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Meeting of the Ecosystem Approach Correspondence Groups on Monitoring (CORMON), Biodiversity and Fisheries

MEDITERRANEAN ACTION PLAN

Videoconference, 10-11 June 2021

Agenda item 3: Monitoring Guidelines/Protocols for IMAP common indicators 1 and 2 on Benthic Habitats

Update of Monitoring Protocols on Benthic Habitats

Note by the Secretariat

Decision IG.23/6 on the 2017 Mediterranean Quality Status Report (COP 20, Tirana, Albania, 17-20 December 2017) recommended, as general directions towards a successful 2023 Mediterranean Quality Status Report (2023 MED QSR), the harmonization and standardization of monitoring and assessment methods of agreed common indicators.

The monitoring protocols on habitats, species and non-indigenous species were reviewed by the Meetings of the Ecosystem Approach Correspondence Group on Monitoring (CORMON), Biodiversity and Fisheries (Marseille, France, 12-13 February 2019¹ and Rome, Italy, 21 May 2019²), the 14th meeting of the SPA/BD thematic Focal points (Portoroz, Slovenia, 18-21 June 2019) ³ and the 7th Meeting of the Ecosystem Approach Coordination Group (Athens, Greece, 9 September 2019)⁴.

These monitoring guidelines/protocols provide detailed methodologies and protocols for monitoring benthic habitats, that can be helpful to national managers and decision makers (e.g., environmental authority representatives, researchers, Marine Protected Area (MPA)'s representatives) for implementing a monitoring programme on Common indicators 1 and 2, on yearly basis, in at least two monitoring areas, one in a low pressure area (e.g. Marine Protected Area/Specially Protected Area of Mediterranean Importance (SPAMI)), or in sites of high conservation relevance (e.g., Natura 2000 sites), and one in a high pressure area due to human activity.

The developed monitoring guidelines/protocols provide information on the monitoring of the agreed biodiversity-related IMAP Common Indicators towards the GES achievement, and address the same common purposes to all monitoring guidelines developed to date:

- (i) Harmonization and standardization of monitoring and assessment methods;
- (ii) Assuring the quality of long time series of data to monitor the trends in the status of the marine environment;
- (iii) Improvement of availability of synchronized datasets for marine environmental state assessment, including data stored in other databases where some of the Mediterranean countries regularly contribute;
- (iv) Improvement of data accessibility and their continuous upgrading, with the view to improving knowledge on the Mediterranean marine environment, to accommodate data submissions for all the IMAP Common Indicators.

The Contracting Parties to the Barcelona Convention reviewed and endorsed the monitoring guidelines/protocols of marine species (marine mammals, sea turtles and sea birds), non-indigenous species and dark habitats. Minor adjustment needs of monitoring parameters on benthic habitats, i.e. marine vegetation and coralligenous and other calcareous bioconstructions, were highlighted. Consequently, they requested the Secretariat to bring them to the attention of respective CORMONs on 2020 and 2021.

The present document is an update of the monitoring protocols on benthic habitats (marine vegetation and coralligenous and other calcareous bioconstructions), that take into consideration the comments highlighted by the Contracting Parties during the 7th Meeting of the Ecosystem Approach Coordination Group and the Integrated CORMON meeting (1-3 December 2020). It outlines the monitoring guidelines of the agreed common indicators 1 and 2 related to marine habitats, namely Common Indicator 1: Habitat distributional range, to consider habitat extent as a relevant attribute and Common Indicator 2: Condition of the habitat's typical species and communities.

¹ UNEP/MED WG.458/4 Guidance on monitoring concerning the biodiversity and non-indigenous species common indicators Monitoring protocols of the Ecosystem Approach Common Indicators 3, 4, 5 and 6

² UNEP/MED WG.474/3 Monitoring protocols of the Ecosystem Approach Common Indicators 1 and 2 related to marine benthic habitats

³ UNEP/MED WG.461/21 Implementation of the first phase (2016-2019) of the Integrated Monitoring and Assessment Programme (IMAP - Biodiversity and non-indigenous species) in the framework of the EcAp Roadmap

⁴ UNEP/MED WG.482/20 Monitoring Protocols for IMAP Common Indicators related to Biodiversity and Non-Indigenous species

The document is organized along two (2) monitoring guidelines/protocols:

- 1. Guidelines/protocols for monitoring marine vegetation;
- 2. Guidelines/protocols for monitoring coralligenous and other calcareous bioconstructions in the upper-circalittoral Mediterranean zone.

3.

In order to highlight the proposed changes and facilitate the review by the meeting, these are reported in track mode for added text and in strikethrough for deletion.

The current update, edited in track mode, is submitted to the present meeting for review and comments prior to be examined by the SPA/BD Focal Points Meeting, in June 2021.

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 sequestration of carboncarbon sequestration (Waycott et al., 2009 and references therein). A majorsignificant economic value of over 17 000 \$ per ha and per annum has been quantified for seagrass meadows worldwide (Costanza et al., 1997).
- 2. Seagrass, like all Magnoliophyta Magnoliophytes, are marine flowering plants of terrestrial origin which that returned to the marine environment approx. 120 to 100 million of years. The global species diversity of seagrass is low when compared to any other marine Phylum or Division, with less than sixty species throughout the world. However, they form extensive meadows that extend for thousands of kilometres kilometers of coastline between the surfaces down to about 50 m depth (according to water transparency) in very clear apparent marine waters and or transitional waters (e.g., estuaries and lagoons). In the Mediterranean region five seagrass species occur: Cymodocea nodosa, Halophila stipulacea (an invasive Lessepsian species), Posidonia oceanica, Zostera marina, and Zostera noltei. The endemic Posidonia oceanica is doubtless the dominant and the most 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 and accelerating decline of seagrass meadows has been was reported in the Mediterranean Sea and mainly in the north-western side of the basin, where many meadows have already 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, which are indicative of a recent trend reversal in seagrass extent and density, thanks to effective adequate management actions (de los Santos et al., 2019).
- 4. Concerns about these declines have prompted efforts to protect legally these habitats 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 ssites 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, their sedentary habit, and their susceptibility to changing environmental conditions, seagrass are habitually used as biological indicators of water quality in accordance with the Water Framework Directive (WFD, 2000/60/EC) and of environmental quality in accordance with the Marine Strategy Framework Directive (MSFD, 2008/56/EC) (Montefalcone, 2009). Due to its recognized ecological importance, *Posidonia oceanica* is considered as the main biological quality element in monitoring programs developed to evaluate the status of marine coastal environment. 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 a-prerequisite knowledge for a-the sustainable use of marine coastal areas. The fFirst 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). Fand a few extentextents of the coastline haves 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 consequence and following an explicit request by managers on the need of for practical guides aimed at harmonizing existing methods for seagrass monitoring and for-subsequent comparison of results obtained by different countries, the Contracting Parties asked the Regional Activity Centre for Specially Protected Areas (RAC/SPA) to improve the existing inventory tools and to propose-a 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 a number of several scientific round tables addressed explicitly specifically addressed 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;
 - A-Sstandardization 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 <u>that most</u> 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 in order toto ease the task of the MPA managers when implementing their monitoring programs. A reviewing process on the scientific literature, taking into account considering the latest techniques and the recent works carried out 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 the north-west Europe within the framework of the European projects MESH (Mapping European Seabed Habitats; MESH, 2007) and EUSeaMap (Vasquez et al., 2021a, b) project, ended in 2008. The mapping procedure includes different actions (Fig. 1), that can be synthesised into three main steps:
 - 1) Initial planning
 - 2) Ground surveys
 - 3) Processing and data interpretation

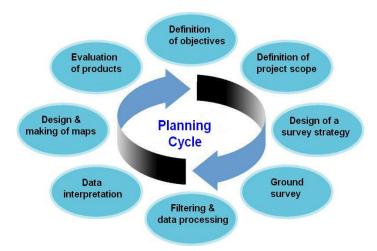


Figure 1: Planning cycle for a habitats' mapping programme (according to the MESH project, 2008).

