Annex VIII

Update of Monitoring Protocols on Benthic Habitats

1. Guidelines for monitoring marine vegetation in the Mediterranean

Introduction

1. 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 carbon sequestration (Waycott et al., 2009 and references therein). A significant economic value of over 17 000 $ per ha and annum has been quantified for seagrass meadows worldwide (Costanza et al., 1997).

2. Seagrass, like all Magnoliophytes, are marine flowering plants of terrestrial origin that returned to the marine environment approx. 120 to 100 million years. The global species diversity of seagrass is low 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 kilometers of coastline between the surface down to about 50 m depth (according to water transparency) in marine and 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 important 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).

3. Physical damages and stressful conditions 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). Biological impact caused by the spread of non-indigenous species (NIS) on seagrass beds must also be considered (Montefalcone et al., 2007). An alarming decline of seagrass meadows was reported in the Mediterranean Sea and mainly in the north-western side of the basin, where many meadows have been lost during the last decades (Boudouresque et al., 2009; Waycott et al., 2009; Pergent et al., 2012; Marbà et al., 2014; Burgos et al., 2017). However, a deceleration in the rate of loss and some signs of local recovery have also been observed, indicative of a recent trend reversal in seagrass extent and density, thanks to adequate management actions (de los Santos et al., 2019).

4. Concerns about these declines have prompted efforts to protect these habitats legally 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 (SACs), 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.

5. Due to their wide distribution 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. Standardized 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 harmonization process undertaken in this document.

6. Detailed spatial information on habitat distribution is prerequisite knowledge for the sustainable use of marine coastal areas. The 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). Few extents of the coastline have 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 standardized monitoring and mapping programs. As a consequence and following an explicit request by managers on the need for practical guides aimed at harmonizing existing methods for seagrass monitoring and 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 standardization 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 several scientific round tables addressed explicitly on this topic.

7. 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;
- Standardization 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 (e.g., dense meadows);
- Implementation of procedures could be difficult in some regions due to the absence of training, competence and/or specific financing.

8. 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 that 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 shoot density, lower limit depth), but the measuring techniques are often very different, and still require a larger effort to reach precise standardization;
- The different monitoring methods available in the Mediterranean countries seem all feasible when appropriate training is undertaken.

9. 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 to ease the task of the MPA managers when implementing their monitoring programs. A reviewing

process on the scientific literature, considering the latest techniques and the recent findings by the scientific community at the international level, has been carried out.

Monitoring methods

a) COMMON INDICATOR 1: Habitat distributional range and extent

Approach

10. 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 north-west Europe within the framework of the European projects MESH (Mapping European Seabed Habitats; MESH, 2007) and EUSeaMap (Vasquez et al., 2021a, b). 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

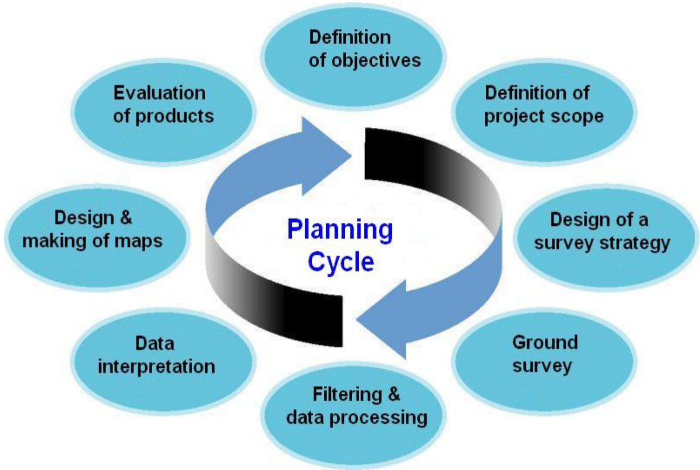

Figure 1: Planning cycle for a habitats' mapping programme (according to the MESH project).

11. Initial planning includes defining the objectives 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.

12. 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 better targeting of the work to be done.

13. 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

14. 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 accuracy used and the surface area to be mapped (Mc Kenzie et al., 2001; Fig. 2).

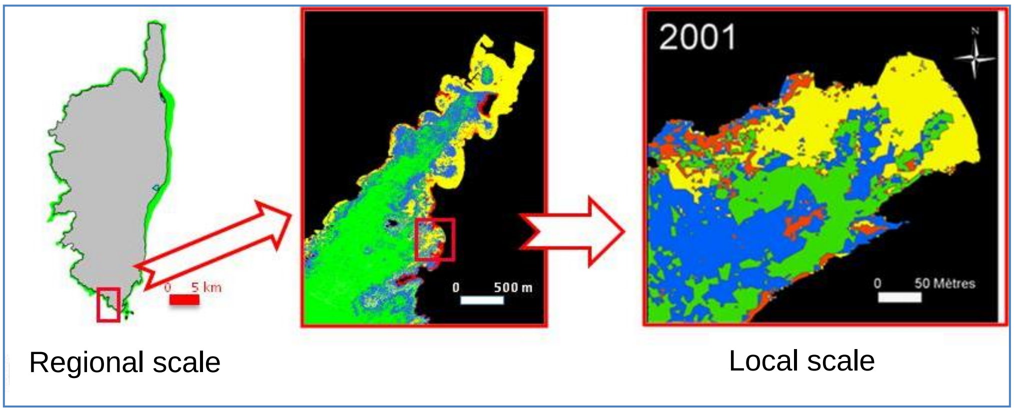

Figure 2: Resolution of a map from regional study to local study (from UNEP/MAP-RAC/SPA, 2015).

15. 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. 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.

16. 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 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 defined period. These high-resolution scales are also used to select sites where monitoring actions must be concentrated. As highlighted by the EU projects, most of the environment management and marine spatial planning activities require a range of habitat maps between these two extremes.

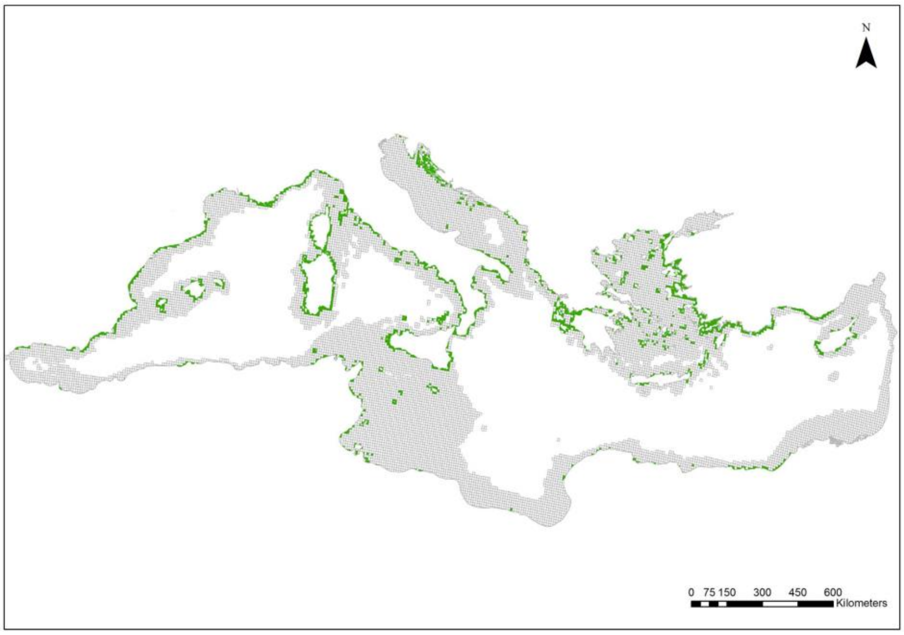

Figure 3: Distribution of *Posidonia oceanica* meadows in the Mediterranean Sea (green areas) (from Giakoumi et al., 2013).

Methods

17. 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 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; Rende et al., 2020; Rowan and Kalacska, 2021). 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, unmanned aerial vehicles). 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).

18. The use of remote sensing allows characterising extensive coastal areas to assess the spatial patterns of seagrass meadows. Itsimultaneously 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 unmanned aerial vehicles (UAVs), and acoustic vessel systems. The power of remote sensing techniques has been highlighted by Mumby et al. (2004), who showed that 20 s of airborne acquisition time would equal six days of field surveys. However, all indirect mapping techniques are intrinsically affected by uncertainties due to manual or automatic supervised classification of spectral or acoustic signatures of seagrass meadows on the images and sonogram, 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). Understanding of remote sensing data requires extensive field calibration and the ground-truthing process remains essential (Pergent et al., 2017). As the interpretation is also time-requiring, several image processing techniques were proposed to rapidly automate the interpretation of images and sonograms and make this interpretation more reliable (Montefalcone et al., 2013 and references therein; Rowan and Kalacska, 2021). These methods allow 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. The human eye, however, always remains the final judge.

19. Satellite telemetry is a valuable tool providing high-resolution regional- to global-scale observations and repeat time-series sampling on seagrass distribution in shallow waters. However, satellite imagery has some disadvantages, such as its reliance on weather conditions, high cost per scene, the revisit period, and the scale of many ecological processes (Ventura et al., 2018). Landsat images have been used successfully for regional mapping of seagrass distribution in many Mediterranean countries. The vast 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. Thanks to emerging technologies, such as long-range transmitters, increasingly miniaturized components for positioning, and enhanced imaging sensors, the collection of images by unmanned aerial vehicles (UAVs), also known as “drones”, coupled with the structure-from-motion (SfM) photogrammetry, offers a rapid and inexpensive tool to produce high-resolution orthomosaic (Ventura et al., 2018). 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 satellite images in seagrass habitats mapping (Pergent et al., 2017).

20. Airborne LIDAR bathymetry (ALB) or airborne light (lazer) detection and ranging (LIDAR) is a remote sensing technique for the bathymetry with an airborne scanning pulsed laser beam (Guenther, 1985). The technique is well suited to nearshore mapping because it provides the three-dimensional data needed to create an accurate digital terrain model (DTM) with 15-cm vertical accuracy (Irish et al., 2000). The LIDAR technology can measure depths up to three times Secchi depths, corresponding to about 60 m in very clear water (Guenther et al., 2000).

21. Once the surveying is completed, data collected needs to be organised to be used in the future by everyone and can be appropriately archived and easily consulted. The resulting dataset can be integrated with similar data from other sources, providing a clear definition of all metadata (MESH, 2007).

22. Despite the increasing number of studies on seagrass mapping with remote sensing instruments, datasets are not often available on digital geographic information system (GIS) platforms. As a final remark, only recently some modeling approaches have been developed to estimate the potential distribution of seagrass meadows in the Mediterranean. The probability of presence of a seagrass 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 *Posidonia oceanica* 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), and the main advantages and limits of each tool are indicated, with some bibliographic 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 400 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 • Not adequate for medium to small coastal dynamics	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 that allows distinguishing 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 • Fine-scale ecological studies	• 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 authorizations for imaging in some countries • Expensive data aquisition	Frederiksen et al. (2004); Kenny et al. (2003); Diaz et al. (2004)
Drone images (UAVs)	From 0 to 10-15 m	Small areas (10 km²)	From 0.1 m	Less than 1km²/hour	• Very high resolution • Manual, direct, and easy interpretation of the images • Availability of automated approaches for data classification • Good identification of boundaries between populations • Low-cost	• Limited to shallow waters characterization • Require permissions to fly over specific areas • Optical refractive distortion effects created by the water surface	Ventura et al. (2017, 2018); Rende et al. (2020)
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	• 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)

					• Quick execution		
Survey tool	**Depth range**	**Surface area**	**Resolution**	**Efficiency**	**Advantages**	**Limits**	**References**
Single-beam acoustic sonar	Below 10 m		From 0.5 m	1.5km²/hour	• Good geo-referencing • Quick execution	• Low discrimination between habitats • Lower reliability compared to satellite techniques	Kenny et al. (2003); Riegl and Purkis (2005)
Multi-beam acoustic sonar	Below 2-8 m	From large (50-100 km²) to small areas (a few hundred square meters)	From 50 cm	0.2 km²/hour	• Possibility to obtain 3D image of a meadow • Data on biomass per surface area unit can be obtained • Huge amount of data collected	• Efficient computer systems for processing and archiving data are needed • 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, according to local rules on scientific diving)	Small areas, usually between 25 m^2 to 100 m² for permanent square	From 0.1 m	0.01 km²/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	• Many working hours • Small areas mapped • Necessity of numerous observers to cover larger areas	Pergent et al. (1995a); Montefalcone et al. (2006)

					• Possibility to do simultaneous monitoring		
Video camera (ROV or towed camera)	Whole bathymetric range of seagrass distribution	Small areas, usually under 1 km²	From 0.1 m	0.2 km²/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, according to local rules on scientific diving)	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 relation to the base, and thus no possibility to work over large areas • Necessity of markers on the seafloor for positioning 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, according to local rules on scientific diving)	Small areas, under 1 km²	Some centimetres		• Same characteristics as for laser-telemetry, but with a greater range (1.5 km)	• Quite difficult technique • Need of many related equipments, and of a team of divers	Descamp et al. (2005)

1) Optical data

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

24. 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 can also be obtained even if their cost is much higher than the rough data. The use of satellite images for mapping seagrass meadows requires knowledge of satellite image analysis software (e.g., ENVI, ErdasGeomatica), mastery in the use of the water column correction algorithm (Lyzenga, 1978), and mastery with pixel-based remote sensing supervised classifiers, for example, the OBIA (Object-Based Image Analysis) classification algorithm.

Table 2: Types of satellites and resolution of the sensors used for mapping seagrass meadows.

Satellite	Resolution	References
LandSat 8	30 m	Dattola et al. (2018)
Sentinel 2A - 2B	10 m	Traganos and Reinartz (2018)
PLANET	3 m	Traganos et al. (2017)
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)

25. Given the changes in 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.

26. Although the spatial resolution of satellite imagery has significantly improved in the last decade, the data collected is still not sufficient for medium to small coastal dynamics. The resolution of the LandSat-8 satellite is not adequate to reach high resolution mappings of seagrass meadows. However, the image LandSat-8 OLI represents a useful tool to estimate the presence/absence of broad seagrass meadows; moreover, LandSat has a historical series of images useful to perform a multitemporal study. For these reasons, it has been suggested to consider the Sentinel-2 satellites of the Copernicus program. The Sentinel-2 satellites have a 13-band multispectral sensor (between visible and near infrared), the spatial resolution varies between 10 and 60 m and the satellite revisiting time in the same area is 5 days (while is 18 days for LandSat). Specifically, for mapping *Posidonia oceanica* meadows, various application tests demonstrated the good applicability of the Sentinel-2 image, at 10 m resolution, for an effective evaluation of the meadows' extent (Dattola et al., 2018; Traganos and Reinartz, 2018). The use of Sentinel-2 images, at the Mediterranean scale, can allow measuring the extent of the *P. oceanica* meadows habitat and verify any possible variations over time. The Sentinel-2 images are also useful for the analysis of pressure and impact drivers.

27. 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., CASI11, Deaedalus Airborne Thematic Mapper; Godet et al., 2009), which provide data in real time, also during unfavourable lighting conditions (Tab. 1). It is possible to create libraries with specific spectral responses to measure values compared to distinct component species and appraise the vegetation cover (Ciraolo et al., 2006; Dekker et al., 2006).

28. Aerial images obtained through various means (e.g., airplanes, ULM) may have different technical characteristics (e.g., shooting altitude, verticality, optical quality). Even though it is more expensive, shooting films from a plane, equipped with an altitude and verticality control system and using large size negatives (24 × 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 (Denis et al., 2003). Given the progress made in the last few decades in terms of shooting (e.g., the quality of the film, filters, lens) and the following processing (e.g., digitalization, geo-referencing), aerial photographs represent today one of the most preferred surveying methods for mapping shallow seagrass meadows (Mc Kenzie et al., 2001).

29. Recent applications of very fine resolution Unmanned Aerial Vehicles (UAVs), usually referred to as "drones", have shown effectiveness for mapping and for detecting changes in small patches and seascape features of seagrass meadows, at the scale and resolution that would not be possible with satellite or aerial photography (James et al., 2020). The application of UAVs for mapping and monitoring of seagrass habitats is limited by the optical characteristic of the water (e.g., turbidity) and environmental conditions (e.g., solar elevation angle, cloud cover, wind speed) during image acquisition (Rende et al., 2020 and references therein), and is therefore limited to shallow waters characterization. Imagery acquired by UAVs coupled with structure-from-motion (SfM) photogrammetry, has recently been extensively tested and validated for the mapping of the upper limits of seagrass meadows, as they offer a rapid and cost-effective tool to produce very high-resolution orthomosaics and maps of coastal habitats (Ventura et al., 2018).

