. For this reason, typical hard bottom species (e.g., *Eunicella verrucosa*) encountered on highly silted environments have to be considered in the analysis
- Structuring species are counted, measured (height expressed in cm) and the density of each structuring species is computed and referred to the hard-bottom surface (as n° of colonies or individuals m^{-2})
- The percentage of colonies with signs of epibiosis, necrosis and directly entangled in lost fishing gears are calculated individually for all structuring anthozoans
- Marine litter is identified and counted. The final density (as n° of items m^{-2}) is computed considering the entire transect (100 m^2).

Within each transect, 20 random high definition photographs targeting hard bottom must be obtained, and for each of them four parameters are estimated, following an ordinal scale. Modal values for each transect are calculated. Evaluated parameters on photos include:

- Slope of the substratum: 0°,<30° (low), 30°-80° (medium),>80°(high)
- Basal living cover, estimated considering the percentage of hard bottom covered by organisms of the basal (encrusting species) and intermediate (erect species but smaller than 10 cm in height) layers: 0, 1 (<30%), 2 (30-60%), 3 (>60%)
- Coralline algae cover (indirect indicator of biogenic reef), estimated considering the percentage of basal living cover represented by encrusting coralline algae: 0, 1 (sparse), 2 (abundant), 3 (very abundant)
- Sedimentation level, estimated considering the percentage of hard bottom covered by sediments: 0%,<30% (low), 30-60% (medium),>60% (high).

All the above listed parameters allow the application of the seascape ecological index namely MACS (Mesophotic Assemblages Conservation Status; Enrichetti et al., 2019). MACS is a new multi-parametric index that is composed by two independent units, the Index of Status (Is) and the Index of Impact (Ii) following a DPSIR (Driving forces – Pressures – Status – Impacts – Response) approach. The Is depicts the biocoenotic complexity of the investigated ecosystem, whereas the Ii describes the impacts affecting it. Environmental status is the outcome of the status of benthic communities plus the amount of impacts upon them: the integrated MACS index measures the resulting environmental status of deep-sea rocky habitats reflecting the combination of the two units and their ecological significance.

Final remarks

Inventorying and monitoring dark habitats in the Mediterranean constitute 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. There is a huge necessity to improve knowledge of dark habitats and their distribution in the Mediterranean Sea, in order to establish international cooperation networks and also to facilitate sharing of experiences among Mediterranean countries. The existing scientific information on the distribution, biodiversity, functioning and connectivity of dark habitats on seamounts, in canyons, caves and escarpments must be continuously improved. Nevertheless, there are still obvious gaps of knowledge with regard to the distribution and diversity of dark habitats from the eastern and the southern parts of the Mediterranean Sea. The available scientific databases must be updated and integrated setting up collaborative tools and/or platforms to help scientists in exchanging data and experience. The assessment of associated ecosystem services should be also undertaken. Common monitoring protocols have to be defined, shared, and applied at the Mediterranean scale. The process of designation of new protected areas aiming at the conservation of deep-sea habitats must be enforced, as well as the 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).

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Annex 1. List of the most common species in Mediterranean marine caves. From SPA/RAC-UN Environment/MAP OCEANA, 2017.

* rare or endangered species

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, 1814)

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, 1814)

Erylus discophorus (Schmidt, 1862)

Fasciospongia cavernosa (Schmidt, 1862)

Geodia cydonium (Linnaeus, 1767)

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)

Lycopodina hypogea (Vacelet & Boury-Esnault, 1996)

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 gelatinosus (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 (Gmelin, 1791)

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

Phyllangia americana mouchezii (Lacaze-Duthiers, 1897)

Polycyathus muellerae (Abel, 1959)

Decapods

Athanas nitescens (Leach, 1813)

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, 1803)

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)

Hydroides pseudouncinata Zibrowius, 1968 [original]

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

Molluscs

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 (Wood, 1841)

Argyrotheca cuneata (Risso, 1826)

Joania cordata (Risso, 1826)

Megathiris detruncata (Gmelin, 1791)

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 in O.F. Müller, 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 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)