- 11. <u>Initial planning</u> includes the definitiondefining of the objectives in order toto 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. <u>Ground survey</u> is the practical phase for data collection. It is often the costliest phase as it generally requires field activities. A prior inventory of the existing data for the area being mapped is recommended, to reduce the amount of work or to have a-better targeting of the work to be done.
- 13. <u>Processing and data interpretation</u> 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 <u>precision_accuracy_used</u> and the surface area to be mapped (Mc Kenzie et al., 2001; Fig. 2).

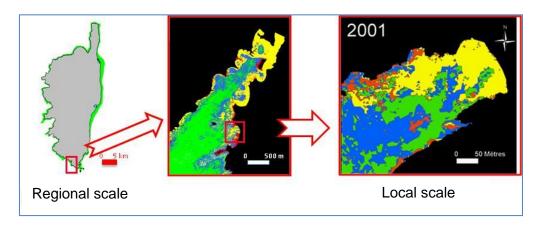


Figure 2: Resolution of a map from regional study to local study (from UNEP/MAP-RAC/SPA, 2015).

- When large surface areas have to be mapped and global investigations carried out, an average precision and a lower detail level can be accepted, which means that the habitat distribution and the definition of its extension limits are often only indicative. Measures of the total habitat extent may be subjected to high variability. as Tthe 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 datapoor 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 m² in local scale studies (Pergent_et al., 1995a). These detailed maps provide an-accurate localisation of the habitat distribution and a precise definition of its extension limits and total habitat extent, all features necessary for future control and monitoring purposes over a defined period of time. These high-resolution scales are also used to select remarkable great sites where monitoring actions must be concentrated. As highlighted by the MESH_EU projects (2008), most of the environment management and marine spatial planning activities require a range of habitat maps between these two extremes.

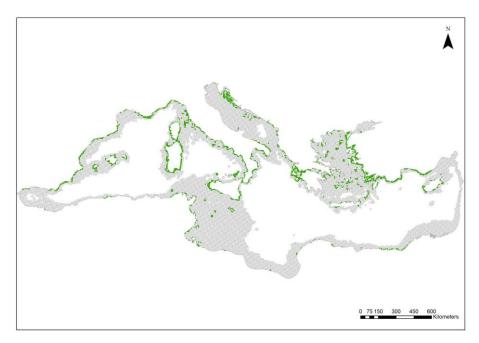


Figure 3: Distribution of *Posidonia oceanica* meadows in the Mediterranean Sea (green areas) (from Giakoumi et al., 2013).

Methods

- Maps of seagrass distribution and extent can be obtained by using indirect instrumental mapping techniques and/or direct field visual surveys (Tab. 1). In the last 50 years the technology in benthic habitat mapping has increased a lot, and several instrumental mapping techniques have been successfully applied to seagrass meadows (see synthesis in Pergent et al., 1995a; McKenzie et al., 2001; Dekker et al., 2006; Hossain et al., 2015; 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, unmanneduncrewed 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 for assessment of to assess the spatial patterns of seagrass meadows. It, and simultaneously can be used to reveal temporal patterns due to the high frequency of the observation. Remote sensing covers a variety of technologies from satellite telemetry, aerial photography, and unmanned aerial vehicles (UAVs), and vessel acoustic vessel systems. The power of remote sensing techniques has been highlighted by Mumby et al. (2004), who highlighted showed that 20 s of airborne acquisition time would equal 6-six days of field surveys. However, all indirect mapping techniques are intrinsically affected by uncertainties due to manual or authomatic automatic supervised classification of spectral or acoustic signatures of seagrass meadows on the images and sonograms, respectively. Errors in images or sonograms interpretation may arise when two habitat types are not easily distinguished by the

observer (e.g., shallow seagrass meadows or dense patch of canopy-forming macroalgae). Interpretation—Understanding of remote sensing data requires extensive field calibration and the ground-truthing process remains essential (Pergent et al., 2017). As the interpretation of images/sonograms—is also time-requiring, several image processing techniques were proposed in order toto 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 a—good discrimination between soft sediments and seagrass meadows, between continuous and patchy seagrass, between a dense seagrass meadow and one exhibiting only limited bottom cover. The hHuman eye, however, always remains the final judge.

- Satellite telemetry is a valuable tool providing <u>high-resolution regional- to global-scale</u> observations and repeat time-series sampling a cost-effective way to easily acquiring large-scale and high resolution on seagrass distribution information in shallow waters. However, satellite imagery has some disadvantages, such as theirits 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 wide 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 longrange 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).
- 19.20. 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).
- <u>20.21.</u> Once the surveying is completed, data collected needs to be organised <u>so that it canto</u> be used in the future by everyone and can be appropriately archived and easily consulted. <u>The rResulting dataset can be integrated with similar data from other sources, providing a clear definition of all metadata (MESH-project, 2007).</u>
- 21.22. Despite the increasing number of studies on seagrass mapping with remote sensing instruments, datasets are not often available in on digital the geographic information system (GIS) platforms. As a final remark, only recently some modelling modeling approaches have been developed to obtain estimation estimate of the potential distribution of seagrass meadows in the Mediterranean. The probability of presence of the 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.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 km² per hour), and the main advantages or and the limits of each tool are indicated, with some bibliographical references.

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Satellite images	From 0 to 10- 15 m	From few km² to large areas (over 100-400 km²)	From 0.5 m	Over 100 km²/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 km² to 5000 km²	From 1 m		 High resolution that allowsing 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 Quick execution 	 Small patches (smaller than 1 m²) or low-density meadows cannot be distinguished Loss of definition at image edge, requiring adjustments between adjacent profiles Possible errors in image interpretation due to large signal amplitude variations (levels of grey) 	Paillard et al. (1993); Kenny et al. (2003); Clabaut et al. (2006)

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-referencingQuick execution	 Low discrimination between <u>habitats</u> 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 m ² 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 Possibility to do simultaneous monitoring 	 Many working hours Small areas mapped Necessity of numerous observers to cover larger areas 	Pergent et al. (1995a); Montefalcone et al. (2006)
Video camera (ROV or towed camera)	Whole bathymetric range of seagrass distribution	Small areas, usually under 1 km ²	From 0.1 m	0.2 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)		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 km ²	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

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

23.24. 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 should 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 image—classifiers, for example, the OBIA systems—(Object-Based Image Analysis): classification algorithm.