2) Only recently the importance to integrate different methodological techniques (i.e., multispectral satellite, drone, multibeam echosounder, underwater towed video camera, autonomous surface vehicle) in a multi-scale approach for mapping seagrass meadows has been highlighted, as it allows for the acquisition of data with very high resolution and accuracy (Rende et al., 2020). An immediate advantage is related to the collection of large-scale remote sense data (with optic and acoustic methods), combined with images from underwater photogrammetry cameras for ground-truth, which ensures very high accuracy in both shallow and deep waters. At present, an integrated approach is the best option for seagrass mapping, as it offers a greater modularity in function of the spatial scales and allows optimizing costs, always maintaining the primary objective of high-resolution seafloor and habitat mapping, from the coastline to deeper water. *Acoustic data*

30. Sonar provides images of the seafloor through the emission and reception of ultrasounds. Among the main acoustic mapping techniques, Kenny et al. (2003) distinguishes: (1) wide acoustic beam systems like the Side Scan Sonar (SSS), (2) single-beam echosounder (3), multiple narrow beam bathymetric system, and (4) multi-beam echosounder.

31. Side Scan Sonar (SSS) 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 help interpreting the data and differentiating among habitats or substrate typologies. 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).

[1]CASI: Compact Airborne Spectrographic Imager

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

33. Multi-beam echosounder 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 are 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. A high-resolution 3D structure of the seafloor is also obtained (the digital elevation model, DEM), where meadows can be visualized and the biomass can be evaluated (Komatsu et al., 2003). Other derived products can be slope, aspect, curvature, and terrain ruggedness maps. Multi-beam echosounders surveys are also limited in very shallow waters, and especially at depths lower than 5 m where vessel navigation might be difficult and dangerous and the swath coverage is very limited (generally, it is 3-4 times the depth of the seabed; Rende et al., 2020).

3) Samplings and visual surveys

34. Field samples and direct underwater 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 (i.e., having a complete coverage of surface areas) obtained through interpolation methods from data collected 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, 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 given the protected character of these species (UNEP/MAP, 2009) and direct underwater samples (e.g., shoot samples) should be limited as much as possible.

35. Observations from the surface can be made by observers on a vessel using, for instance, a bathyscope, or underwater by using visual techniques such as photography and video recording. Video-photography plays a valuable role in seagrass research, as a non-destructive technique and especially in fine and meso-scale studies. Photographic equipment and video cameras can also be mounted on a platform structure (sleigh) or within the remotely operated vehicle (ROV). The camera on the platform is submerged at the back of the vessel and is towed by the vessel that advances very slowly (under 1 knot), allowing for the collection of long video transects; on the contrary the ROVs have their propulsion system and are remotely controlled from the surface and allow recording comparatively shorter video transects. Recent development in underwater photogrammetry and 2D photo mosaicing (i.e., merging several images of the same scene into a single and larger composite image photo mosaic by aligning and stitching photographs together) provided an ultrafine scaling methodology for micro-chartography and for monitoring activities in the short term to assess current regression/progression of individual meadows, such as using permanent squares or for monitoring the meadow boundaries (Rende et al., 2015). To acquire overlapping pictures, ensuring about 75% of shared coverage between two consecutive photos, the vessel needs to maintain a speed of about 1 knot/h. 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. This preliminary video survey may also be 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 improve the results obtained with the camera, joint acquisition modules integrating the depth and images of the seafloor with geographical positioning have been developed (UNEP/MAP-RAC/SPA, 2015).

36. *In situ* direct underwater observations by scuba diving represent the most reliable, although time-consuming, surveying technique. Surveys can be done along lines (transects), or over

small surface areas (permanent square frames, i.e., quadrates) positioned on the seafloor and located to follow the habitat limits. The transect consists of a marked line wrapped on a rib and laid on the bottom from fixed points and in a precise direction, typically perpendicular or parallel 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).

37. Marking the limits of a meadow also allows obtaining a distribution map. Laser-telemetry is a valuable 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 differential GPS 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 recorded their arrival times. 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 also be fixed on a submarine scooter driven by a diver. The maximum distance of the pinger in relationship to the center of the polygon formed by the 4 buoys can be approx. 1500 m (UNEP/MAP-RAC/SPA, 2015).

38. Freediving monitoring with a differential GPS can also be envisaged to locate the upper limits of the meadows. The diver precisely follows the contours of the limits and the GPS continuously records the diver’s geographical position. 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 a depth transect perpendicular to the coastline (© Monica Montefalcone).

Data interpretation

39. The recent EU projects on habitat mapping (MESH, 2007; Vasquez et al., 2021a, b) identified four essential stages to produce a habitat map:

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

40. During the processing, analysis and classification stage, pixels in the image (obtained from both optical and acoustic methods) are given a thematic label as belonging to groups that have either been defined by the user or generated by algorithm models to automate the classification process (Rowan and Kalacska, 2021). Object-Based Image Analysis (OBIA) differs from traditional pixel-based classification methods (maximum likelihood classifiers) because these latter techniques group similar, neighboring pixels into distinct image objects within designated parameters. A typical OBIA workflow involves firstly image segmentation (sequence of processes that are executed in a defined order including segmentation parameters that create meaningful objects made up of multiple neighbouring pixels sharing similar spectral values) and secondly classification of the segmented data through a multiresolution segmentation algorithm that generates objects with similar information by using only the most important features identified (Rende et al., 2020). OBIA methodology allows classifying also underwater cover classes in a rapid, accurate and cost-effective

way, and represents to date an effective tool to obtain robust thematic maps of benthic communities. An automatic classification approach can also be applied to underwater photogrammetry (Marre et al., 2020). Images must be georeferenced and before performing the 3D processing, an image enhancement technique should be performed to minimize the effect of the water column on the underwater images. After the image enhancement step, a Structure-from-Motion (SfM) 3D reconstruction is performed using any commercial software available (Rende et al., 2020). Finally, a Multiview Stereo (MVS) algorithm can be used to produce a dense 3D point cloud from the refined intrinsic orientation and ground-referenced camera exterior orientation.

41. To label and classify benthic habitats on resulting maps, a standardised classification system must be used to ensure the uniformity and the readability of maps. The two recently updated lists of benthic marine habitat types should be consulted, which are: 1) the European Nature Information System (EUNIS) proposed for the European seas (available at https://www.eea.europa.eu/data-and-maps/data/eunis-habitat-classification; Evans et al., 2016); and 2) the Barcelona Convention classification of marine benthic habitat types adopted for the Mediterranean region by the Contracting Parties (available at https://www.rac-spa.org/sites/default/files/doc_fsd/habitats_list_en.pdf; SPA/RAC-UN Environment/MAP, 2019a, b; Montefalcone et al., 2021). As seagrass assemblages are often small, they can only be identified with high (metric) precision mapping. The updated lists identify 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. The first original description of habitat types for the Mediterranean has been revised in 2015 (UNEP/MAP-RAC/SPA, 2015b), but a newly updated interpretation manual of all the updated reference habitat types for the Mediterranean region is under elaboration, which also provides the criteria for their identification. Habitats dominated by seagrass species listed in the updated Barcelona Convention classification system are the following (SPA/RAC-UN Environment/MAP, 2019a, b):

LITTORAL

MA3.5 Littoral coarse sediment

- MA3.52 Midlittoral coarse sediment
  - MA3.521 Association with indigenous marine angiosperms
  - MA3.522 Association with *Halophila stipulacea*

MA4.5 Littoral mixed sediment

- MA4.52 Midlittoral mixed sediment
  - MA4.521 Association with indigenous marine angiosperms
  - MA4.522 Association with *Halophila stipulacea*

MA5.5 Littoral sand

- MA5.52 Midlittoral sand
  - MA5.521 Association with indigenous marine angiosperms
  - MA5.522 Association with *Halophila stipulacea*

MA6.5 Littoral mud

- MA6.52 Midlittoral mud
  - MA6.52a Habitats of transitional waters (estuaries and lagoons)
  - MA6.521a Association with halophytes or marine angiosperms

INFRALITTORAL

MB1.5 Infralittoral rock

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

MB1.541 Association with marine angiosperms or other halophytes

MB2.5 Infralittoral biogenic habitat

MB2.54 *Posidonia oceanica* meadow

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, stripped meadow, atoll)

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 (estuaries and lagoons)

MB5.541 Association with marine angiosperms or other halophytes

MB6.5 Infralittoral mud sediment

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

MB6.511 Association with marine angiosperms or other halophytes

42. 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).

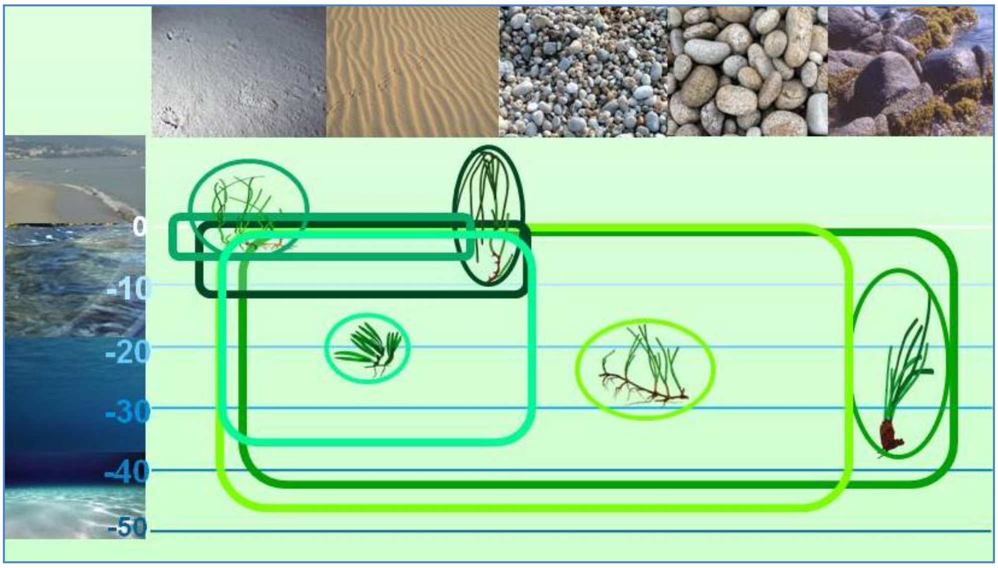

Figure 5: Distribution of seagrass species depending on the nature of the substrate and the depth in the Mediterranean (from UNEP/MAP-RAC/SPA, 2015a).

43. The data integration and modeling stage will differ depending on the survey tools and acquisition strategy used. Due to its acquisition rapidity, aerial techniques usually allow for a complete coverage of the littoral and shallow infralittoral zones and this dramatically reduces interpolation of data. On the contrary, surveys from vessels are often limited because of time and costs involved, and only rarely allow obtaining a complete coverage of the area. Coverage under 100% automatically means that it is impossible to get high resolution maps and therefore interpolation procedures must be used, so that from partial surveys a lower resolution map can be obtained (MESH, 2007; Fig. 6). Spatial interpolation is a geostatistical 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 and algorithms (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 the field and the percentage of interpolations.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 boundaries (upper and lower limits) of the meadow. The description between these two limits could be reduced to occasional field investigations leaving the interpolation to play its part (Pasqualini et al., 1998).

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

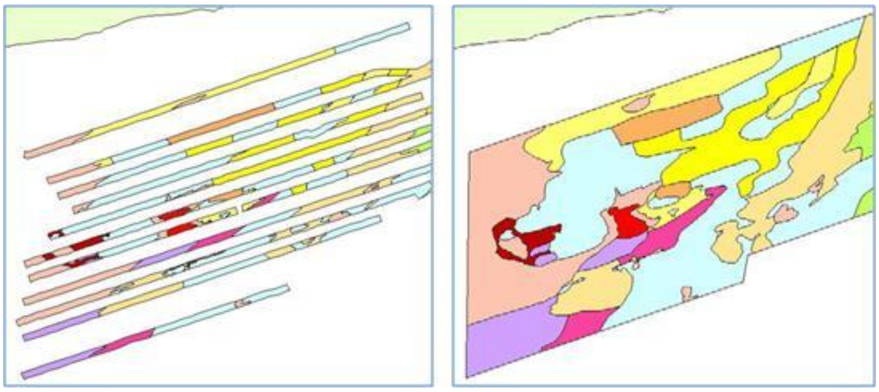

Figure 6: Example of partial coverage survey (left) and the output of the final map produced through interpolation (right). The area surveyed is about 20 km wide (from UNEP/MAP-RAC/SPA, 2015a).

45. To facilitate comparison among maps, standardized symbols and colors should be used for the graphic representation of the main seagrass assemblages (Meinesz and Laurent, 1978; Fig. 7). According to the newly updated classification of marine benthic habitat types for the Mediterranean region adopted by the Contracting Parties of the Barcelona Convention (available at https://www.rac-spa.org/sites/default/files/doc_fsd/habitats_list_en.pdf; SPA/RAC-UN Environment/MAP, 2019a, b; Montefalcone et al., 2021), all the habitats dominated by seagrass can be represented on maps using specific symbols and/or colors that can be labeled in the legend using their relative codes (e.g., code MB2.54: *Posidonia oceanica* meadow; code MB5.531: Association with indigenous marine angiosperms on fine sand in sheltered waters). When the cartographical detail is good enough, it is possible also to represent discontinuous meadows that are characterised by a cover below 50%, or the two main species that constitute a mixed meadow (the color 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 the map.

46. 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 also be compared with previous historical available data from the literature to evaluate any changes experienced by meadow over time (Mc Kenzie et al., 2001). Using the overlay vector methods on GIS, a diachronic analysis can be done, where temporal changes are measured in terms of percentage gained or lost in the meadow extension, through the creation of concordance and discordance maps (Barsanti et al., 2007).

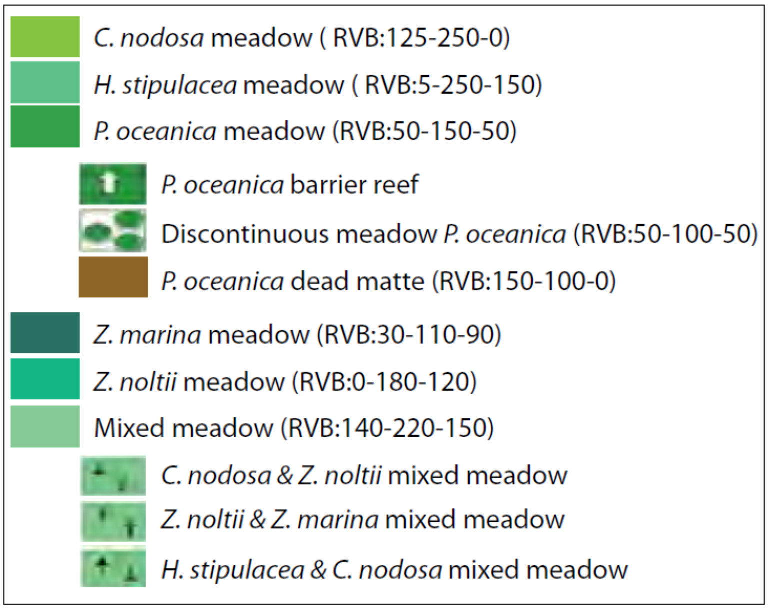

Figure 7: Examples of symbols and colours used for the graphic representation of the main seagrass assemblages. RVB: values in red, green, and blue for each type of meadow (from UNEP/MAP-RAC/SPA, 2015a).

47. The reliability of the map produced should also be evaluated. Several evaluation scales for reliability have already been proposed and may be helpful for seagrass meadows. Pasqualini (1997) proposed a reliability scale about 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, 2015a). 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 restitution scale needs to be adapted.

48. Denis et al. (2003) proposed a reliability index for 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, 2015a). The reliability index ranges from 0 to 20 and can vary from one point to another on the map, depending on the bathymetry and the survey technique used.

49. 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

50. Seagrasses are 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 Marine Strategy Framework Directive (MSFD, 2008/56/EC) and the IMAP CI2related to EO1 "biodiversity". The CI2 is aimed at providing information about the condition (i.e., ecological status) of seagrass meadows.

51. Monitoring the ecological status of seagrass meadows is today mandatory and is even an obligation for numerous Mediterranean countries since:

- Four out of the five species present in the Mediterranean (*Cymodocea nodosa*, *Posidonia oceanica*, *Zostera marina*, and *Z. noltei*) are listed in the Annex II (list of endangered or threatened species) of the Protocol concerning Specially Protected Areas and Biological Diversity (SPA/BD protocol, 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 I (strictly protected flora species) of the Bern Convention concerning the Mediterranean geographical region;
- Seagrass meadows are defined as priority natural habitats by the European Directive No. 92/43 (EEC, 1992).