Table 2: Types of satellites and resolution of the sensors used for mapping seagrass meadows. $\frac{\text{n.a.}}{\text{not available.}}$

Satellite	Resolution	References
LandSat 8	30 m	Dattola et al. (2018)
Sentinel 2A - 2B	10 m	Traganos and Reinartz (2018)
PLANETSPOT 5	<u>3 m</u> 2.5 m	Traganos et al. (2017) Pasqualini et al. (2005)
SPOT 5	<u>2.5 m</u>	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)

24.25. <u>In view of Given</u> the changes <u>of in</u> 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.

25.26. 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. In particular, resolutionThe rResolution of the LandSat_8 satellite is not adequate to have reach high resolution mappings of seagrass meadows. However, the image LandSat_8 OLI represents a valid 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_2A and 2B satellites of the Copernicus programme. The Sentinel_2A and 2B satellites have a 13-band multispectral sensor (between visible and near infrared), the spatial resolution varies between 10, 20 and 60 m and the satellite revisiting time in the same area is 5 days (whilest 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_2A and 2B 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_2A and 2B images are also useful for the analysis of pressure and impact drivers.

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, and 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). It is possible to create libraries with specific spectral responses, so that measured values can be compared to distinct component species and appraise the vegetation cover (Ciraolo et al., 2006; Dekker et al., 2006).

27.

- Aerial images obtained through various means (e.g., airplanes, drones, ULM) may have different technical characteristics (e.g., shooting altitude, verticality, optical quality). Even though it is more expensive, shooting films from a plane that is equipped with an altitude and verticality control system and using large size negatives (24 × 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). In view of Given the progress made in the last few decades in terms of shooting (e.g., the quality of the film, filters, lens) and in the following processing (e.g., digitalization, geo-referencing), aerial photographs represents today one of the most preferred surveying methods for mapping shallow seagrass meadows (Mc Kenzie et al., 2001). Imagery acquired by unmanned aerial vehicles (UAVs), usually referred to as "drones", coupled with structure from motion 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 coatal habitats (Ventura et al., 2018).
- 29. Recent applications of very fine resolution Unmanned Aerial Vehicles (UAVs), usually referred to as "drones", have showned 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 unmanned aerial vehicles (UAVs), usually referred to as "drones", 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 eoatalcoastal habitats (Ventura et al., 2018).
- 27. 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.

2) Acoustic data

28.30. Sonar provides images of the seafloor through the emission and reception of ultrasounds. Among the main acoustic mapping techniques, Kenny et al. (2003) distinguishedistinguishes: (1) wide acoustic beam systems like the Sside sScan sSonar (SSS), (2)

¹CASI: Compact Airborne Spectrographic Imager

single_beam soundersechosounder (3), multiple narrow beam bathymetric systems, and (4) multibeam soundersechosounder.

- 29.31. Side sean-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 be helpful in interpretinghelp interpreting the data and to differentiatein 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).
- 30.32. Single-beam sounder is based on the simultaneous emission of two frequencies separated by several octaves (38 kHz and 200 kHz) to obtain the seafloor characterisation 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.
- 31.33. <u>Multi-beam sounderechosounder</u> 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 <u>form a beamare</u> 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. <u>A high-resolution</u> 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). <u>Other derived products can be slope</u>, aspect, curvature, and terrain ruggedness maps. <u>Multi-beam echosounders surveys are also limited in very shallow waters</u>, 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

- 32.34. Field samples and direct <u>underwater</u> 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 (<u>i.e.</u>, <u>having a complete</u> coverage of surface areas) obtained <u>through interpolation methods</u> from data <u>collected</u> 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, <u>and</u> also in view of the heterogeneity of the habitats. In the case of meadows of *Cymodocea nodosa*, *Posidonia oceanica*, *Zostera marina* or *Zostera noltei*, destructive sampling (using dredger buckets, core samplers, trawls, dredgers) are forbidden in viewgiven of the protected character of these species (UNEP/MAP, 2009) and direct underwater samples (e.g., shoot samples) should be limited as much as possible.
- 33. Observations from the surface can also be made by observers on a vessel using, for instance, a bathyscope, or <u>underwater</u> by using <u>imagery visual</u> techniques such as photography and video <u>recording</u>. 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 <u>camerasvideo cameras</u> can <u>also</u> be mounted on a <u>vertical-platform</u> structure (sleigh) or within <u>the remotely operated vehicle</u> (ROV). The camera on <u>a the vertical structureplatform</u> is submerged at the back of the vessel and is towed by the vessel that advances very slowly (under 1 knot), <u>allowing for the collection of long video transects</u>; on the contrary <u>whilst</u> the ROVs have their <u>own</u>-propulsion system and are remotely controlled from the surface <u>and allow recording comparatively shorter video transects</u>. 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.

34.35. The use of towed video cameras (or ROVs) during surveys makes it possible to see the images on the screen in real time, to identify specific features of the habitat and to evaluate any changes in the habitat or any other characteristic element of the seafloor., and Tthis preliminary video survey may be 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 to improve the results obtained with the camera, joint acquisition modules integrating the depth, and images of the seafloor and with geographical positioning have been developed (UNEP/MAP-RAC/SPA, 2015).

35.36. *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 limits of 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 with respect to the coastline (Bianchi et al., 2004). Any changes in the habitat and in the substrate typology, within a belt at both sides of the line (considering a surface area of about 1-2 m per side), are recorded on underwater slates (Fig. 4). The information registered allows precise and detailed mapping of the sector studied (Tab. 1).

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

37.38. Free-diving monitoring with a differential GPS can also be envisaged to locate the upper limits of the meadows. The diver <u>precisely</u> follows <u>precisely</u> the contours of the limits and the DGPS continuously records the diver's geographical <u>dataposition</u>. 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 <u>a depth</u> transect perpendicular <u>on-to</u> the coastline (© Monica Montefalcone).

Data interpretation

38.39. The recent MESH EU projects (2008) on habitat mapping (MESH, 2007; Vasquez et al., 2021a, b) identified four important essential stages for the production ofto 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 modelling 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 therefore its reliability.

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, neighbouring 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 onto 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.

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.

During the processing analysis and classification stage, tThe 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 should be consulted (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 UNEP/MAP SPA/RAC, 2019) to recognize any specific habitat type (i.e., seagrass species). As seagrass assemblages are often small in sizesmall, they can only be identified with high (metric) precision mapping. The updated lists identifyies the specific "seagrass meadow" habitats that are also listed in the annex of the Habitats Directive (Directive 92/43/EEC), and which must be taken into consideration within the framework of the NATURA 2000 programs. A complete The first original description of these 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 s and the criteria for their identification are available in Bellan-Santini et al. (2002). Habitats dominated by seagrass species that must be represented on maps listed in the updated Barcelona Convention classification system are the following (SPA/RAC-UN Environment/MAP, 2019a, bUNEP/MAP-SPA/RAC, 2019):

LITTORAL

MA3.5 Littoral coarse sediment

MA3.52 Medidolittoral coarse sediment

MA3.521 Association with indigenous marine angiosperms

MA3.522 Association with Halophila stipulacea

¹The updated list of benthic marine habitat types for the Mediterranean region is in a draft stage. It was endorsed by the Meeting of Experts on the finalization of the Classification of benthic marine habitat types for the Mediterranean region and the Reference List of Marine and Coastal Habitat Types in the Mediterranean (Roma, Italy 22-23 January 2019). The draft updated list will be examined by the 14th Meeting of SPA/BD FocalPoints (Portoroz, Slovenia, 18-21 June 2019) and submitted to the MAP Focal Points meeting and to the 21st Ordinary Meeting of the Contracting Parties, for adoption.

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MA4.5 Littoral mixed sediment

MA4.52 Medidelittoral mixed sediment

MA4.521 Association with indigenous marine angiosperms

MA4.522 Association with Halophila stipulacea

MA5.5 Littoral sand

MA5.52 Meidiolittoral sands

MA5.521 Association with indigenous marine angiosperms

MA5.522 Association with Halophila stipulacea

MA6.5 Littoral mud

MA6.52 Medidiolittoral mud

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

MA6.521a Association with halophytes (Salicornia spp.) or marine

angiosperms (e.g. Zostera noltei)

INFRALITTORAL

MB1.5 Infralittoral rock

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

MB1.541 Association with marine angiosperms or other halophytesa

MB2.5 Infralittoral biogenic habitat

MB2.54 Posidonia oceanica meadows

MB2.541 Posidonia oceanica meadow on rock

MB2.542 Posidonia oceanica meadow on matte

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

sediment

MB2.544 Dead matte of Posidonia oceanica

MB2.545 Natural monuments/Ecomorphoses of Posidonia oceanica

(fringing reef, barrier reef, stripped meadow, atollls)

MB2.546 Association of Posidonia oceanica with Cymodocea nodosa or

Caulerpa spp.

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

MB5.5 Infralittoral sand

MB5.52 Well sorted fine sand

MB5.521 Association with indigenous marine angiosperms

MB5.522 Association with Halophila stipulacea

MB5.53 Fine sand in sheltered waters

MB5.531 Association with indigenous marine angiosperms

MB5.532 Association with Halophila stipulacea

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

MB5.541 Association with marine angiosperms or other halophytesa

MB6.5 Infralittoral mud sediment

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

MB6.511 Association with marine angiosperms or other halophytahalophytes

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).

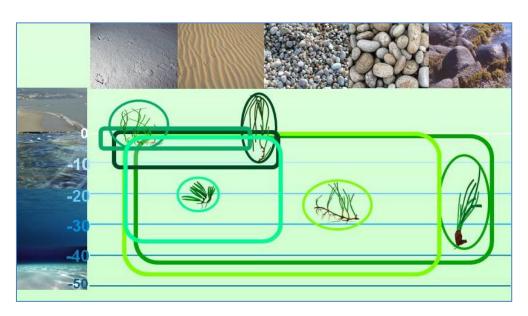


Figure 5: Distribution of seagrass species depending on the nature of the substrate and the depth in the Mediterranean (from UNEP/MAP-RAC/SPA, 2015a).

40. The <u>data integration and modelling stage</u> will differ depending on the survey tools and acquisition strategy used. Due to its acquisition rapidity, aerial techniques usually allow to for a complete coverage completely of the littoral and shallow infralittoral zones and this greatly dramatically reduces interpolation of data. On the contrary, surveys from vessels are often limited because of time and costs involved, and only rarely allow to obtaining a complete coverage of the area. Coverage under 100% automatically means that it is impossible to obtain-get high resolution maps and therefore interpolation procedures have to must be used, so that from partial surveys a lower resolution map can be obtained (MESH-project, 20078; 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. - run.

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

42.44. The processing and digital analysis of data (optical or acoustic) on GIS allows to creating charts where each tonality of grey is associated to with a specific texture representing a type of population/habitat, also on the basis of 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 substrates 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).

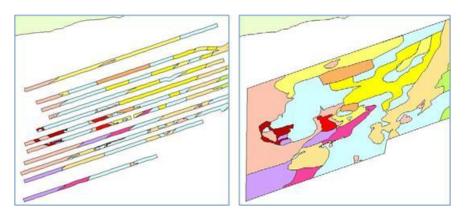


Figure 6: Example of partial coverage survey (left) and the output of the final map produced through interpolation (right). The area surveyed is about 20 km wide (from UNEP/MAP-RAC/SPA, 2015a).

43.45. To facilitate-a comparison among maps, standardized symbols and colours 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 colours that can be labelled 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 indicate alsoalsorepresent the discontinuous meadows that are characterised by a cover below 50%, or the two main species that constitute a mixed meadow (the colour of the patches allows identification of the species concerned). To represent some typical forms of *Posidonia oceanica* meadows (e.g., striped, atolls) no specific symbols are available being these forms (bands and circular structures, respectively) easily identifiable on 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 be also be compared with previous historical available data from the literature to evaluate any changes experienced by meadow over time a period of time (Mc Kenzie et al., 2001). Using the overlay vector methods on GIS, a diachronic analysis can be done, where temporal changes are measured in terms of percentage gained or losts of in the meadow extension, through the creation of concordance and discordance maps (Barsanti et al., 2007).

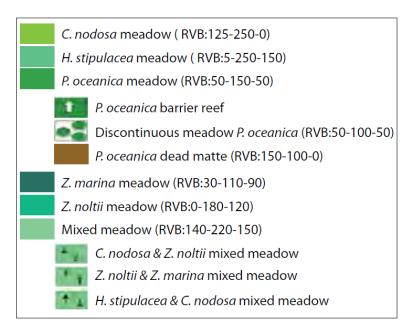


Figure 7: Examples of ssymbols and colours used for the graphic representation of the main seagrass assemblages. RVB: values in red, greengreen, and blue for each type of meadow (from UNEP/MAP-RAC/SPA, 2015a).

44.47. The <u>reliability</u> of the <u>map</u> produced should also be evaluated. Several evaluation scales <u>of for</u> reliability have already been proposed and may be <u>useful-helpful</u> for seagrass meadows. Pasqualini (1997) propose<u>ds</u> a reliability scale <u>in relation to about</u> the image processing of the aerial photos, which can also be applied to satellite images, or another scale in relation to the processing of sonograms (UNEP/MAP-RAC/SPA, 2015<u>a</u>). 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 <u>restitution</u> scale needs to be adapted.

45.48. Denis et al. (2003) proposed a reliability index of 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 of on the map, depending on the bathymetry or and the survey technique used.