52. 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 seagrass 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).

53. Defined and standardized procedures for monitoring the status of seagrass meadows, comparable to those 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.

54. The <u>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.

55. The <u>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 the 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 should be planned during a favourable season, also because some of the parameters chosen for monitoring purposes must be collected during the same period due to the seasonality in seagrass growth. This phase might be quite long, especially if numerous sites have to be monitored.

56. <u>Monitoring over time and data analysis</u> phase seems to be easy being the data acquisition a routinary 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, to be useful, must be medium time at least. This phase often constitutes the key element of the monitoring system as it makes possible to:

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

57. Monitoring of seagrass meadows is linked with the conservation targets and with their use as ecological indicators of the quality of marine environment. The main aims of seagrass monitoring are generally:

- Preserve and conserve the heritage of marine priority habitats, with the aim of ensuring that seagrass meadows are in a satisfactory ecological status (GES) and to identify as early as possible any degradation of these priority habitats or any change in their distributional range and extent. Assessment of the ecological status of meadows allows measuring the effectiveness of local or regional environmental 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 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 environments;
- Evaluate effects of any coastal activity and construction likely to impact seagrass meadows during environmental impact assessment (EIA) procedures. This particular kind of monitoring aims to establish the condition of the habitat at the time “zero” (i.e., before the beginning of activities), then the state of health of the meadow is monitored during the development of the work phase or at the end of the phase, to check for any impact on the environment evaluated as changes in the meadow state of health. The EIA procedure is not intended as a typical monitoring activity, although it provides the state of the system at the “zero” time, which can be very useful in the time series obtained during a monitoring programme. Unfortunately, most of the EIA studies are qualitative and are often performed by environmental consultants without specialized personnel, using unspecific guidelines and without following any standardised procedure, which prevent their use in effective monitoring programs.

58. The objective(s) of the monitoring system 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.

59. 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 standardized 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).

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 depth of its upper and lower 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 every 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 sites and 1 impact site, all representative of the coastal area	• Physical descriptors of the quality of environment (e.g., water turbidity, enhancement in nutrients, nitrogen content of leaves and rhizomes, chemical contamination, trace metals in plant) • Descriptors of the state of health of meadow (e.g., cover, shoot density, lower limit depth)	• Medium term (5 to 8 years) • Data acquisition is variable depending on the species concerned (every 1-3 years)
Environmental impact assessment (EIA)	The site subject to coastal development or interventions. The selection of 2 reference/control sites might be also useful for comparison	• Specific descriptors to be defined depending on the possible effects of human activities on seagrass	• 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

Methods

60. 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 standardized 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 a detailed description of the sampling tools and methodologies can be found.

61. The many descriptors available for monitoring seagrass habitat (see Table 4) work at different ecological complexity levels (Montefalcone, 2009), which are from the highest to the lowest: the seascape (i.e., the whole habitat), the ecosystem, the associated community (e.g., leaf

epiphytes), the population (i.e., the meadow), the species (i.e., the plant), the cellular or physiological/biochemical level. At each ecological level, a pool of different descriptors and indices can be selected. The selection of the most appropriate descriptor/index should be made considering the specificity of the monitoring program and of its objectives, the means (also funds) available, and the duration of the activities. The best choice would be to combine two or more descriptors/indices to capture the various responses of the system to environmental conditions and to accurately define the health status of seagrass (Oprandi et al., 2019). Some ecological indices (see next section) working at the highest ecological levels have been recently developed. At the seascape level there are, for instance, the Conservation Index (Moreno et al., 2001), the Substitution Index and the Phase Shift Index (Montefalcone et al., 2007), and the Patchiness Index (Montefalcone et al., 2007); at the ecosystem level there is the EBQI (Personnic et al., 2014), while other ecological indices integrate different ecological levels, such as for instance the PREI (Gobert et al., 2009), the BiPo (Lopez y Royo et al., 2009), and the POMI (Romero et al., 2007).

62. Descriptors listed in Table 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 depth of the limits); ii) *in situ* observations and measures by scuba diving (e.g., lower limit type, cover, rhizome baring, and shoot density); 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 considered 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 inside MPAs. However, when the monitoring objective is the assessment of environmental quality, descriptors capable to link the influence of pressures with the health status of the plants are necessary, which usually require the collection of shoots (e.g., descriptors working at the physiological/biochemical level). An effective monitoring should be done at intervals over a fixed period, even if it would mean a reduced number of sites and a reduced number of descriptors being monitored. Number of adopted descriptors should be adequate 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; Oprandi et al., 2019). The nature of the descriptor is less important than its reproducibility, reliability and the precision of the method used for its acquisition.

63. *In situ* observations and samples must be done over defined and, possibly, standardized 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 species (the plant), cellular or physiological/biochemical level, and most of the analyses at the community level (i.e., the associated organisms of leaves and rhizomes) require collection of shoots. For *Posidonia oceanica*, the mean number of sampled shoots ranges between a minimum of 9 to a maximum of 18-21 shoots collected at each sampling station (Pergent-Martini et al., 2005). At each station, an equal number of shoots should be collected in three distinct areas tens of meters apart (e.g., 3 to 6 shoots per area, for a total of 9 to 18 shoots per station).

64. Among all the descriptors listed in Table 4, the shoot density is the most adopted, standardized and 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 proved effective in revealing environmental alterations (Montefalcone, 2009). Meadow seascape is often patchy (at large spatial scale), but the meadow distribution within patches (medium to small spatial scales) can also be highly heterogeneous (Bacci et al., 2015). The size of the quadrate and the criteria used for randomly placing it on the bottom are crucial to standardize the method to measure shoot density. For measuring *P. oceanica* shoot density, two sizes of the quadrate are usually adopted: 40 cm × 40 cm and 20 cm × 20 cm. The use of a larger surface area (1600 cm^2) incorporate the small-scale meadow heterogeneity, increasing the variability between replicates and thus decreasing the sensibility of statistical test to detect differences between stations. The use of the

20 cm × 20 cm quadrate (400 cm^2) can reduce this small-scale variability increasing the probability to detect clear spatial patterns. The overall time required for data acquisition increases according to the quadrate size: counting shoots in a 40 cm × 40 cm quadrate is at least four times more time-consuming than in a 20 cm × 20 cm one (Bacci et al., 2015). Smaller quadrates are also easier to use and counting errors are less likely to happen. On the other hand, smaller quadrates require a larger number of replicates to catch the natural shoot density variability. Many studies showed that the use of the 20 cm × 20 cm quadrate is more effective than the use of the 40 cm × 40 cm or larger quadrates, as it allows reaching a better accuracy level given the same sampling effort (Charbonnel et al., 2000; Bacci et al., 2015). To speed the count of shoot density in very dense *P. oceanica* meadows (as usually occur in correspondence of the upper limit), as well as in very sparse meadows (in correspondence of the lower limits), the use of the smaller quadrate 20 cm × 20 cm is recommended. Similarly, the 20 cm × 20 cm quadrate is generally used to measure shoot density of other smaller seagrass species (e.g., *Cymodocea nodosa*, *Zostera noltei*). A minimum of 3 independent replicated counts should be done in each of the three distinct areas tens of meters apart, totalising 9 counts per station that are enough to catch the natural within patches variability. The 3 replicated quadrates in each area must be randomly located within homogeneous seagrass patches with maximum coverage. On the contrary, in the case of a patchy meadow, quadrates must be positioned randomly using a stratified sampling procedure on the vegetated patches, and the number of replicates can be increased with 6 replicated quadrates in each area, totalising 18 mesurements per sampling station.

65. Measuring the depth and defining the typology of both the upper and the lower limits of the meadow (Fig. 8), as well as monitoring over time their bathymetrical position with permanent marks (i.e., *balises*) are other commonly adopted procedures to assess the evolution of the meadow in term of stability, improvement or regression that is linked to water transparency, water movement, sedimentary balance, and human activities along the coastline.

66. An adequate number of sampling stations must be localised randomly within the meadow according to its extent, and usually in correspondence of the meadow upper limit, the meadow lower limit and at intermediate depth. As stated before, at each depth (i.e., station) 3 sampling areas must be selected, tens of meters apart. To assess the overall ecological condition of the meadow and to reduce the number of sampled shoots, shoots can be collected only at the intermediate depth of the meadow, which is usually located at about 15 m depth, where the meadow is expected to find the optimal conditions for its development (Buia et al., 2004). When the aim of the monitoring program includes biochemical measurements, a sampling station in the deepest portion of the meadow should also be included, since many sources of pressure are usually displaced to deep areas (e.g., wastewater treatment plants, fish farms). Due to the seasonality of most of the descriptors (especially for those linked with leaves growth), sampling activities should be carried out during the late spring or early summer season (Gobert et al., 2009).

67. Following the requirements of the WFD and the MSFD in the European countries, the ecological quality of the environment must be defined according to classification scales. For *P. oceanica* shoot density the absolute scale proposed for its classification (Pergent-Martini et al., 2005) has been adapted with the creation of five classes of ecological quality (bad, poor, moderate, good, and high; Annex 1) and can be used at the Mediterranean wide spatial scale, although it has been elaborated using data from *P. oceanica* meadows of France and Corsica. The absolute classification scale for the lower limit depth (Annex 1) is another valid tool to assess the meadow ecological status. Although all the existing absolute scales proposed for *P. oceanica* represent important standardized tools to classify the ecological status of meadows in the frame of the IMAP procedure and allow for the comparisons among regions, they could require some adaptations according to the specific geographical area and the morphodynamics setting of the site. It is more than likely that the threshold values fixed between classes are not valid at the whole Mediterranean scale: regional and even more local sub-regional scales should be defined (Montefalcone et al., 2007), providing the same methodologies and intercalibration procedures. For instance, in many *P. oceanica* meadows of the Ligurian Sea (NW Mediterranean), along the Spanish coast (NW Mediterranean), and of the North Aegean Sea (NE Mediterranean) (Marbà et al., 2014; Oprandi et al., 2019; Gerakaris

et al., 2021), the lower limit rarely reaches depths greater than 20-25 m, due to natural constrains (e.g., substrate typology, seafloor topography). Adopting the absolute scale proposed for the lower limit depth, all these meadows would be classified from moderate to bad ecological status, even in the case of low human pressure. Also the nitrogen (N) content in leaves is highly variable within meadows and shows a high natural variability among meadows in the Mediterranean. Each country/region is thus suggested to define proper local regional scales for the classification of each descriptor, which should also be compared with the absolute scales for the Mediterranean Sea to point out geographical patterns (Annex 1)

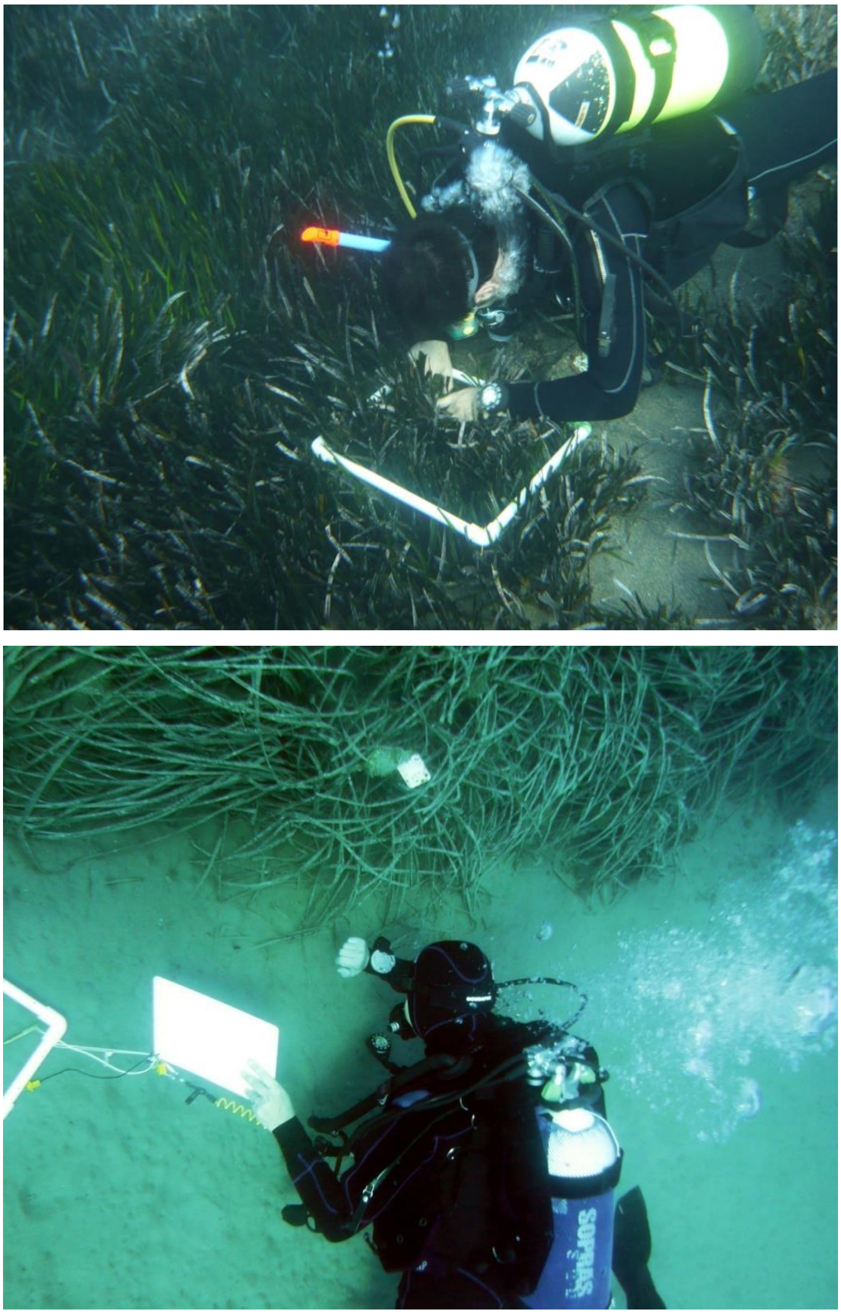

Figure 8: *In situ* measurement of *Posidonia oceanica* shoot density using a quadrate of 40 cm × 40 cm (upper panel, © Monica Montefalcone) and monitoring over time of the meadow lower limit position with permanent marks (lower panel, © Annalisa Azzola).