46.49. 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

47.50. 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 (MSFD, 2008/56/EC) and the IMAP Ecap CI2-related to EO1 "biodiversity". The CI2 is aimed at providing information about the condition (i.e., ecological status) of seagrass meadows.

48.51. Monitoring the ecological status of seagrass meadows is today mandatory and is even an obligation for numerous Mediterranean countries due to the fact that 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 <u>I</u> (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).
- 49.52. 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 seagrasses will then reflect the Good Environmental Status (GES) pursued by the Contracting Parties to the Barcelona Convention under the Ecosystem Approach (EcAp) and under the Marine Strategy Framework Directive (MSFD).
- 50.53. AdDefined 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.
- 51.54. 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.
- 52.55. 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 <u>the</u> 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 <u>must thus should</u> be planned during a favourable season, also because some of the parameters chosen for monitoring purposes must be collected during the same period <u>due to the seasonality in seagrass growth</u>. This phase might be quite long, especially if numerous sites have to be monitored.
- 53.56. Monitoring over time and data analysis phase seems to be easy being the data acquisition a routinarye operation, with no major difficulties if the previous two phases had been carried out correctly. Data analysis needs clear scientific competence. Duration of the monitoring, in order toto be useful, must be medium time medium time at least. This phase often constitutes the key element of the monitoring system as it makes it-possible to:

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

54.57. The objectives of the Mmonitoring of seagrass meadows is can linked cover with the conservation targets of seagrass meadows and also with their use as an ecological indicators of the quality of the marine environment. The main aims of seagrass monitoring are generally:

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

55.58. The objective(s) ehosen 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.

56.59. 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 depths of their 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 nonpersistent 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 impacted site, all representative of the coastal area	Physical Ddescriptors of the quality of the environment (e.g., water turbidity, depth of lower limit, 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 consequences 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

57.60. 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 descriptions of the sampling tools and methodologies can be found.

61. The many available descriptors available for monitoring seagrass habitat (see Table 4) work at each of the different ecological complexity levels of seagrass (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 individual species (i.e., the plant), the physiological or cellular or physiological/biochemical level, and the associated community (especially leaf epiphytes). 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 specifityspecificity 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) have been developed to working at the highest ecological levels have been recently developed.s, At i.e. 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 ; Personnic et al., 2014), while . Some recent other ecological indices integrate different ecological levels, such as (e.g., for instance the PREI (, Gobert et al., 2009), the BiPo (Lopez y Royo et al., 2009), and ; the POMI (Romero et al., 2007).

58.

59.62. 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 depths 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 taken into account 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 within-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 period of timea fixed period, even if it could would mean a reduced number of sites and a reduced number of descriptors being monitored. Number of adopted descriptors should be adequate enoughadequate 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 descriptors is less important than its reproducibility, reliability and the precision of the method used for its acquisition.

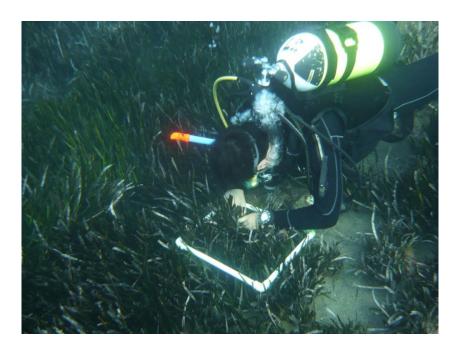
63. In situ observations and samples must be done over defined and, possibly, standardized surface areas, and the number of replicates must be adequate for the descriptor involved and high enough to catch the heterogeneity of the habitat. The analyses at the individual species (the plant), cellular or physiological/biochemical level or cellular, and most of the analyses associated 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 and measured shoots ranges between a minimum of 10-9 to a maximum of 18-210 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).

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 a standardized sizes of the quadrate are usually adopted: surface area is settled at $40 \text{ cm} \times 40 \text{ cm}$ and $20 \text{ cm} \times 20 \text{ cm}$. 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 $20 \text{ cm} \times 20 \text{ cm}$ 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 $40 \text{ cm} \times 40 \text{ cm}$ quadrate is at least four times more time-consuming than in a $20 \text{ cm} \times 20 \text{ cm}$ 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*). with a A minimum of 3-3 independent replicated eounts-counts should be done per in each station 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, samples of shoots can be performed collected only at the intermediate meadow 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), and sampling activities should be carried out during the late spring or early summer season (Gobert et al., 2009).
- 60.67. Among all the descriptors listed in Table 4, the shoot density can be viewed as 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 proves effective in revealing environmental alterations (Montefalcone, 2009). 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 existing 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. This seale provides a tool to classify the ecological status of the meadow that can be used in the frame of the IMAP under the EcAp. Evaluating depth and typology of both the upper and the lower limits of the meadow and monitoring over time their positions with permanent marks (i.e., balises) are commonly adopted procedures to assess the evolution of the meadow in term of stability, improvement or regression that is linked to water transparency, hydrodynamic regimes, sedimentary balance and human activities along the coastline (Fig. 8). The absolute classification scale of 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 under the EcAp, 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 in of the Ligurian Sea (NW Mediterranean), along the Spanish coast (NW Mediterranean), and or 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)



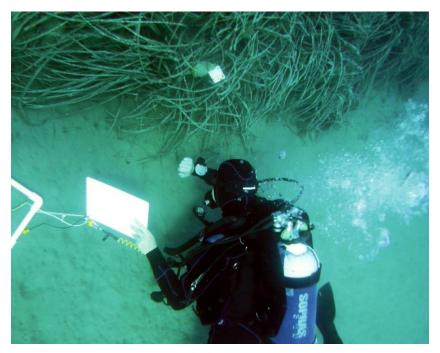


Figure 8: *In situ* measurement of *Posidonia oceanica* shoot density using the <u>a standard square frame</u> <u>quadrate</u> of 40 cm × 40 cm (upper <u>imagepanel</u>, © <u>Monica Montefalcone</u>) and monitoring over time of the meadow lower limit position with permanent marks (lower <u>imagepanel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

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 the 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, individual species, physiological cell, community).

Descriptor	Method	Expected response/factors	Destr	Target species	Advantages	Limits	References
Population Sea	scape (meadow) level	<u> </u>	1	<u> </u>			
Meadow extent (i.e. surface area)	Mapping (Cf. Part "a" of this document) and/or identification definition of the meadow boundaries position of limits	 Reduction of the total meadow extent Coastal development, turbidity, mechanical impacts 	No	All	 Informative of many aspects of the meadow Usable everywhere in view of the many techniques available Cover the whole depth range of meadow distribution 	 For slow growing species (Po) needs of prepositioning 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 (me	<u>eadow) level</u>						
Bathymetric position of the meadow upper limit (in m) and its morphology	A detailed mapping of the seagrass extension 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 at-during the same season for on all sites Fixed markers (balises) might disappear if the 	Pergent et al. (1995); Montefalcone (2009)

Descriptor	Method	Expected response/factors	Destr	Target species	Advantages	site is strongly frequented Limits	References
Bathymetric position of the meadow lower limit (in m)	A detailed mapping of the seagrass extension 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 Tturbidity 	No	All	 Easily measured (also by scuba diving) Absolute cClassification scale available for Po 	 For Cn, Hs and Zn, strong seasonal variability, requiring periodical monitoring or observations at during the same season fon all sites Beyond 30 m depth, underwater acquisition surveys is 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 typemorphology	In situ visual observations	Change in morphology Water tTurbidity, mechanical impacts damages (e.g., trawling)	No	Po	•	Well known descriptor Several types morphologies described CAbsolute classification scale for Po	•	Good knowledge of Po meadows necessary to identify some of the morphologiestypes Beyond 30 m depth, underwater surveys are difficult and costly (limited diving time, need for experienced divers, numerous dives requested) Difficult and costly the assessment at great depths (>30 m)	Boudouresque and Meinesz (1982); Pergent et al. (1995); Montefalcone (2009); Annex 1
<u>Descriptor</u>	<u>Method</u>	Expected response/factors	<u>Destr</u>	Target species		<u>Advantages</u>		<u>Limits</u>	References
Presence of inter-matte channels and dead matte areas	High resolution and ly detailed mapping of the area (Cf. Part "a" of this document, permanent square frames) and/or in situ observations	 Increase in the extent Mechanical impacts damages (e.g., anchoring, fishing gear) 	No	Po	•	Surface areas <u>can be easily</u> <u>measured</u> <u>can be measured</u> on maps	•	Dead matte areas are natural components intrinsic to in some typologies of types 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 <i>P. oceanica</i> the most adopted sizes are 40 cm	Reduction Water Tturbidity, mechanical impacts 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 Cclassification 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	Duarte and Kirkman (2001); Pergent-Martini et al. (2005); Pergent et al. (2008); Bacci et al. (2015); Annex 1

	× 40 cm and 20 cm × 20 cm					is inexperienced; b) high density; c) small sized species. In this latter case in situ counting can be replaced by sampling over a given area and the counting can be done in the laboratory -(but becoming a destructive technique)	
Descriptor 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 measure estimate the cover in situ by divers or in laboratory- (from photos or video, visual estimation). Variable observation surface area (0.16 to 625 m²), visualised by a quadrate or a transparent plate	Expected response/factors Reduction Water tTurbidity, mechanical damages	No No	Target species All	 Advantages 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 	References 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 in a given on a defined surface area (e.g., 40-20 cm × 40-20 cm, which can be visualised by a quadrate)	 Increase Mechanical impacts damages (e.g., anchoring, fishing gear) 	No	Cn, Po	 Easy, rapid, and low-cost Absolute c Classification scale available for Po 	Mainly used at shallow depths (0-20 m)	Boudouresque et al. (2006); Annex 1
Individual Spec	<u>ies (</u> plant) <u>level</u>	L	l		1	1	
Leaves surface area (cm² · shoot), and other phenological measures	Counting and measuring the length and width of the different types of leaves in each shoot (10 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 nutriments enhancement 	Yes	All	 Easy, rapid and low-cost Possibility to measure the length of adult leaves (the most external leaves) in situ to avoid sampling Classification 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
<u>Descriptor</u>	Method	Expected response/factors	<u>Destr</u>	Target species	<u>Advantages</u>	<u>Limits</u>	References
Necrosis on leaves (in %)	Percentage of leaves with necrosis, through observation in laboratory.	IncreaseIncreased contaminants concentration	Yes	Po	• Easy, rapid, and low-cost	Necrosis is very rare in some sectors of the Mediterranean (e.g.,	Romero et al. (2007)
		concentration				Corsica littoral) • Destructive sampling	
State of the apex	Percentage of leaves with broken apex	Increase Overgrazing, mechanical impacts (e.g., anchoring)	No	Po	 Easy, rapid, and low-cost Specific marks of left by the bit of some animals are easily recognizable 	 Destructive sampling Not informative onf the grazing pressure in the 	Boudoresque and Meinesz (1982)

(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 markings or by using the relationship bases length/leaves growth (Zm)	interspecific competition	No (Zm)		 Absolute Cclassification scale available For Zm the relationship bases length/leaves growth allows in situ non destructive measuring 	 Monthly monitoring, or at least for every4 season, is 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 Classification_Absolute classification_scale available for Po	Increase in the Interpretation sometimes difficult as rrhizome production increase can be also be observed in reference sites in the absence of human impacts Destructive sampling	Pergent et al. (2008); Annex 1
<u>Descriptor</u>	Method	Expected response/factors	Destr	Target species	Advantages	<u>Limits</u>	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 	<u>No</u>	All	 Easily measured in situ Not destructive and low-cost Independent from the season 		Boudoresque et al. (2006)
Burial or baring of the rhizomes	Measuring the degree of burial or baring of rhizomes in situ, or the	• Increase in burial for increased sedimentation (e.g.,	No	All	 Eas measure in situ Not destructive and low-cost 		Boudoresque et al. (2006)

(in mm) Cellular or phys	percentage of buried or bared shoots on a given surface area	coastal development, dredging) Increase in baring for deficit in the sediment load			Independent from season		
Nitrogen and phosphorus content (in % dry weight) in plant tissues Descriptor	Dosage through mass spectrometry and plasma torch in different plant tissues (both leaves and rhizomes) after acid mineralisation (e.g., in rhizome for Po) Method	 Increase Nutriments enhancement Expected response/factors 	YesD estr.	AllTar get species	 Short response time to environmental changes Absolute classification scale for PoAdvantages 	 Very expensive Analytical equipment and specific competence necessary Destructive sampling Limits 	Romero et al. (2007); Annex 1References
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)	ReductionHuman 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
Physiological (c) Trace metal content (in µg · g 1) Nitrogen and phosphorus content in plant (in % dry weight)	Dosage through spectrometry in different plant tissues (both leaves and rhizomes) after acid mineralisationDosage through mass spectrometry and plasma torch in different plant tissues after acid	 Increase Increased concentration of metallic contaminantsIncrea se Nutriments enhancement 	Yes¥ es	<u>All</u> All	 Short response time to environmental changes Absolute classification scale for Po Short response time to environmental changes Classification scale for Po 	 Very expensive Analytical equipment and specific competence necessary Destructive samplingVery expensive Analytical equipment and specific competence necessary Destructive sampling 	Salivas-Decaux (2009); Annex 1Romero et al. (2007); Annex