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

Descriptor	Method	Expected response/factors	Destr	Target species	Advantages	Limits	References
Seascape level							
Meadow extent (i.e. surface area)	Mapping (Cf. Part “a” of this document) and/or definition of the meadow boundaries	• 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)
Population (meadow) level							
Bathymetric position of the meadow upper limit (in m) and its morphology	A detailed mapping of the seagrass upper 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 and direct destruction	No	All	• Easily measured (also by scuba diving) • Morphology of this limit may reflect environmental conditions	• For Cn, Hs and Zn, strong seasonal variability, requiring periodical monitoring or observations during the same season on all sites • Fixed markers (*balises*) might disappear if the	Pergent et al. (1995); Montefalcone (2009)

						site is strongly frequented	
Descriptor	**Method**	**Expected response/factors**	**Destr**	**Target species**	**Advantages**	**Limits**	**References**
Bathymetric position of the meadow lower limit (in m)	A detailed mapping of the seagrass lower 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 • Water turbidity	No	All	• Easily measured (also by scuba diving) • Absolute classification scale available for Po	• For Cn, Hs and Zn, strong seasonal variability, requiring periodical monitoring or observations during the same season on all sites • Beyond 30 m depth, underwater surveys are difficult and costly (limited diving time, need for experienced divers, numerous dives requested) • Fixed markers (*balises*) might disappear (e.g., by trawling) • For slow growing species (Po) long time required to see any progress (several years)	Pergent et al. (2008); Annex 1

Meadow lower limit morphology	*In situ* visual observations	• Change in morphology • Water turbidity, mechanical damages (e.g., trawling)	No	Po	• Well known descriptor • Several morphologies described • Absolute classification scale for Po	• Good knowledge of Po meadows necessary to identify some of the morphologies • Beyond 30 m depth, underwater surveys are difficult and costly (limited diving time, need for experienced divers, numerous dives requested)	Boudouresque and Meinesz (1982); Pergent et al. (1995); Montefalcone (2009); Annex 1
Descriptor	**Method**	**Expected response/factors**	**Destr**	**Target species**	**Advantages**	**Limits**	**References**
Presence of inter-matte channels and dead matte areas	High resolution and detailed mapping of the area (Cf. Part "a" of this document, permanent square frames) and/or *in situ* observations	• Increase in the extent • Mechanical damages (e.g., anchoring, fishing gear)	No	Po	• Surface areas can be easily measured on maps	• Dead matte areas are natural components intrinsic in some typologies of meadows (e.g., striped meadows) and do not reflect systematically human influence	Boudouresque et al. (2006)
Density (shoots · m^{-2})	No. of shoots counted underwater within a square frame (a quadrate of fixed dimension) by divers. The square size depends on the seagrass species and on the meadow density. For *P. oceanica* the most adopted sizes are 40 cm × 40 cm and 20 cm × 20 cm	• Reduction • Water turbidity, mechanical damages (e.g., anchoring)	No	All	• Easily measured • Low-cost • Can be measured at all depths that can be safely reached by scuba diving • Absolute classification scale available for Po	• Strong variability with depth • Long acquisition time for densities over 800 shoots per square meter • Many replicates necessary to evaluate meadow heterogeneity • Considerable risk of error if: a) the surveyor is inexperienced; b) high	Duarte and Kirkman (2001); Pergent-Martini et al. (2005); Pergent et al. (2008); Bacci et al. (2015); Annex 1

						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 laboratory (but becoming a destructive technique)	
Descriptor	**Method**	**Expected response/factors**	**Destr**	**Target species**	**Advantages**	**Limits**	**References**
Cover (in %)	Average percentage of the surface area occupied (in vertical projection) by meadow in relation to the surface area observed. Various methods to visual estimate the cover *in situ* by divers or in laboratory (from photos or video). Variable observation surface area (0.16 to 625 m²), visualised by a quadrate or a transparent plate	• Reduction • Water turbidity, mechanical damages	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 spatial 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)

Percentage of plagiotropic rhizomes	Counting of plagiotropic rhizomes on a defined surface area (e.g., 20 cm × 20 cm, which can be visualised by a quadrate)	• Increase • Mechanical damages (e.g., anchoring, fishing gear)	No	Cn, Po	• Easy, rapid, and low-cost • Absolute classification scale available for Po	• Mainly used at shallow depths (0-20 m)	Boudouresque et al. (2006); Annex 1
Species (plant) level							
Leaves surface area ($cm^2 \cdot$ shoot), and other phenological measures	Counting and measuring the length and width of the different types of leaves in each shoot (9 to 18-20 shoots according to the sampling design)	• Reduction of leaves surface area (Po) for overgrazing and human impacts • Increase in the length of leaves (Po, Cn) for nutrients enhancement	Yes	All	• Easy and low-cost • Possibility to measure the length of adult leaves (the most external leaves) *in situ* to avoid sampling • Absolute classification scale available for Po	• Strong seasonal variability • Strong individual variability and necessity to measure (and sample) an adequate number of shoots • Destructive sampling	Giraud (1977, 1979); Lopez y Royo et al. (2010b); Orfanidis et al. (2010); Annex 1
Descriptor	**Method**	**Expected response/factors**	**Destr**	**Target species**	**Advantages**	**Limits**	**References**
Necrosis on leaves (in %)	Percentage of leaves with necrosis, through observation in laboratory	• 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) • Destructive sampling	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 left by the bit of some animals are easily recognizable	• Not informative on the grazing pressure in the case of strong water movement and on old leaves	Boudoresque and Meinesz (1982)
Foliar production	For Po possibility, thanks to lepidochronology, to	• Reduction • Nutrients deficit, increase in	Yes (Po)	All	• For Po lepidochronology allows assessments at all depths	• Long time to analyse	Pergent (1990) ; Gaeckle et al.

(in mg dry weight · shoot^{-1} yr^{-1})	reconstruct number of leaves produced in one year, at present or in the past. For other species, measuring leaves through marking or by using the relationship bases length/leaves growth (Zm)	interspecific competition	No (Zm)		• Absolute classification scale available • For Zm the relationship bases length/leaves growth allows *in situ* non destructive measuring	• Monthly monitoring, or at least every season, is necessary • Destructive sampling for Po	(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 • Absolute classification scale available for Po	• Increase in the rhizome production can also be observed in reference sites in the absence of human impacts • Destructive sampling	Pergent et al. (2008); Annex 1
Descriptor	**Method**	**Expected response/factors**	**Destr**	**Target species**	**Advantages**	**Limits**	**References**
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	• Easily measured *in situ* • Not destructive and low-cost • Independent from the season		Boudoresque et al. (2006)
Cellular or physiological/biochemical level							
Nitrogen and phosphorus content (in %	Dosage through mass spectrometry and plasma torch in	• Increase • Nutriments enhancement	Yes	All	• Short response time to environmental changes	• Very expensive	Romero et al. (2007); Annex 1

dry weight) in plant tissues	different plant tissues (both leaves and rhizomes) after acid mineralisation (e.g., in rhizome for Po)				• Absolute classification scale for Po	• Analytical equipment and specific competence necessary • Destructive sampling	
Carbohydrate content (in % dry weight) in plant tissues and sediments	Dosage through spectrophotometry after alcohol extraction in different plant tissues (e.g., in rhizome for Po)	• Reduction • Human impacts	Yes	All	• Short response time to environmental changes • Absolute classification scale for Po	• Very expensive • Analytical equipment and specific competence necessary • Destructive sampling	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 (both leaves and rhizomes) after acid mineralisation	• Increase • Increased concentration of metallic contaminants	Yes	All	• Short response time to environmental changes • Absolute classification scale for Po	• Very expensive • Analytical equipment and specific competence necessary • Destructive sampling	Salivas-Decaux (2009); Annex 1
Descriptor	**Method**	**Expected response/factors**	**Destr**	**Target species**	**Advantages**	**Limits**	**References**
Nitrogen isotopic relationship ($d^{15}N$ in ‰)	Dosage through mass spectrometer in different plant tissues after acid mineralisation (e.g., in 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 • Destructive sampling	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 • Analytical equipment and specific competence necessary	Romero et al. (2007)

						• Destructive sampling	
Community							
Epiphytes biomass (in mg dry weight · $shoots^{-1}$ or % dry weight · $shoots^{-1}$) and epiphytes cover (in %) on the leaves	Measure of biomass (µg · $shoots^{-1}$) after scraping, drying and weighing; 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	• Easily measured • Low-cost (biomass and cover) • Absolute classification scale available for Po • Early-warning indicator	• Time-consuming • Strong seasonal and spatial variability • Specific analytical equipment (nitrogen content) necessary • Destructive sampling	Morri (1991); Pergent-Martini et al. (2005); Romero et al. (2007); Fernandez-Torquemada et al. (2008); Giovannetti et al. (2008, 2015)

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

69. At the regional spatial scale, two main monitoring systems have been developed: 1) the seagrass monitoring system (SeagrassNet), which has been established at a 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 for 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 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 standardized approach for the *P. oceanica* monitoring network has been tested and validated (UNEP/MAP-RAC/SPA, 2009). The main differences between the 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.

70. SeagrassNet allows compare the data obtained in the Mediterranean with the data obtained in other regions of the world, having a world-wide coverage on over 80 sites distributed in 26 countries (available at www.seagrassnet.org). However, this monitoring system is not suitable for large-size species (such as *Posidonia* genus) and for meadows where the 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 comparing different meadows in the Mediterranean, and evaluating the plant's vitality and the quality of the environment where it grows. Other monitoring system, such as permanent transects with seasonal monitoring, or acoustic surveys, can be used in specific situations like the monitoring of lagoons (Pasqualini et al., 2006) or for the study of relict meadows (Descamp et al., 2009).

71. The sampling technique and the chosen descriptors define the nature of the monitoring (e.g., monitoring of chemical contamination in 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 monitoring 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	×		
Temperature	×		×
Salinity	×		
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

72. As a final remark, the IMAP should also consider the long-term organic carbon stored in seagrass sediments from both *in situ* production by photosynthetic activity and sedimentation of particulate carbon from the water column, known as “Blue Carbon” (Nellemann et al., 2009). The estimation of the Blue Carbon should consider above and below ground living and dead biomass and soil fine and coarse carbon. Recent findings, however, suggested clearly that most of the carbon stored in seagrass is in the soil, being the fractions stored as living tissue virtually negligible. Hence, soil stocks rather than biomass stocks should be the focus of assessment in Mediterranean seagrass. International guidelines had been provided for this estimation from the Blue Carbon Initiative and IUCN (Howard et al., 2014, IUCN, 2021). Following this, soil carbon is determined by soil depth, bulk density and % of organic carbon in the first meter of the soil. Advanced techniques for large scale Blue Carbon inventories using high resolution sub-bottom profilers have been recently developed in the Mediterranean (Monnier et al., 2020). In the case additional carbon sequestration would like to be estimated, the methodology proposed by lepidochronology (i.e., the ‘retro-datation’ of *Posidonia* rhizomes) will provide estimations on the plant growth and accretion rates over a short timescale (although it is often very variable). The sequestration rate calculated using the accretion rate should be determined using C^{14} to date the age at which soil was laid down. The following parameters are useful for the estimation of carbon contents in plant tissues:

- Leaf Biomass Index (Leaf Standing Crop) (dry weight · m^{-2}): it is calculated by multiplying the average leaf biomass per shoot by the density of the meadow reported per square meter;
- Leaf Surface Index (Leaf Area Index) ($m^2 \cdot m^{-2}$): it is calculated by multiplying the average leaf area per shoot by the density of the meadow reported per square meter;
- Height of the leaf canopy to be estimated by means of acoustic, optical, and *in situ* measurements.

73. Monitoring activities should also be planned on key typical species associated to seagrass meadows, such as for instance the bivalves *Pinna* spp. Given the critical situation of *P. nobilis* in the Mediterranean and the apparent incipient expansion of *P. rudis* within *P. oceanica* meadows, visual censuses of these species in monitored meadows should be seriously considered.

Data processing and interpretation

74. Measurements made *in situ* must be analysed 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 classification 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.

75. The huge increase of studies on *Posidonia oceanica* (over 2700 publications indexed in the Web of Science on April 2021) 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; Montefalcone, 2009; Salivas-Decaux et al., 2010; Tab. 4).

76. 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 and the "Seagrass Atlas of Spain" available at http://www.ieo.es/es/atlas-praderas-marinas), 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.

<u>Ecological indices</u>

77. Ecological synthetic indices are today widespread for measuring the ecological status of ecosystems given 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 seagrass monitoring programs in the past, e.g., the Leaf Area Index (Buia et al., 2004), the Epiphytic Index (Morri, 1991). Following the requirements of the WFD, the MSFD, and the EcAp in the European countries, many synthetic indices have been set up to provide, based on 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 (Tab. 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)

78. Most of the ecological indices integrate different ecological levels (Tab. 6). The POSWARE index is based on 6 descriptors working at the population and species levels. 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, species and cellular levels. 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 in plant tissues), thus showing little usage in the *P. oceanica* monitoring programs (Pergent-Martini et al., 2005). The Valencian CS index integrates 9 descriptors from species to community level. The PREI index is based on 5 descriptors working at the population, species and community levels. The BiPo index is based only on 4 non-destructive descriptors at the population and species levels and is particularly well suited for the monitoring of protected species or within MPAs.

79. Some not-destructive ecological indices have been developed to work at the seascape ecological level, such as the Conservation Index (CI; Moreno et al., 2001), the Substitution Index and the Phase Shift Index (SI and PSI, respectively; Montefalcone et al., 2007), and the Patchiness Index (PI; Montefalcone et al., 2010). 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., water movement). 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. The 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. The PI has been developed to evaluate the degree of fragmentation of the habitat and uses the number of patches for measuring the fragmentation of seagrass meadows. All these seascape indices are useful tools for assessing the quality of coastal environments in their whole (as requested by the MSFD), not only for assessing the quality of the water bodies (as requested by the WFD).

80. One of the most recently proposed indices works at the ecosystem level (EBQI; Personnic et al., 2014). This index has been developed based on 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 by selecting 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 but also complex formulation makes this index more time-consuming when compared to other indices.

81. Intercalibration trials between the POMI and the POSID indices have shown that there is coherence in the classification of the sites studied (Pergent et al., 2008). Applying the BIPO index to 9 Catalonia sites yielded an identical classification to that obtained with the POMI index (Lopez y Royo et al., 2010c). Concurrent application of the POMI, PREI, BiPo, and Valencian CS in the Eastern Mediterranean Sea showed high comparability among indices (Gerakaris et al., 2017). Finally, using both the POSID and the BiPo indices within the framework of the “MedPosidonia” program, similar classifications of the meadows studied were found (Pergent et al., 2008). A recent exercise to compare several descriptors and ecological indices working at different ecological levels (species, 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 compared descriptors (Karayali, 2017; Oprandi et al., 2019). In view of this result, a concurrent use of more descriptors and indices, covering different levels of ecological complexity, should be preferred in any monitoring programme.

82. 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 over several sites and to start wide intercalibration processes. As a general comment, those indices based on a high number of descriptors imply excessive costs in terms of acquisition time and budget required (Fernandez-Torquemada et al., 2008), although the use of a comparatively lower number of descriptors can lead to an oversimplification, particularly in those situations where specific pressures should be linked to the meadow state of health.

Table 6: Descriptors used in the mostly adopted synthetic ecological indices in the regional/national monitoring programs to evaluate the environmental quality based on the "seagrass" biological quality element. The ecological complexity level at which each descriptor works is also indicated (i.e., cellular, species, population, community, ecosystem, seascape).

Index	Cellular	Species	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	Leaf 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	Leaf surface; Coefficient A; rhizomes elongation	Shoot density; meadow cover; percentage of plagiotropic rhizomes; depth of the lower limit	Epiphytes biomass		
Valencian CS		Leaf surface; percentage of foliar necrosis	Shoot density; meadow and dead matte cover; percentage of plagiotropic rhizomes; rhizome baring/burial	Herbivore pressure; leaf epiphyte's biomass		
PREI		Leaf surface; leaf biomass	Shoot density; lower limit depth and type	Leaf epiphytes biomass		
BiPo		Leaf 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

Index	**Cellular**	**Species**	**Population**	**Community**	**Ecosystem**	**Seascape**
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

Absolute 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 (species 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

Cell (physiological/biochemical level): 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%

Cell (physiological/biochemical level): 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 the upper circalittoral Mediterranean zone

Introduction

1. The calcareous formations of biogenic origin in the Mediterranean Sea are represented by coralligenous reefs, vermetid reefs, reefs of *Sabellaria* spp., serpulid reefs, cold water corals reefs in deep waters, encrusting Corallinales concretions/trottoirs made by *Lithophyllum byssoides*, *Titanoderma trochanter*, and *Tenarea tortuosa*, banks formed by the corals *Cladocora caespitosa*, *Astroides calycularis*, *Phyllangia americana mouchezii*, *Polycyathus muellerae,* reefs formed by the stylasteridae *Errina aspera*, bryozoan nodules and biostalactites within semi-dark and dark caves, and rhodoliths seabeds. Among all, coralligenous reefs (Fig. 1) and rhodoliths seabeds (Fig. 2) are the two most typical and abundant bioconstructed habitats that develop in the Mediterranean upper circalittoral zone (sometimes also in the lower littoral 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 standardization.

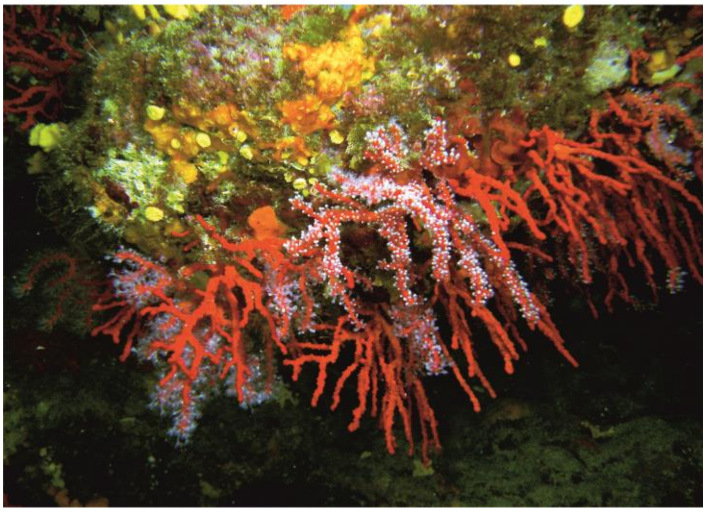

Figure 1: Coralligenous habitat dominated by the gorgonian *Paramuricea clavata* (upper panel © Simone Musumeci), and facies with *Corallium rubrum* in enclave in the coralligenous (lower panel © Monica Montefalcone).