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	mineralisation (e.g., rhizomes for Po)						
<u>Descriptor</u>	Method	Expected response/factors	Destr	Target species	<u>Advantages</u>	<u>Limits</u>	References
Nitrogen isotopic relationship (d ¹⁵ N in ‰) Carbohydrate content (in % dry weight) in plant and sediments	Dosage through mass spectrometer in different plant tissues after acid mineralisation (e.g., in rhizomes for Po)Dosage through spectrophotometry after alcohol extraction in different plant tissues (e.g., rhizomes for Po)	 Increase for nutriments enhancement from farms and urban effluents Reduction for nutriments enhancement from fertilizers Reduction Human impacts 	Yes¥ es	<u>Po</u> All	 Short response time to environmental changes Short response time to environmental changes Classification scale for Po 	 Very expensive Analytical equipment and specific competence necessary Destructive sampling Very expensive Analytical equipment and specific competence necessary Destructive sampling 	Romero et al. (2007)Alcoverr o et al. (1999, 2001); Romero et al. (2007); Annex 1
Trace metal content (in µg · g · l)	Dosage through spectrometry in different plant tissues after acid mineralisation	 Increase Increased concentration of metallic contaminants 	Yes	All	 Short response time to environmental changes Classification scale for Po 	 Very expensive Analytical equipment and specific competence necessary Destructive sampling 	Salivas Decaux (2009); Annex 1
Sulphur isotopic relationship (d ³⁴ S in <u>%0)</u> Nitrogen isotopic relationship (d ¹⁵ N in %0)	Dosage through mass spectrometer in different plant tissues (e.g., rhizomes of Po) Dosage through mass spectrometer in different plant tissues after acid mineralisation (e.g., rhizomes for Po)	 Reduction Human impacts Increase for nutriments enhancement from farms and urban effluents Reduction for nutriments enhancement from fertilizers 	Yes¥ es	<u>Po</u> Po	Short response time to environmental changesShort response time to environmental changes	Very expensive Analytical equipment and specific competence necessary Destructive sampling Very expensive Analytical equipment and specific competence necessary Destructive sampling	Romero et al. (2007)Romero et al. (2007)
<u>Community</u> De	Method	Expected	Destr	Target	Advantages	Limits	References

biomass (in mg dry weight · shoots-1 or % dry weight · shoots-1) and epiphytes cover (in %) on the leaves Sulphur isotopic relationship (d³4S in %)	· shoots ⁻¹) after scraping, drying and weighing; estimate the epiphytes cover on leaves under a binocular; indirect estimation of biomass from epiphytes cover Dosage through mass spectrometer in different plant tissues (e.g., rhizomes of Po)	 Increase Nutriments enhancement from rivers, high touristic frequentationReduc tion Human impacts 	es		 Easilyy to-measured Low-cost (biomass and cover) Absolute classification scale available for Po Early-warning indicator Short response time to environmental changes 	 Time-consuming Strong seasonal and spatial variability Specific analytical equipment (nitrogen content) necessary Destructive sampling Very expensive Analytical equipment and specific competence necessary 	Pergent-Martini et al. (2005); Romero et al. (2007); Fernandez- Torquemada et al. (2008); Giovannetti et al. (2008, 2015)Romero et al. (2007)
Community							
Epiphytes biomass (in mg dry weight -shoots-+ or % dry weight - shoots-+) and epiphytes cover (in %) of leaves	Measure of biomass (µg -shoots ¹) after scraping, drying and weighingMeasure of nitrogen content (in % dry weight). Measure using simple CHN analyser Estimate the epiphytes cover on leaves under a binocular Indirect estimation of biomass from epiphytes cover	 Increase Nutriments enhancement from rivers, high touristic frequentation 	Yes	All	 Eas measure Low-cost (biomass and cover) Classification scale available for Po Early warning indicator 	 Time consuming Strong seasonal and spatial variability Specific analytical equipment (nitrogen content) necessary Destructive sampling 	Morri (1991); Pergent Martini et al. (2005); Romero et al. (2007); Fernandez Torquemada et al. (2008); Giovannetti et al. (2008, 2015)

- 61.68. The setting-up phase is the concrete operational phase of the monitoring program that starts with the data acquisition. The observations and samplings during the acquisition phase or data validation of the cartographical surveys, could surveys may also constitute an output of a the monitoring system (Kenny et al., 2003), and cartography could also represent a monitoring tool (Tab. 4; Boudouresque et al., 2006).
- 62.69. At the regional spatial scale, two main monitoring systems have been developed: 1) the seagrass monitoring system (SeagrassNet), which was has been established at the 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 to-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 in over 350 sites (Buia et al., 2004; Boudouresque et al., 2006; Romero et al., 2007; Fernandez-Torquemada et al., 2008; Lopez y Royo et al., 2010a). After the work carried out within the framework of the Interreg IIIB MEDOCC programme "Coherence, development, harmonization and validation of evaluation methods of the quality of the littoral environment by monitoring the *Posidonia oceanica* meadows", and the "MedPosidonia" programme set up by RAC/SPA, an updated and standardized approach for the *P. oceanica* monitoring network has been tested and validated (UNEP/MAP-RAC/SPA, 2009). The main differences between the former-two monitoring systems are:
 - Within the framework of SeagrassNet, monitoring is done along three permanent transects, laid parallel to the coastline and positioned respectively (i) in the most superficial part of the meadow, (ii) in the deepest part, and (iii) at an intermediate depth between these two positions. The descriptors chosen (Short et al., 2002; Tab. 5) are measured at fixed points along each transect and every three months.
 - Within the framework of the "Posidonia" monitoring network, measurements are taken (i) in correspondence of fixed markers placed along the lower limit of the meadow, (ii) at the upper limit, and (iii) at the intermediate and fixed depth of 15 m. The descriptors (Tab. 5) are measured every three years only if, after visual surveys, no visible changes in the geographical position of the limits are observed.
- 63.70. SeagrassNet allows to-compareing the data obtained in the Mediterranean with the data obtained in other regions of the world, having a world-wide coverage one 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 also and evaluating the plant's vitality and the quality of the environment in which where it grows. Other monitoring system, such as permanent transects with seasonal monitoring, or acoustic surveys, can be used in particular specific situations like the monitoring of lagoons environments (Pasqualini et al., 2006) or for the study of relict meadows (Descamp et al., 2009).
- The sampling technique and the chosen descriptors define the nature of the monitoring (e.g., monitoring of chemical contamination of 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 the monitoring of seagrass meadows, but rather a great diversity of efficient and complementary tools. They must be chosen depending on the objectives, the species present and the local context. Independently from the descriptors selected, particular attention must be paid to the validity of the measurements made (acquisition protocol, precision of the measurements, reproducibility; Lopez y Royo et al., 2010a). The following data processing and interpretation phase is thus fundamental to ensure the good quality of the monitoring programme.

<u>Table 5: Descriptors measured within the framework of the SeagrassNet, the "Posidonia" monitoring Network, and the MedPosidonia monitoring programs (Pergent et al., 2007).</u>

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

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 (Howard et al., 2014). 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 C¹⁴ to date the age at which soil was laid down. The following parameters are useful for the estimation of carbon contents in plant tissues:

64. Estimating the production of carbon obtained by photosynthetic activity from *P. oceanica* meadows (above and belowground production) at the Mediterranean basin scale

requires the following parameters (essential for the calculation of the Blue Carbon) from the lepidochronological analyses:

- 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.