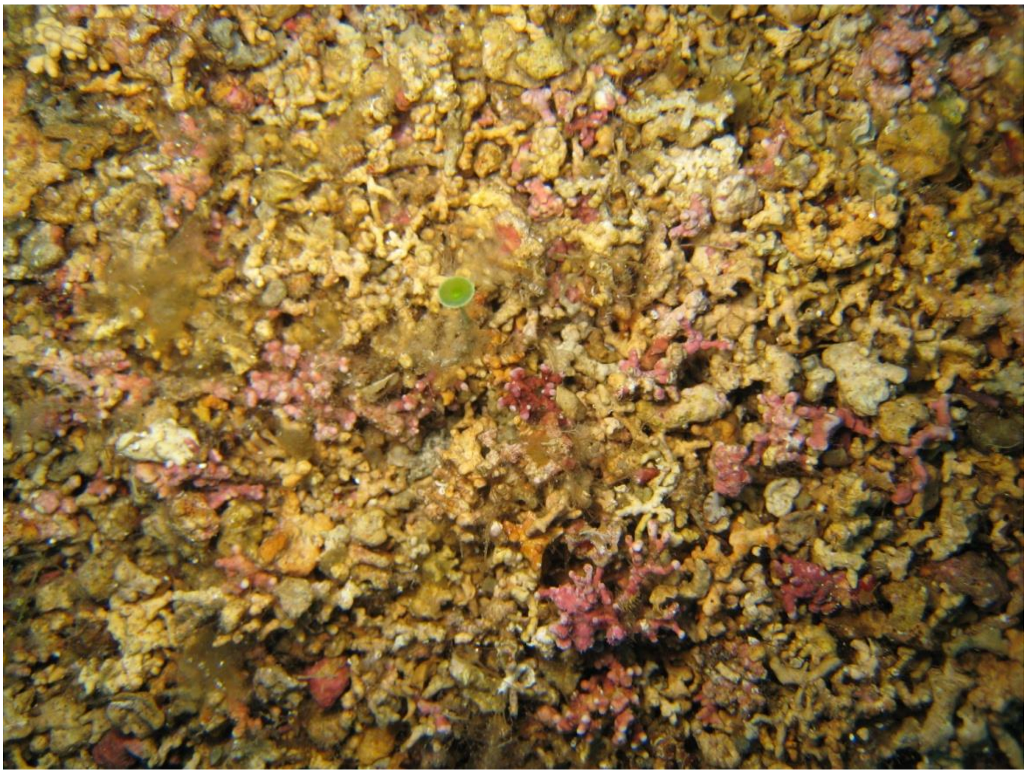

Figure 2: Rhodoliths habitat (photo from UNEP/MAP-RAC/SPA, 2015).

2. The most important and widespread bioconstruction in the Mediterranean Sea is represented by coralligenous reefs (UNEP/MAP-RAC/SPA, 2008), an endemic and characteristic habitat considered as the climax biocoenosis in the upper 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 with high economic values (Cánovas-Molina et al., 2014). Construction of coralligenous reefs started during the post-Würm transgression, about 15000 years ago, and developed on rocky and biodetritic bottoms in relatively stable conditions of temperature, currents, and salinity.

3. Coralligenous reefs are distributed both on rocky and soft bottoms, developing different morphologies: i) coralligenous developing on the upper circalittoral rocks and at the entrance of caves with cliffs, outcrops, banks, rims, atolls; and ii) coralligenous developing over circalittoral soft/detritic bottoms creating biogenic platforms (Bonacorsi et al., 2012; Piazzi et al., 2019b). Coralligenous habitat results from the dynamic equilibrium between bioconstruction, mainly made by encrusting calcified Rhodophyta belonging to Corallinales and Peyssonneliales (such as species belonging to 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 can 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 about 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.

4. 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. They create a biogenic, unstable, three-dimensional habitat typically exposed to bottom currents, which harbors greater biodiversity compared to surrounding bottoms, and thus are viewed as biodiversity hotspots. Rhodoliths beds mainly occur on coastal detritic bottoms in the upper circalittoral 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 include 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., 2016). The name maërl should be restricted to those rhodoliths beds that are composed of non-nucleated, unattached growths of branching, twig-like coralline algae.

5. Coralligenous reefs provide different ecosystem services to humans (Paoli et al., 2017), such as provisional (food, materials, habitat), regulating (carbon sequestration, nutrient recycling), and cultural services. They are vulnerable to global and local pressures. Coralligenous is threatened by direct human activities, such as trawling, pleasure diving, illegal exploitation of protected species, artisanal and recreational fishery, aquaculture, and is also vulnerable to the indirect effects of climate change and global warming (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, by forming dense carpets or by increasing sedimentation rate.

6. Despite the occurrence of many species with high ecological value (some of which are also legally protected, e.g., *Savalia savaglia*, *Spongia (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 this Directive, and appear also in the Bern Convention. This implies that the most important Mediterranean bioconstruction remains without formal protection as it is not included within the list of Special Areas of Conservation (SACs). Few years after the adoption of the Habitat Directive, coralligenous reefs were listed among the “special habitat types” needing rigorous protection by the protocol concerning the Special Protected Areas and Biological Diversity (SPA/BD Protocol) of the Barcelona Convention (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 in 2008 and updated in 2016, the legal conservation of coralligenous assemblages has been encouraged by the establishment of marine protected areas and the need for standardized programs for its monitoring has been emphasized. Coralligenous has also been included in the European Red List of marine habitats by IUCN, where the lower infralittoral coralligenous bioconcretions (code A5.6x) are classified as “near-threatened”, and the circalittoral coralligenous bioconcretions (code A5.6y) 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 Status of the marine environment. Biogenic structures, such as coralligenous reefs, have thus been recognized as important biological indicators of environmental quality.

7. Similarly, rhodolith seabeds are expected to be damaged by dredging, heavy anchors and mooring chains, and trawling and are 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 priority habitats of the Directive and therefore is given protection through the designation of Special Areas of Conservation (SACs). Moreover, a special plan for the legal protection of Mediterranean rhodoliths beds has been adopted within the framework of the "Action Plan for the Conservation of Coralligenous and other Mediterranean bio-constructions" (UNEP/MAP-SPA/RAC, 2017). Rhodolith seabeds have also been included in the Natura 2000 sites and in the Red List of Mediterranean threatened habitats by IUCN.

8. The Action Plan (UNEP/MAP-SPA/RAC, 2017) 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 on the composition (list of species) of these habitats;

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

9. Detailed information on habitat geographical distribution and bathymetrical ranges is prerequisite for the sustainable use of marine coastal areas. Coralligenous and rhodoliths distribution maps are a fundamental prerequisite to any conservation action on these habitats and their associated species (Azzola et al., 2021). The scientific knowledge concerning several aspects of biogenic concretions (e.g., taxonomy, processes, functioning, biotic relationships, and dynamics) is currently increasing. However, it is still far away from the knowledge we have on 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 spatial-temporal studies on their geographical and depth distribution both at regional level and basin-wide scale. This information is essential to know the real extent of these habitats in the Mediterranean Sea and to implement appropriate management measures to guarantee their conservation (UNEP/MAP-SPA/RAC, 2017). Inventory and monitoring of coralligenous and rhodoliths raise several problems, due to their large bathymetric distribution and the consequent sampling constraints, the often-limited accessibility, heterogeneity, and the lack of standardized 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 exist, common methods for monitoring rhodoliths are comparatively less documented.

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

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

- If underwater scuba diving is recommended for mapping and monitoring at small spatial scales and at shallower depths, it becomes unsuitable when the study area and/or the depth increase (usually at depths >40 m);

Acoustic survey methods (side scan sonar or multibeam echosounder) coupled with underwater visual observation systems (ROV, towed camera), which provide ground-truth data, becomes then dispensable at depths greater than 40 m.

12. 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 rely mainly on underwater scuba diving activities but given the above listed constraints, using other tools of investigation (e.g., ROV, towed camera) should be also considered because they allow monitoring on larger areas and at greater depths;
- Although the use of underwater photography or videorecording may be relevant, the presence of specialists in taxonomy with a good experience in surveying methods is often essential given the complexity of these habitats. Abundance or coverage of specific taxa can be visually estimated underwater on defined surfaces or along transects through standardized indices. The presence of broken individuals and of areas of necrosis are other factors to be considered;
- Monitoring of coralligenous habitat starts with the realisation of micro-mapping and then applying descriptors and/or ecological indices. However, these descriptors vary widely from one team to another, as well as their measurement protocols;
- Monitoring of rhodolith habitats can be done by underwater scuba diving and visual inspection using ROVs or towed cameras and collecting samples using dredges, grabs, and box corers. At present, there is not any standardized method yet that has been widely accepted for monitoring rhodoliths, also because the action of water movement may cause a shift of these habitats on the seabed making their inventory rather difficult.

13. In the framework of the Barcelona Convention Ecosystem Approach (EcAp) implementation and based on the recommendations raised during the meeting of the Ecosystem Approach Correspondence Group on Monitoring (CORMON), Biodiversity and Fisheries (Madrid, Spain, 28 February - 1 March 2017), the Contracting Parties requested SPA/RAC to develop standardized monitoring protocols to be used in the context of the Integrated Monitoring and Assessment Programme (IMAP), to ease the task for the countries when implementing their monitoring programmes. The two guidelines published by SPA/RAC, the ‘Standard methods for inventorying and monitoring coralligenous and rhodoliths assemblages’ (UNEP/MAP-RAC/SPA, 2015) and the ‘Guidelines for inventorying and monitoring of dark habitats in the Mediterranean Sea’ (SPA/RAC-UN Environment/MAP, OCEANA, 2017), have been considered in the elaboration of this document. A reviewing process on the available scientific literature, considering the latest techniques and the recent works carried out by the scientific community at the international level, has been also carried out. If standardized protocols for seagrass mapping and monitoring exist and are well-implemented, and several 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 some of the most adopted descriptors for inventorying and monitoring the coralligenous and rhodoliths in the Mediterranean are described, with the relative advantages, restrictions, and conditions for their use. Some of the monitoring methods for coralligenous have already been compared or cross-calibrated and results are briefly reported here. A standardized procedure recently proposed for coralligenous monitoring is also described.

Monitoring methods

a) COMMON INDICATOR 1: Habitat distributional range and extent

Approach

14. The CI1 aims to provide information about the geographical area in which 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 the Mediterranean" for major details):

1) Initial planning, which includes the definition of the objectives 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, it is the costliest phase as it generally requires field activities;

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

Resolution

15. 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 critical 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 can be accepted, which means that the habitat distribution and the definition of its boundaries are often only indicative. When smaller areas have to be mapped, much higher precision and resolution are required and it is easily achievable thanks to the high-resolution mapping techniques (e.g., multibeam echosounder) available to date. However, obtaining detailed maps is costly, thus practically impossible when time or resources are limited (Giakoumi et al., 2013). These detailed maps provide accurate localisation of the habitat distribution and a precise definition of its boundaries and total habitat extent, all features necessary for future control and monitoring purposes over time. These high-resolution scales are also used to select remarkable sites where monitoring actions must be concentrated.

16. 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).

17. Although we have an overall knowledge about the composition and occurrence of coralligenous and rhodoliths habitats in the Mediterranean (Ballesteros, 2006; Relini, 2009; Relini and Giaccone, 2009; UNEP-MAP-RAC/SPA, 2009), the scarceness of fine-scale cartographic data on the geographical 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 Mediterranean 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-standardized legends, even within the same country. Updated data have also been collected in the last few years in some countries, thanks to the new monitoring activities afferent to the MSFD, and this information will become available in the coming years (see for instance Aguilar et al., 2018; SPA/RAC-UNEP/MAP, 2020).

18. 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 were 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 on the presence of coralligenous formations in the southern and the eastern coasts of the Levantine Sea,

although recent information has become available from Lebanon (Aguilar et al., 2018; SPA/RAC-UNEP/MAP, 2020). 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 the southern and the 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.

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

20. These low-resolution global maps on coralligenous and rhodoliths distribution are still incomplete being the available information highly heterogeneous due to the high variability in 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, these global maps 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.

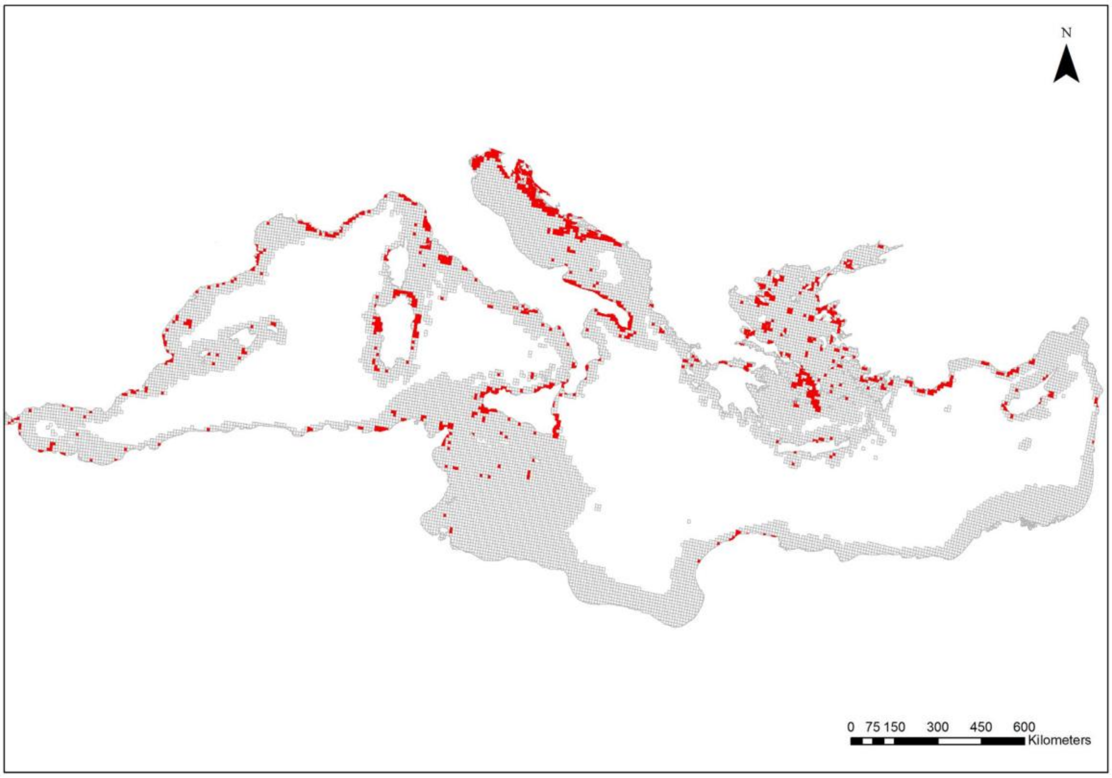

Figure 3: Global scale distribution of coralligenous habitat in the Mediterranean Sea (red areas) (from Giakoumi et al., 2013).

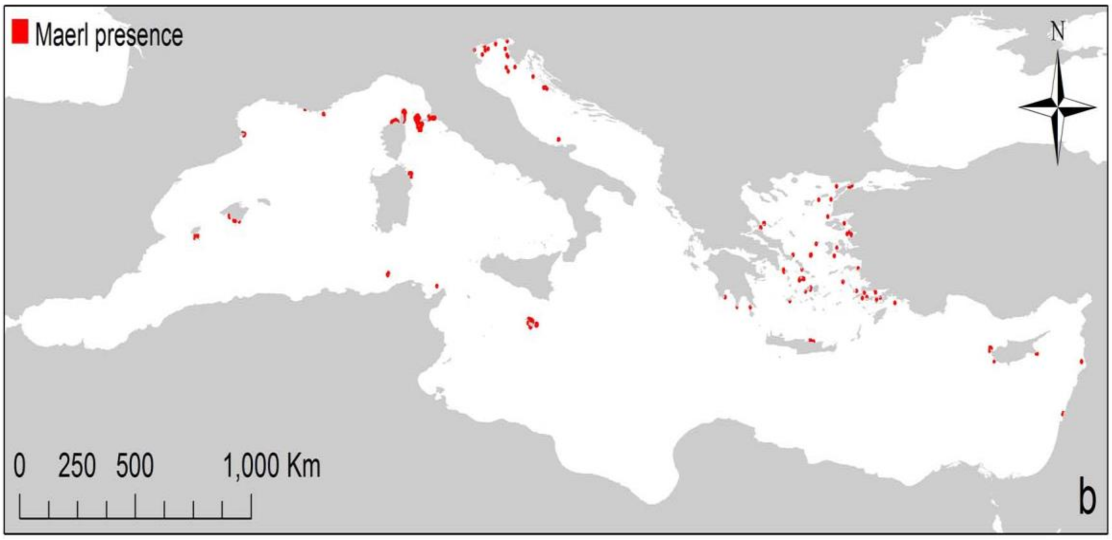

Figure 4: Global scale distribution of rhodoliths/maërl habitat in the Mediterranean Sea (red areas) (from Martin et al., 2014).

Methods

21. Definition of distributional boundaries and extent of coralligenous and rhodolith habitats requires “traditional” habitat mapping techniques, like those used for seagrass meadows in deep waters (Tab. 1). Remote sensing mapping techniques and/or underwater visual surveys must be used and are often integrated. The simultaneous use of two or more mapping methods makes it possible to optimise the results being the information obtained complementary. The strategy to be adopted will depend on the study’s aim and the area concerned, means, and time available.

<u>Underwater observations and sampling methods</u>

22. Although underwater direct observation by scuba diving (e.g., visual assessments along transects) is often used for mapping small areas, this method of investigation quickly shows its limits when the study area and depth increase significantly, even if the assessment can be improved through the integration with video transects. Direct underwater observations provide discrete punctual data that are vital for ground-truthing the instrumental surveys, and for the validation of modelled/interpolated continuous information (i.e., 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 and rhodoliths habitats.

23. *In situ* underwater observations represent the most reliable, although time-consuming, mapping technique of coralligenous habitat up to 30-40 m depth, according to local rules for safe scientific diving (Tab. 1). Surveys can be done along lines (transects) or over small surface areas (permanent quadrates) positioned on the seafloor and located to follow the limits of the habitat. A transect consists of a marked line wrapped on a rib and laid on the bottom from fixed points and in a precise direction, typically perpendicular or parallel to the coastline (Bianchi et al., 2004a). Any change in the habitat and the substrate typology, within a belt at both sides of the line (considering a surface area of about 1-2 m per side), is recorded on underwater slates. The information registered allows precise and detailed mapping of the sector studied (Tab. 1).

24. Scuba diving is also suggested as a safe and cost-effective tool to obtain a visual description and sampling of shallow rhodoliths beds up to 30-40 m depth, according to local rules for scientific diving (Tab. 1). Underwater observations are effective for a first characterisation of the aboveground facies of this habitat, while describing 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 varying thickness of dead material and finer sediment. There is 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 substrate 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 covered 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 ranges, areal extent, occurrence of sedimentary structures on the seafloor (such as ripples, mega-ripples, and underwater dunes), thickness of live layer, mean percentage cover of live thalli, live/dead rhodoliths ratio, dominant morphologies of rhodoliths(see Fig. 5).

25. Recently an innovative tool, namely the BioCube, a 1 m high device that enables the acquisition of 80 cm × 80 cm frame photo-quadrates, has been implemented to charecterise the aboveground detritic and rhodoliths seabottoms 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 seascape for complementary information on demersal fish and extent of assemblages.

26. Sampling methods from vessel 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 (to ground-truth the acoustic data) and for a complete taxonomical and structural 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 substrate stratification. The use of destructive sampling methods from vessel for characterizing rhodoliths beds should be, however, as much as possible discouraged, in order to minimize the impact of the investigation.

27. The potential contribution of citizen science networks for mapping and monitoring coralligenous habitat should be mentioned (Gerovasileiou et al., 2017), especially for assessing mass mortality events linked with global warming and heat waves (Garrabou et al., 2019). See for instance the initiatives available at http://cs.cigesmed.eu/en and https://t-mednet.org/mass-mortality/mass-mortality-events). The CIGESMED protocol, in particular, has already been applied in different parts of the Mediterranean (David et al., 2014; Çinar et al., 2020).

Remote sensing surveys

28. Being the biogenic coralligenous and rhodoliths habitats mainly distributed down to 30 m depth, the remote sensing acoustic techniques (side scan sonar and multibeam echosounder) and the underwater video recording (through ROVs and towed cameras) are usually recommended (Georgiadis et al., 2009). The use of remote sensing allows characterising extensive coastal areas to define the overall spatial patterns of coralligenous and rhodoliths habitats. From maps obtained through remote sensing surveys, the presence/absence of the habitat, its bathymetrical ranges, its boundaries, and the total habitat extent can be obtained. Acoustic methods are presently the most convenient technique for mapping rhodoliths beds, associated with ground-truthing by ROV and/or 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 geolocation system, allows monitoring change in the extent of rhodoliths habitat over time (Bonacorsi et al., 2010).

29. Visual observations from the surface can be made by using imagery techniques such as photography and videorecording. Photographic equipment and cameras can be mounted on a vertical structure (sleigh or platform) 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), while 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, identify specific features of the habitat, and evaluate any change in the habitat or in other characteristic elements of the seafloor. This preliminary video survey may be also useful to locate specific monitoring stations. Recorded images are then reviewed to obtain a cartographical restitution on a GIS platform for each area surveyed. To facilitate and 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).

30. 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 the multibeam 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 associated with substrate types on sonograms. Errors in sonogram 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 time-requiring, several automatic supervised processing techniques have been recently proposed to rapidly automate the interpretation and the classification of acoustic signatures and to make this interpretation more reliable (Montefalcone et al., 2013 and references therein; Viala et al., 2021), also considering that current technology provides systems of neural networks and artificial intelligence to support these operations. These classification methods allow for good discrimination between soft sediments and rocky reefs. Human eye, however, always remains the final judge.

Modelling

31. 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, water movement, nutrient concentrations, seabed types), which are typically easier to record and map at regional and global scales, in contrast to data on species and habitats. A recent study showed the correlation between wind-wave energy at the bottom and the rhodoliths bed presence (Agnesi et al., 2020). It also provided the confidence interval of this environmental variable associated with the probability of rhodoliths beds to occur, therefore informing on the wave energy values required for the modelling in the off-shore continental shelf. Despite inherent limitations and associated uncertainties, predictive modelling is a cost-effective alternative to field surveys as it can help identifying and mapping areas 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 have been defined in the North African coast, where there are no available cartographic data to date. For rhodoliths, phosphate concentration, geostrophic velocity of sea surface current, silicate concentration, and bathymetry were the four main contributors to the model. Given the lack of occurrence data for this habitat across the Mediterranean, and especially in the North African coast and the southern Levantine coast, the model output is relatively informative in highlighting several suitable areas where no cartographic data are available to date.

32. A recent application of predictive spatial modelling was done starting from a complete acoustic coverage of the seafloor combined with sea-truthing underwater observations 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* observations. The model allowed recognising and mapping the coralligenous habitat within the MPA and showed that the distribution of the habitat was mainly driven by the distance from coast, the depth, and the lithotypes. Other examples of habitat predictions can be found in Zapata-Ramírez et al. (2016) and Rossi et al. (2021).

Table 1: Synthesis of the 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 limits of each tool are indicated, with some bibliographic references.

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Underwater diving and visual surveys	0 m up to 40 m, according to local rules on safe scientific diving	Small areas, less than 250 m^2	From 0.1 m	0.0001 to 0.001 km^2/hour	• Very great precision in 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 scientific divers required (safety constraints) • Variable geo-referencing of the dive site	Piazzi et al. (2019a, and references therein)
Sampling from vessels with blind grabs, dredges, or box corers	0 m to about 50 m (until the lower limit of the rhodoliths bed)	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 identified • Possibility of *a posteriori* identification • Low cost, easy to implement	• Destructive method • Small area inventoried • Need of sampling materials • Analyses on samples very time-consuming • Limited operational depth • Difficulty in collecting representative samples	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)	<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) • Quick execution • Very big mass of data • Non-destructive	• Flat (2D) picture to represent 3D complex habitats • Possible errors in sonograms interpretation • Acquisition of field data necessary to validate sonograms • High cost • Not effective for mapping vertical slopes	Cánovas-Molina et al. (2016b)
Survey tool	**Depth range**	**Surface area**	**Resolution**	**Efficiency**	**Advantages**	**Limits**	**References**
Multibeam echosounder	2 m to over 120 m (until the lower limit of the coralligenous habitat)	From small areas (a few hundred square meters) to large areas (50-100 km²)	From 50 cm (linear) and lower than few centimetres	0.5 to 6 km²/hour	• Possibility to obtain 3D representation of the seafloor • Double information collected (bathymetry and seafloor image) • Very precise and wide bathymetric range • Quick execution • Very big mass of data • Non-destructive	• Less precise recognition of the nature of the seabed than side scan sonar • Acquisition of field data necessary to validate the interpretation of acoustic data • 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 to collect pictures • Good identification of habitat and conspicuous species • Wide bathymetric range	• High cost	Cánovas-Molina et al. (2016a); Enrichetti et al. (2019)

Towed camera	2 m to over 120 m (until the lower limit of the coralligenous habitat)	Intermediate-large areas (some km^2)	From 1 m to 10 m	0.025 to 1 km^2/hour	• Easy to implement and possibility to collect pictures • Good identification of habitat and conspicuous species • Non-destructive • Large area covered	• Limited to homogeneous and horizontal bottoms • Slow recording and processing of information • Variable positioning (georeferencing) • Water transparency • Hard to handle in the case of heavy nautical traffic	UNEP/MAP-RAC/SPA (2015)

Data interpretation

33. Once the surveying is completed, data collected need to be organized in order to 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 to ensure future integration with similar data from other sources. To produce a habitat map, four important steps must be followed:

a. Processing, analysis and classification of biological data and their correct and precise geolocation, through a process of interpretation of acoustic images when available;

b. Selecting the most appropriate physical layers (e.g., substrate, bathymetry, hydrodynamics);

c. Integration of biological data and physical layers, and use of statistical modelling to predict habitat distribution and interpolate punctual information;

d. The map produced must then be evaluated for its accuracy, i.e. its capacity to represent reality, and its reliability.

34. During the first processing analysis and classification step, a standardised classification system must be used to label and classify benthic habitats on resulting maps and to ensure the uniformity and the readability of the final maps. The two recently updated lists of benthic marine habitat types should be consulted, which are: 1) the EUropean Nature Information System (EUNIS) proposed for the European seas (available at http://eunis.eea.europa.eu; Evans et al., 2016); and 2) the Barcelona Convention classification of marine benthic habitat types adopted for the Mediterranean region by the Contracting Parties (available at https://www.rac-spa.org/sites/default/files/doc_fsd/habitats_list_en.pdf; SPA/RAC-UN Environment/MAP, 2019a, b; Montefalcone et al., 2021). The two updated lists identify the specific coralligenous and rhodolith habitats that may be found from the infralittoral zone to the circalittoral zone, with their main characteristic associations and facies. The first original description of habitat types for the Mediterranean has been revised in 2015 (UNEP/MAP-RAC/SPA, 2015b), but a new updated interpretation manual of all the updated reference habitat types for the Mediterranean region is under elaboration, which also provides the criteria for their identification. Habitats of coralligenous and rhodoliths listed in the updated Barcelona Convention classification system are the following (SPA/RAC-UN Environment/MAP, 2019a, b):

INFRALITTORAL

MB1.5 Infralittoral rock

- MB1.55 Coralligenous (enclave of circalittoral)

CIRCALITTORAL

MC1.5 Circalittoral rock

- MC1.51 Coralligenous cliffs
  - MC1.51a Algal-dominated coralligenous
    - MC1.511a Association with encrusting Corallinales
    - MC1.512a Association with Fucales or Laminariales
    - MC1.513a Association with sciaphilic algae (except Fucales, Laminariales, encrusting Corallinales, and Caulerpales)
    - MC1.514a Association with non-indigenous Mediterranean *Caulerpa* spp.
  - MC1.51b Invertebrate-dominated coralligenous

MC1.511b Facies with small sponges

MC1.512b Facies with large and erect sponges

MC1.513b Facies with Hydrozoa

MC1.514b Facies with Alcyonacea

MC1.515b Facies with Ceriantharia

MC1.516b Facies with Zoantharia

MC1.517b Facies with Scleractinia

MC1.518b Facies with Vermetidae and/or Serpulidae

MC1.519b Facies with Bryozoa

MC1.51Ab Facies with Ascidiacea

MC1.51c Invertebrate-dominated coralligenous covered by sediment

See MC1.51b for examples of facies

MC1.52 Continental shelf rock

MC1.52a Coralligenous outcrops

MC1.521a Facies with small sponges

MC1.522a Facies with Hydrozoa

MC1.523a Facies with Alcyonacea

MC1.524a Facies with Antipatharia

MC1.525a Facies with Scleractinia

MC1.526a Facies with Bryozoa

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

MC1.522c Facies with Alcyonacea

MC1.523c Facies with Scleractinia

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

MC2.515 Facies with large and erect sponges

MC2.516 Facies with Hydrozoa

MC2.517 Facies with Alcyonacea

MC2.518 Facies with Zoantharia

MC2.519 Facies with Scleractinia

MC2.51A Facies with Vermetidae and/or Serpulidae

MC2.51B Facies with Bryozoa

MC2.51C Facies with Ascidiacea

MC3.5 Circalittoral coarse sediment

MC3.51 Coastal detritic bottoms

MC3.511 Association with Laminariales

MC3.512 Facies with large and erect sponges

MC3.513 Facies with Hydrozoa

MC3.514 Facies with Alcyonacea

MC3.515 Facies with Pennatulacea

MC3.516 Facies with Polychaeta (*Salmacina*-*Filograna* complex included)

MC3.517 Facies with Bivalvia

MC3.518 Facies with Bryozoa

MC3.519 Facies with Crinoidea

MC3.51A Facies with Ophiuroidea

MC3.51B Facies with Echinoidea

MC3.51C Facies with Ascidiacea

MC3.52 Coastal detritic bottoms with rhodoliths

MC3.521 Association with maërl

MC3.522 Association with Peyssonnelia spp.

MC3.523 Association with Laminariales

MC3.524 Facies with large and erect sponges

MC3.525 Facies with Hydrozoa

MC3.526 Facies with Alcyonacea

MC3.527 Facies with Pennatulacea

MC3.528 Facies with Zoantharia

MC3.529 Facies with Ascidiacea

35. 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 rhodolith habitats, reducing the processing time. However, it is still of

little use as only few physical parameters are able to clearly predict the distribution of these two habitats, e.g., bathymetry, slope of the seafloor, nutrient input and phosphate concentration for coralligenous, geostrophic velocity of sea surface current, silicate concentration, and bathymetry for rhodoliths (Martin et al., 2014).

36. The data integration and modelling are often necessary because indirect visual or remote sensing surveys from vessel are limited due to time and costs involved, and only rarely allow obtaining a complete coverage of the study area. Coverage under 100% automatically means that it is impossible to get high resolution maps and therefore interpolation procedures must 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 locations where data have been collected. 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.

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

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

39. Finally, reliability of the map produced should be evaluated. No evaluation scales of reliability have been proposed for coralligenous and rhodoliths habitat mapping; however, scales of reliability evaluation available for seagrass meadows can be adapted also for these two habitats (see the “Guidelines for monitoring marine vegetation in the Mediterranean” for further details). These scales usually consider 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

40. Monitoring is 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 good ecological status. The good state of health of both coralligenous and rhodolith 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).

41. Monitoring the condition (i.e., the ecological status) of coralligenous and rhodolith 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 rhodolith seabeds are listed among the "special habitat types" needing rigorous protection by the protocol concerning the Specially Protected Areas and Biological Diversity in the Mediterranean (SPA/BD Protocol) of the Barcelona Convention.

42. According to the EcAp, the CI2 fixed by the Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP) guidelines and related to "biodiversity" (EO1) is aimed at providing information about the condition (i.e., ecological status) of coralligenous and rhodolith habitats, as they represent two 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 coralligenous reefs and rhodolith seabeds, have been recognized as important biological indicators of environmental quality.

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

a. Initial planning, to define objective(s), duration, sites to be monitored, descriptors to be evaluated, sampling strategy, human, technical and financial needs;

b. 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 repeat monitoring during the same season;

c. Monitoring over time and data analysis. During these activities, robust scientific competences are needed because the acquired data must be interpreted. Duration of the monitoring, to be useful, must be medium time at least.

44. The objectives of the monitoring are primarily linked with the conservation of biogenic 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, to ensure that coralligenous and rhodolith habitats are in a good ecological status (GES), and identify as early as possible any degradation of these habitats or any change in their distributional range and extent. Assessment of the ecological status of these habitats allows measuring 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 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.

45. Evaluate effects of any coastal activity and construction likely to impact coralligenous and rhodolith habitats during environmental impact assessment (EIA) procedures. This specific kind of monitoring aims to establish the condition of the habitat at the time "zero" (i.e., before the beginning of activities), then the state of health of the habitat is monitored during the development of the work phase or at the end of the phase, to check for any impact on the environment evaluated as changes in the habitat state of health. The EIA procedure is not intended as a typical monitoring activity, although it provides the state of the system at the "zero" time, which can be very useful in the time series obtained during a monitoring programme. Unfortunately, most of the EIA studies are qualitative and are often performed by environmental consultants without specialized personnel, using unspecific guidelines and without following any standardised procedure, which prevent their use in effective monitoring programs.

46. The objective(s) of the monitoring system will influence the choices in the following steps (e.g., duration, sites to be monitored, descriptors, and sampling methods; Tab. 2). The duration of the monitoring should be at least medium-long term (minimum 5-10 years long) for heritage conservation and for monitoring environmental quality. The interval of data acquisition could be annual, as most of the typical species belonging to coralligenous assemblages and to rhodolith 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) and possibly also areas with high pressure related to human activities for comparison. 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 monitoring 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.

47. To ensure the sustainability of the monitoring system, the following final remarks must be considered:

- 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 standardized 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

48. Following the preliminary definition of the distributional range and extent of coralligenous and rhodolith habitats (the previous CI1), the assessment of the condition of the two

habitats starts with an overall descriptive characterisation of the typical species and assemblages occurring within each habitat. Monitoring of these two habitats relies on underwater diving activities, although this technique gives rise to many operational constraints due to the conditions of the environment in which these habitats develop (e.g., great depths, weak luminosity, low temperatures, presence of currents, etc.). Underwater surveys must be done by confirmed and expert scientific divers (for safety), within a limited range of depths (from the surface down to the maximum depths of 30-40 m, according to local rules on safe scientific diving), and over a limited underwater time (Bianchi et al., 2004b; Tetzaff and Thorsen, 2005). Adopting alternative visual investigation tools (e.g., ROVs) allows for a less precise assessment but over larger spatial scales. A first characterisation of the habitat (e.g., species present, abundance, vitality, etc.) can be done by direct visual underwater inspections, indirect ROVs or towed camera video recordings, or sampling procedures with dredges, grabs or box corers in the case of rhodolith seabeds. The acoustic methods described above are totally inoperative for detailed characterisations of these habitats, especially for coralligenous. The survey method depends greatly on the scale of the work and the spatial resolution requested (Tab. 2). The complementarity of these techniques must be considered when planning an operational strategy (Cánovas-Molina et al., 2016b). A list of the main conspicuous species/taxa or morphological groups recognisable underwater, or on images, in the two habitats is presented in the Annex 1. This list is not exhaustive but includes species/taxa frequently reported from coralligenous and rhodoliths at the Mediterranean scale. Each Contracting Party can regularly improve these lists and chose the most appropriate species/taxa according to its geographical situation.

49. The use of ROVs or towed cameras can be useful to optimise information obtained and sampling effort (in term of working time) and become essential for monitoring deep coralligenous assemblages and rhodolith seabeds that develop in the upper mesophotic zone (down to 40 m depth), where scuba diving procedures are usually not recommended. High quality videos and photographs recorded by ROV or towed camera 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). Videos and photographs can then be archived to create temporal datasets.

50. At shallower depths (up to about 30-40 m, and according to local rules for scientific diving), direct underwater visual surveys by scuba diving are strongly recommended. Good experience in underwater diving is requested to operate an effective work at these depths. Scientific divers annotate on their slates the list of the main conspicuous species/taxa characterising the assemblages. Given the complexity of the coralligenous habitat (3D structure 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 into 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.

51. For a rough and rapid characterisation of coralligenous assemblages, semi-quantitative evaluations often give sufficient information (Bianchi et al., 2004b): it is possible to estimate the abundance (usually expressed as % cover) by standardized indices directly *in situ* or using photographs (UNEP/MAP-RAC/SPA, 2008). However, a high-quality and fine characterisation of the assemblages often requires square frames (quadrates) of defined surface or transects (with or without photographs; Piazzi et al., 2018) to collect quantitative data on the assemblages composition. The sampling by scraping of all the organisms present over a given area and further laboratory analyses (Bianchi et al., 2004b) represents an alternative destructive procedure, which should be avoided to preserve coralligenous habitat. *In situ* observation and sample must be done over defined and, possibly, standardized surface areas (Piazzi et al., 2018), and the number of replicates must be adequate and high enough to catch the heterogeneity of the habitat.

52. As well as the presence and abundance of a given species, assessing its vitality seems a particularly interesting parameter. The presence of broken individuals (especially of branching colonies occurring in the intermediate and upper layers of coralligenous, such as bryozoans and gorgonians) and of signs of necrosis and bleaching are important elements to be taken into consideration to assess specific pressures, such as mechanical damages or effects of thermal anomalies (Garrabou et al., 1998, 2001, 2019; Gatti et al., 2012). Finally, the nature of the substrate (silted up, roughness, interstices, exposure, slope), the temperature of the water, the vagile fauna associated, the coverage by epibiont, and the presence of invasive species must also be considered to give a clear characterisation of bioconstructed habitats (Harmelin, 1990; Gatti et al., 2012).

Table 2: Synthesis of the main methods used to characterise coralligenous and rhodolith habitats in the Mediterranean, as the first necessary step for defining the Common Indicator 2_Condition of the habitat's typical species and communities. 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 and the limits of each tool are indicated, with some bibliographic references.

Methods	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Remote Operating Vehicle (ROV) or towed camera	From 2 m to over 120 m	Small-Intermediate areas of about 1 km^2 (larger areas in the case of towed camera)	From 1 m to 10 m	0.025 to 0.01 km²/hour	• Non-destructive method • Possibility of collecting pictures • Wide bathymetric range • Good identification of facies and associations • Possibility of semi-quantitative/quantitative evaluation • Possibility to collect samples (for ROV)	• High cost, major means out at sea • Difficulty of observation and access according to the complexity of the habitat (multilayer assemblages) • Quali-quantitative assessments only on conspicuous species/taxa	Cánovas-Molina et al. (2016a); Enrichetti et al. (2019); Piazzi et al. (2019b)
Underwater visual observation	0 m up to 40 m, according to local rules for scientific diving	Small areas (less than 250 m^2)	From 1 m	0.0001 to 0.001 km²/hour	• Non-destructive • Good precision in the identification (taxonomy) and characterisation of the habitat (also its 3D) • Low cost, easy to implement • Possibility to collect samples • Data already available after dive	• Small area inventoried • Very time-consuming underwater activities • Limited operational depths • Highly qualified scientific divers required • Subjectivity of the observer • Quali-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 sampling by scraping or collection	0 m up to 40 m, according to local rules for scientific diving	Small areas (less than 10 m^2)	From 1 m	0.0001 to 0.001 km²/hour	• Very good precision in the identification (taxonomy) and characterisation of the habitat • All species identified • *A posteriori* identification • Easy to implement	• Destructive method, usually not recommended • Very small area inventoried • Sampling material needed • Limited operational depths • Highly qualified scientific divers required • Very time-consuming underwater activities • Analysis of samples in laboratory very time-consuming • Involvement of many taxonomists	Bianchi et al. (2004b)
Underwater photography or video recording	0 m up to 40 m, according to local rules for scientific diving	Small areas (less than 250 m^2)	From 0.1 m	0.0001 to 0.001 km²/hour	• Non-destructive • Good precision in the identification (taxonomy) and characterisation of the habitat • *A posteriori* identification possible • Low cost, easy to implement • Possibility to collect samples • Possibility to create archives	• Small area inventoried • Photograph and video analysis very time-consuming • Limited operational depths • Highly qualified scientific divers required • Tools to collect photo/video necessary • Quali-quantitative assessments only on conspicuous species/taxa Only 2D observation	Gatti et al. (2015b); Montefalcone et al. (2017); Piazzi et al. (2017a, 2019a); Çinar et al. (2020)

Methods	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Sampling from vessel with blind grabs, dredges, or box corers	0 m to about 120 m (until the lower limit of the rhodolith habitat)	Intermediate areas (a few km^2)	From 1 to 10 m	0.025 to 0.01 km^2/hour	• Very good precision in the identification (taxonomy) and characterisation of the habitat • All species identified • *A posteriori* identification • Easy to implement	• Destructive method, usually not recommended • Small area inventoried • Sampling material needed • Samples analysis in laboratory very time-consuming and costly • Difficulty in collecting representative samples	UNEP/MAP-RAC/SPA (2015a)

53. Effective monitoring should be done at defined intervals over time, even if it could mean fewer sites being monitored. The reference "zero-state" will be contrasted with data coming from subsequent monitoring periods, always assuring reproducibility of data over time. Thus, the experimental design and protocol have capital importance. The 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 marks underwater (with fixed pickets into the rock) for positioning the quadrates or transects in the exact original position (García-Gómez et al., 2020). Finally, even if it cannot be denied that there are logistical constraints linked to the underwater observation of coralligenous and rhodolith 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).

54. Although destructive methods (scraping of the substrate with all the organisms present over a given area, dredges, grabs, or box-corers) 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 monitoring (UNEP/MAP-RAC/SPA, 2008), and especially within MPAs. Moreover, identification of all 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 favor non-destructive methods, like photographic sampling, ROV survey, or direct underwater observation in given areas (using quadrates or transects) to collect quali-quantitative data. These methods do not require sampling of organisms and are therefore appropriate for long-term monitoring. The different methods can be used either separately or together, according to the objective of the study, the area inventoried, and means available (Tab. 3). Non-destructive methods have been increasingly used and, mainly for video and photographic sampling, enjoy significant technological advances.

Table 3: Comparison among 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, depth-limitations when done by divers
Use	Studies integrating a strong taxonomical element
Video or photography	
Advantages	Objective evaluation, can be reproduced, reference samples, can be automated, speedy diving work, large area inventoried, non-destructive method, no depth-limitations
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, depth-limitations
Use	Exploratory studies, monitoring of populations, bionomic studies

55. Differently from seagrass, the descriptors used to evaluate the status of coralligenous assemblages vary greatly from one team to another and from one region to another, as well as their measuring protocols (Piazzi et al., 2019a and references therein). A first standardized sheet for coralligenous monitoring was created in the context of the Natura 2000 programs, which solved only partially the issues about comparability among data (Fig. 5). However, methods and descriptors

considered must be the subject of a standardized protocol. Although many disparities among data acquisition methods still occur, an integrated and standardized procedure named STAR (STAndaRdized coralligenous evaluation procedure) for monitoring the condition of coralligenous reefs has recently been proposed (Piazzi et al., 2019a; Gennaro et al., 2020).

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*…	

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

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

- **Observation :**

Photos quadrats et paysagères à réaliser

Figure 5: Example of a standardized 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 reefs

56. The protocol STAR (STAndaRdized coralligenous evaluation procedure) (Piazzi et al., 2019a; Gennaro et al., 2020) has been proposed for monitoring the ecological status of coralligenous reefs to obtain information about most of the descriptors adopted in the different ecological indices

that have been developed to date, through a single sampling effort and data analysis. The CIGESMED protocol, applied in different parts of the Mediterranean (David et al., 2014; Çinar et al., 2020), should also be mentioned.

57. Monitoring plans should at 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). Deep coralligenous reefs can be surveyed only by means of ROV inspections.

58. 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 modify 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 than once per year.

59. Depth and slope: the depth range where coralligenous reefs can develop changes with latitude and characteristics of the water. Moreover, different kinds 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. To define a standardized 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.

60. 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.

61. 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 must 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 (Kipson et al., 2011, 2014; Deter et al., 2012; Teixidó et al., 2013; Cecchi et al., 2014; Piazzi et al., 2015; Sartoretto et al., 2017) or in a square design (3 × 3 square structure) (Çinar et al., 2020). A comparison between these 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 for the analysis of data through both seascape and biocenotic approaches (see the 'Ecological Indices' paragraph below).

62. <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 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 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 to the photographic technique, such as the size of colonies of erect species and the thickness and consistency of the calcareous accretion (see the 'Descriptors' paragraph 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.

63. <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 the manual contouring techniques through appropriate software may be useful to reduce the subjectivity of the operator's estimate.

64. <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 a 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 on photographic samples, as this method showed consistent results with those obtained through underwater measurements of the 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 located tens of metres apart. For each measure, the hand-held 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 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 measure 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 bioconstruction 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 substrates 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 local 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 species. 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 located 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 based on 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 on 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 the distribution of organisms decreases, and their 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 as the distance from centroids (Piazzi et al., 2017a) through permutational analysis of multivariate dispersion (PERMDISP). Thus, any change in the compositional variability displayed by PERMDISP may be directly interpretable as changes in the β-diversity.

Protocol for monitoring deep water mesophotic (down to 40 m depth) coralligenous reefs

65. 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.

66. 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 multibeam maps when available. Two parallel laser beams (90° angle) can provide a scale for size reference. To guarantee the best quality of video footages, ROV is 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 ending 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.

67. 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 substrate (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-bottom 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 in 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).

68. 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 substrate: 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 rhodolith beds

69. A standardized and common sampling method for monitoring rhodolith beds is not available to date (UNEP/MAP-RAC/SPA, 2008). Mediterranean rhodolith beds seem to display more diverse assemblages of coralline and peyssonneliacean algal species 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 of the Atlantic Ocean cannot be applied as such and require calibration to the Mediterranean specificities.

70. A recent proposal of protocol for monitoring rhodolith beds can be found in Basso et al. (2016). Monitoring of rhodolith habitats can be done by underwater diving and direct visual observation, with sampling and following taxa identification in laboratory, as well as by blind sampling from vessel using grabs, dredges, and box corers (Tab. 4). Surveys using ROVs and towed cameras are also effective because of the great homogeneity of this habitat, although they do not provide a complete quantitative information on composition and abundance of rhodolith community as that provided by destructive sampling techniques. Monitoring should address all the variables already described for the first descriptive characterisation of the habitat, with the addition of a full quantitative description of the rhodolith community composition, through periodical surveys, including number of typical or indicator species. A decrease in rhodolith beds extent, live/dead rhodoliths ratio, live rhodoliths percentage cover, associated with changes in the composition of the macrobenthic community (calcareous algal engineers and associated taxa) may reveal potential negative impacts acting on rhodolith 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: 1) compact and nodular pralines; 2) larger and vacuolar box work rhodoliths; and 3) unattached branches (Fig. 7). Each of the three end-members within rhodoliths morphological

variability corresponds to a typical (but not exclusive) group of composing coralline algal species and associated biota and it is possibly correlated with environmental variables, among which substrate instability (mainly due to water movement) and sedimentation rate are the most obvious. Thus, the indication of the cover (in %) by the three live rhodoliths categories at the surface of each rhodolith bed is a proxy of the rhodolith habitat structural and ecological complexity. The high species diversity hosted by rhodolith beds requires time-consuming and expensive laboratory analysis for species identification. Videos and photos allow for a less fine assessment on the composition of rhodolith community due to the absence of conspicuous, easy-to-detect species. Moreover, since most coralline algal species belong to few genera only, the use of taxonomic ranks higher than species is not useful.

Table 4: Comparison among four traditional methods used to monitor rhodolith 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, and box corers)	
Advantages	Easy to implement, taxonomical precision, reference samples, analysis on the substrate (granulometry, calcimetry, % of organic matter), large depth-range investigated
Limits	Low precision of observation, several replicates needed, limited area inventoried, destructive method, high costs for taxonomic analysis
Use	Localised studies integrating a taxonomical element, validation of acoustic methods
ROV and towed camera	
Advantages	Objective evaluation, reference samples (images), large area inventoried, non-destructive method, information on the distribution of conspicuous species, large depth-range investigated
Limits	High cost, low taxonomical precision, problem of *a posteriori* interpretation of images, observation only of the superficial layer, little information on the substrate and on the basal layer
Use	Studies on distribution and temporal change, validation of acoustic methods
Acoustic methods	
Advantages	Very large areas inventoried, information on water movement (sedimentary figures), can be reproduced, non-destructive method, large depth-range investigated
Limits	High cost, uncertainties in the sonograms interpretation, additional validation (inter-calibration), observation only of the superficial layer, no taxonomical information
Use	Studies over large spatial scales, monitoring of populations, bionomic studies

71. When necessary, for a detailed characterization of rhodolith communities, a minimum of three box-cores with opening ≥0.16 m^2 should be collected in each rhodolith bed at the same depth, and to a depth of about 20 cm of sediment. One additional box-corer sample must be collected within the rhodolith area with the highest percentage of live cover (based on preliminary ROV surveys that remain necessary to pilot blind samplings from vessel), and the others as far as possible from it, following the depth gradient in opposite directions of the maximum rhodolith 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 rhodolith morphologies characterising the sample (Fig. 7); 4) measurement of the thickness of the live rhodoliths layer. According to the specific objective of investigation, the sediment sample can then be 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 could be identified and quantified, to detect variability in space and time, and for any change 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 rhodolith morphotypes should be air-dried, and then preserved in silica gel. The sediment sample should be analysed for grain-size (mandatory), and carbonate content.

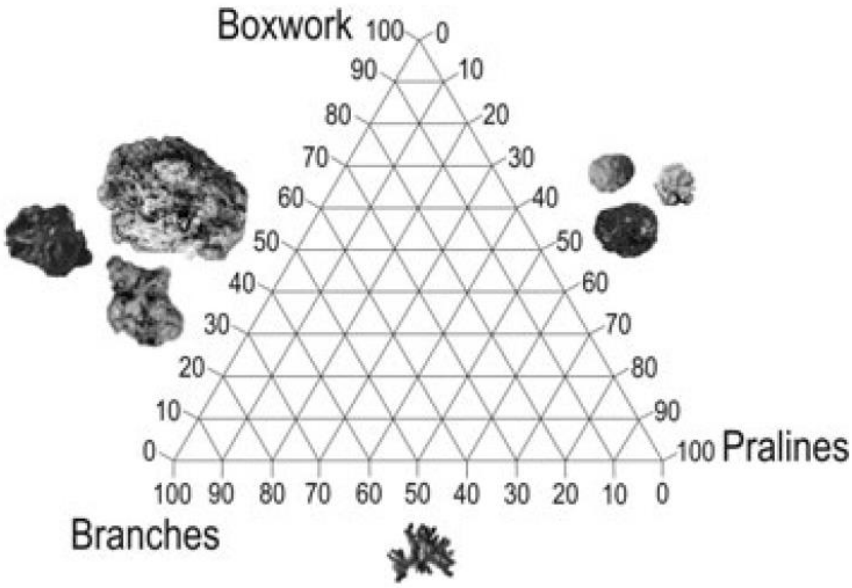

Figure 7: Ternary diagram for the description of the rhodoliths tridimensionality. The percentage cover of each rhodolith morphotype, relative to the total rhodoliths cover, can be plotted on the correspondent axis. The three main rhodolith morphotypes (box work rhodoliths, pralines, and unattached branches) are intended as focal points of a continuum, to which any possible rhodolith morphology can be approximately assigned. From Basso et al. (2016).

Ecological indices

72. At present, an ecological index to evaluate the status of rhodolith beds has not been proposed yet. On the contrary, 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., 2019a), which are summarised in Table 5. Most of the ecological indices available for monitoring shallow (up to about 40 m depth) coralligenous reefs require underwater surveys by scuba diving. These indices adopt distinct descriptors and sampling techniques, thus hampering the comparison of data and results, and requiring inter-calibration procedures. However, as described before, the protocol STAR (STAndaRdized coralligenous evaluation procedure; Piazzi et al., 2019a; Gennaro et

al., 2020) has been recently proposed as an effective procedure to obtain standardized data on most of the descriptors adopted in the different ecological indices through a single sampling effort and a shared data analysis. Detailed descriptions of the sampling tools and the methodologies needed to apply each ecological index listed in Table 5 can be found in the relative bibliographic references.

73. ESCA (Ecological Status of Coralligenous Assemblages; Cecchi et al., 2014; Piazzi et al., 2015, 2017a, 2021), 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 of percentage cover of mud and builder organisms (i.e., Corallinales, bryozoans, and scleractinians) for CAI.

74. 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.

75. COARSE (COralligenous Assessment by ReefScape Estimate; Gatti et al., 2012, 2015a) uses a seascape approach to provide information about the structure of coralligenous reefs 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.

76. 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 state of coralligenous assemblages.

77. 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, 2021), 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 is thus 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 the main differences among indices are linked to the different approaches used, with ESCA and ISLA showing the highest consistency 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.

78. Comparatively fewer efforts have been made to propose ecological 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 of 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 MACS 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 biocenotic complexity of the investigated ecosystem, whereas the *Ii* describes its impacts. Environmental status is the outcome of the status of benthic communities plus the effects 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 and subjected to different environmental conditions and levels of human pressures.

Final remarks

79. Inventorying and monitoring the condition of coralligenous reefs and rhodolith 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.

80. A standardized approach must be encouraged for monitoring the condition of coralligenous reefs and rhodolith seabeds, and in particular:

- Knowledge on coralligenous reefs and rhodolith seabeds distribution should be continuously enhanced at the Mediterranean scale, especially in the eastern basin, 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 standardized protocols here proposed should be applied to the entire Mediterranean both on coralligenous reefs and rhodolith seabeds.

Table 5: Descriptors used in the ecological indices mostly adopted in the regional/national monitoring programs to evaluate environmental quality of shallow water (up to 40 m depth) coralligenous reefs 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. Sensitivity Level to stress (SSL) and Sensitivity Level to disturbance (DSL)
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* observations 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 the Rapid Visual Assessment (RVA): 3 replicated visual estimations over an area of about 2 m^2 each		9 descriptors, 3 per each layer: Basal layer: % cover of encrusting calcified rhodophyta, non-calcified encrusting algae, encrusting animals, turf-forming algae and sediment; amount 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

Index	**Method**	**Image analysis**	**Descriptors**
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 are 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 water (from about 40 m to about 120 m depth) coralligenous reefs occurring in the 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 seconds 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; substrate 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 of coralligenous and rhodolith habitats (from UNEP/MAP-RAC/SPA, 2015). Each Contracting Party can regularly improve these lists and chose the most appropriate species according to its geographical situation.

Coralligenous
(*invasive; **disturbed or stressed environments, when abundant; * protected species)**

Builders
Algal builders
Lithophyllum cabiochae (Boudouresque & Verlaque) Athanasiadis, 1999
Lithophyllum stictiforme (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
Adeonella spp. Canu & Bassler, 1930
Myriapora truncata Pallas, 1766
Pentapora fascialis Pallas, 1766
Rhynchozoon neapolitanum Gautier, 1962
Schizomavella spp.
Schizoretepora serratimargo (Hincks, 1886)
Smittina cervicornis Pallas, 1766
Turbicellepora spp.

Polychaeta
Serpula spp.
Protula tubularia (Montagu, 1803)
Spirobranchus polytrema Philippi, 1844
Spirorbis sp.

Cnidaria
Caryophyllia (Caryophyllia) inornata (Duncan, 1878)
Caryophyllia (Caryophyllia) smithii Stokes & Broderip, 1828
Cladocora caespitosa Linnaeus, 1767
Dendrophyllia ramea Linnaeus, 1758
Dendrophyllia cornigera Lamarck, 1816
Hoplangia durotrix Gosse, 1860
Leptopsammia pruvoti Lacaze-Duthiers, 1897
Madracis pharensis (Heller, 1868)
Polycyathus muellerae Abel, 1959
Phyllangia americana mouchezii Lacaze-Duthiers, 1897

Bioeroders
Sponges
Clionidae (*Cliona*, *Pione*)

Echinoids
Echinus melo Lamarck, 1816
Sphaerechinus granularis (Lamarck, 1816)

Molluscs
Hiatella arctica Linnaeus, 1767
Lithophaga lithophaga Linnaeus, 1758***
Petricola lithophaga (Retzius, 1788)
Rocellaria dubia (Pennant, 1777)

Polychaetes
Dipolydora spp.
Dodecaceria concharum Örsted, 1843
Polydora spp.

Sipunculids
Aspidosiphon (Aspidosiphon) muelleri muelleri Diesing, 1851
Phascolosoma (Phascolosoma) stephensoni Stephen, 1942

Other relevant species
Algae
Green algae
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**

Flabellia petiolata (Turra) Nizamuddin, 1987
Halimeda tuna (J. Ellis & Solander) J.V. Lamouroux, 1816
Palmophyllum crassum (Naccari) Rabenhorst, 1868

Brown algae
Acinetospora crinita (Carmichael) Sauvageau, 1899**
Cystoseira dubia Valiante, 1883***
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
Laminaria rodriguezii Bornet, 1888***
Phyllariopsis brevipes (C. Agardh) E.C. Henry & G.R. South, 1987
Stictyosiphon adriaticus Kützing, 1843**
Stilophora tenella (Esper) P.C. Silva in P.C. Silva, Basson & Moe, 1996**
Stypopodium schimperi (Kützing) M. Verlaque & Boudouresque, 1991*

"Yellow" algae (Pelagophyceae)
Nematochrysopsis marina (J. Feldmann) C. Billard, 2000**

Red algae
Acrothamnion preissii (Sonder) E.M. Wollaston, 1968*
Asparagopsis taxiformis (Delile) Trevisan de Saint-Léon, 1845*
Cryptonemia lomation (Bertoloni) J. Agardh, 1851
Gloiocladia spp.
Halymenia spp.
Kallymenia spp.
Leptofauchea coralligena Rodríguez-Prieto & De Clerck, 2009
Lophocladia lallemandii (Montagne) F. Schmitz, 1893*
Osmundaria volubilis (Linnaeus) R.E. Norris, 1991
Peyssonnelia spp. (non calcareous)
Phyllophora crispa (Hudson) P.S. Dixon, 1964
Ptilophora mediterranea (H.Huvé) R.E. Norris, 1987
Rodriguezella spp.
Sebdenia spp.
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.***
Calyx nicaeensis (Risso, 1827)
Chondrosia reniformis Nardo, 1847
Clathrina clathrus Schmidt, 1864
Cliona viridis (Schmidt, 1862)
Crambe crambe (Schmidt, 1862)
Dysidea spp.
Fasciospongia cavernosa (Schmidt, 1862)
Haliclona (Reniera) mediterranea Griessinger, 1971
Haliclona (Soestella) mucosa Griessinger, 1971
Haliclona (*Halichoclona*) *fulva* (Topsent, 1893)
Hemimycale columella Bowerbank, 1874
Ircinia oros Schmidt, 1864
Ircinia variabilis Schmidt, 1862
Oscarella spp.
Petrosia (Petrosia) ficiformis (Poiret, 1789)
*Phorbas tenacior*Topsent, 1925
Sarcotragus foetidus Schmidt, 1862
Sarcotragus spinosulus Schmidt, 1862
Spirastrella cunctatrix Schmidt, 1868
Spongia (Spongia) officinalis Linnaeus, 1759***
Spongia (Spongia) lamella Schulze, 1879***

Cnidaria
Aglaophenia kirchenpaueri (Heller, 1868)
Alcyonium acaule Marion, 1878
Alcyonium palmatum Pallas, 1766
Antipathes spp.***
Callogorgia verticillata Pallas, 1766
Cerianthus lloydii Gosse, 1859
Cerianthus membranaceus (Gmelin, 1791)
Corallium rubrum Linnaeus, 1758***
Desmophyllum dianthus (Esper, 1794)
Ellisella paraplexauroides Stiasny, 1936
Eunicella spp.
Leptogorgia sarmentosa Esper, 1789
Madracis pharensis (Heller, 1868)
Paramuricea clavata Risso, 1826
Parazoanthus axinellae Schmidt ,1862
Savalia savaglia Bertoloni, 1819***

Polychaeta
Filograna implexa Berkeley, 1835
Sabella spallanzanii Gmelin, 1791
Salmacina dysteri Huxley, 1855
Protula spp.

Bryozoans

Chartella tenella Hincks, 1887
Hornera frondiculata (Lamarck, 1816)***
Margaretta cereoides Ellis & Solander, 1786

Tunicates
Aplidium spp.
Cystodytes dellechiajei (Della Valle, 1877)
Halocynthia papillosa Linnaeus, 1767
Herdmania momus (Savigny, 1816)
Microcosmus sabatieri Roule, 1885
Pseudodistoma cyrnusense Pérès, 1952

Molluscs
Cerithium scabridum Philippi, 1848*
Charonia lampas Linnaeus, 1758***
Charonia variegata Lamarck, 1816
Luria lurida Linnaeus, 1758***
Naria spurca (Linnaeus, 1758)
Pinna rudis Linnaeus, 1758***

Decapoda
Dardanus arrosor (Herbst, 1796)
Maja squinado Herbst, 1788***
Palinurus elephas Fabricius, 1787***
Pilumnus hirtellus (Linnaeus, 1761)
Scyllarides latus Latreille, 1803***

Echinodermata
Antedon mediterranea Lamarck, 1816
Centrostephanus longispinus Philippi, 1845***
Diadema setosum (Leske, 1778)*
Echinaster (*Echinaster*) *sepositus* (Retzius, 1783)
Hacelia attenuata Gray, 1840
Holothuria (Panningothuria) forskali Delle Chiaje, 1823
Holothuria (Platyperona) sanctori Delle Chiaje, 1823
Synaptula reciprocans (Forsskål, 1775)

Pisces
Anthias anthias (Linnaeus, 1758)
Coris julis (Linnaeus, 1758)
Chromis chromis (Linnaeus, 1758)
Epinephelus spp.***
Mycteroperca rubra Bloch, 1793
Pterois miles (Bennett, 1828)*
Sargocentron rubrum (Forsskål, 1775)*
Seriola dumerili (Risso, 1810)
Siganus luridus (Rüppell, 1829)*
Siganus rivulatus Forsskål & Niebuhr, 1775*
Sparisoma cretense (Linnaeus, 1758)
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, when abundant; * protected species. Species that can be dominant or abundant are preceded by #)**

Algae
Red algae (calcareous)
Lithophyllum cabiochae (Boudouresque et Verlaque) Athanasiadis
#*Lithophyllum racemus* (Lamarck) Foslie, 1901
Lithophyllum stictiforme (J.E. Areschoug) Hauck, 1877
#*Lithothamnion corallioides* (P.L. Crouan & H.M. Crouan) P.L. Crouan & H.M. Crouan, 1867***
Lithothamnion minervae Basso, 1995
#*Lithothamnion valens* Foslie, 1909
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 crispate* Boudouresque & Denizot, 1975
Peyssonnelia heteromorpha (Zanardini) Athanasiadis, 2016
#*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
Sporolithon ptychoides Heydrich, 1897
#*Tricleocarpa cylindrica* (J. Ellis & Solander) Huisman & Borowitzka, 1990

Red algae (non-builders)
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
#*Osmundaria volubilis* (Linnaeus) R.E. Norris, 1991
Peyssonnelia spp. (non-calcareous)
#*Phyllophora crispa* (Hudson) P.S. Dixon, 1964
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*

Green algae
Caulerpa cylindracea Sonder, 1845*
Caulerpa taxifolia (M. Vahl) C. Agardh, 1817*
Codium bursa (Olivi) C. Agardh, 1817

Flabellia petiolata (Turra) Nizamuddin, 1987
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
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
Laminaria rodriguezii Bornet, 1888***
Lobophora variegata (J.V. Lamouroux) Womersley ex E.C.Oliveira, 1977
Nereia filiformis (J. Agardh) Zanardini, 1846
Phyllariopsis brevipes (C. Agardh) E.C. Henry & G.R. South, 1987
Spermatochnus paradoxus (Roth) Kützing, 1843
Sporochnus pedunculatus (Hudson) C. Agardh, 1817
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
Adamsia palliata (Müller, 1776)
Alcyonium palmatum Pallas, 1766
Aglaophenia spp.
Calliactis parasitica Couch, 1838
Cereus pedunculatus Pennant 1777
Cerianthus membranaceus (Gmelin, 1791)
Eunicella verrucosa Pallas, 1766
Funiculina quadrangularis Pallas, 1766
Leptogorgia sarmentosa Esper, 1789
Nemertesia antennina Linnaeus, 1758

Paramuricea macrospina Koch, 1882
Pennatula spp.
Veretillum cynomorium Pallas, 1766
Virgularia mirabilis Müller, 1776

<u>Polychaetes</u>
Aphrodita aculeata Linnaeus, 1758
Sabella pavonina Savigny, 1822
Sabella spallanzanii Gmelin, 1791

<u>Bryozoans</u>
Cellaria fistulosa Linnaeus, 1758
Hornera frondiculata (Lamarck, 1816)
Pentapora fascialis Pallas, 1766
Turbicellepora spp.

<u>Tunicates</u>
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

<u>Echinodermata</u>
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

<u>Pisces</u>
Mustelus spp.
Pagellus acarne (Risso, 1827)
Pagellus erythrinus (Linnaeus, 1758)
Raja undulata Lacepède, 1802
Scyliorhinus canicula (Linnaeus, 1758)
Squatina spp.***
Trachinus radiatus Cuvier, 1829