65.73. The methodological approaches for estimating Blue Carbon consider both the use of satellite images, acoustic surveys (multibeam, single beam, and sub bottom profiler), optical acquisitions, and measurements in situ and in the laboratory. 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.

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

Data processing and interpretation

66.74. Measurements made *in situ* must be <u>analyzedanalysed</u> 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 <u>classification</u> 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.

67.75. The huge increase of studies on *Posidonia oceanica* (over 2400–2700 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).

68.76. 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.

Ecological indices

69.77. Ecological synthetic indices are today widespread for measuring the ecological status of ecosystems in viewgiven of the Good Environmental Status (GES) achievement or maintenance. Ecological indices succeed in "capturing the complexities of the ecosystem yet remaining simple enough to be easily and routinely monitored" and may therefore be considered "user-friendly" (Montefalcone, 2009 and references therein). They are anticipatory, integrative, and sensitive to stress and disturbance. Many ecological indices had been employed in the seagrass monitoring programsmes 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, on the basis of 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)

70.78. Most of the ecological indices integrate different ecological levels (Tab. 6). The POSWARE index is based on 6 descriptors working at the population and individual 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, individual 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 individual species to community level. The PREI index is based on 5 descriptors working at the population, individual species and community levels. The BiPo index is based only on 4 non-destructive descriptors at the population and individual species levels and is particularly well suited for the monitoring of protected species or within MPAs.

Some not-destructive ecological indices have been developed to work at the seascape ecological level, such as the 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., hydrodynamismwater 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 level-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).

72.80. One of the most recently proposed indexindices works at the ecosystem level (EBQI; Personnic et al., 2014). This index has been developed on the basis of 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 through the selection by selecting of one or two specific descriptors (most of them not destructive) and the final index value integrates all compartment scores. Being an ecosystem-based index, it complies with the MSFD and the EcAp requirements. However, its complete and thus but also complex formulation makes this index more time-consuming when compared to other indices.

73.81. 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 Mediterranean sites yieldeds an identical classification of the Catalonia sites as the 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" programmme, a similar classifications of

the meadows studied wereas found (Pergent et al., 2008). A recent exercise to compare a number of several descriptors and ecological indices working at different ecological levels (individual 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, the a combined concurrent use of more descriptors and indices, covering different levels of ecological complexity, should be preferred in any monitoring programme.

74.82. 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 <u>over several</u> 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 the 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 <u>mostly adopted</u> synthetic ecological indices <u>mostly adopted</u> in the regional/national monitoring programs to evaluate <u>the</u> environmental quality based on the "seagrass" biological quality element. The ecological complexity level at which each descriptor works is also indicated (i.e., <u>physiologicalcellular, individualspecies</u>, population, community, ecosystem, seascape).

Index	Physiological Cellular	Individual 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; δ^{15} N and δ^{34} S isotopic ratio in rhizomes; Cu, Pb, and Zn content in rhizomes	Leafves 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	Leafves surface; Coefficient A; rhizomes elongation	Shoot density; meadow cover; percentage of plagiotropic rhizomes; depth of the lower limit	EpiphytesEpip hytes biomass		
Valencian CS		Leafves 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		Lea <u>fves</u> surface; lea <u>fves</u> biomass	Shoot density; lower limit depth and type	Leaf epiphytees biomass		
BiPo		Lea <u>fves</u> surface	Shoot density; lower limit depth and type			
CI			Meadow and dead matte cover			Relative proportion between <i>Posidonia</i> oceanica and dead matte
SI			Meadow cover	Substitutes cover		Relative proportion between <i>P. oceanica</i> and substitutes

Index	Cellular	Species	Population Population	Community	Ecosystem	Seascape
PSI			Meadow and dead matte cover	Substitutes cover		Relative proportion of <i>P. oceanica</i> , 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 1.1

<u>Absolute c</u>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 \cdot m²) (Pergent-Martini et al., 2005)

Depth (m)	I	High	G	lood		Mo	der	ate		Poor	•		Bad
1	>	1133	1133	to	930	930	to	727	727	to	524	<	524
2	>	1067	1067	to	863	863	to	659	659	to	456	<	456
3	>	1005	1005	to	808	808	to	612	612	to	415	<	415
4	>	947	947	to	757	757	to	567	567	to	377	<	377
5	>	892	892	to	709	709	to	526	526	to	343	<	343
6	>	841	841	to	665	665	to	489	489	to	312	<	312
7	>	792	792	to	623	623	to	454	454	to	284	<	284
8	>	746	746	to	584	584	to	421	421	to	259	<	259
9	>	703	703	to	547	547	to	391	391	to	235	<	235
10	>	662	662	to	513	513	to	364	364	to	214	<	214
11	>	624	624	to	481	481	to	338	338	to	195	<	195
12	>	588	588	to	451	451	to	314	314	to	177	<	177
13	>	554	554	to	423	423	to	292	292	to	161	<	161
14	>	522	522	to	397	397	to	272	272	to	147	<	147
15	>	492	492	to	372	372	to	253	253	to	134	<	134
16	>	463	463	to	349	349	to	236	236	to	122	<	122
17	>	436	436	to	328	328	to	219	219	to	111	<	111
18	>	411	411	to	308	308	to	204	204	to	101	<	101
19	>	387	387	to	289	289	to	190	190	to	92	<	92
20	>	365	365	to	271	271	to	177	177	to	83	<	83
21	>	344	344	to	255	255	to	165	165	to	76	<	76
22	>	324	324	to	239	239	to	154	154	to	69	<	69
23	>	305	305	to	224	224	to	144	144	to	63	<	63
24	>	288	288	to	211	211	to	134	134	to	57	<	57
25	>	271	271	to	198	198	to	125	125	to	52	<	52
26	>	255	255	to	186	186	to	117	117	to	47	<	47
27	>	240	240	to	175	175	to	109	109	to	43	<	43
28	>	227	227	to	164	164	to	102	102	to	39	<	39
29	>	213	213	to	154	154	to	95	95	to	36	<	36
30	>	201	201	to	145	145	to	89	89	to	32	<	32
31	>	189	189	to	136	136	to	83	83	to	30	<	30
32	>	179	179	to	128	128	to	77	77	to	27	<	27
33	>	168	168	to	120	120	to	72	72	to	24	<	24
34	>	158	158	to	113	113	to	68	68	to	22	<	22
35	>	149	149	to	106	106	to	63	<	63			
36	>	141	141	to	100	100	to	59	<	59			
37	>	133	133	to	94	94	to	55	<	55			
38	>	125	125	to	88	88	to	52	<	52			
39	>	118	118	to	83	83	to	48	<	48			
40	>	111	111	to	78	78	to	45	<	45			

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

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

Plant (individual 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 levelphysiological level): environment contamination

Argent <u>c</u>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 <u>c</u>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 <u>c</u>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 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, Lithophyllumbyssoidesconcrationsencrusting 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, sabellariid and serpulid worm reefs, and rhodoliths_seabeds. Among all, coralligenous reefs (Fig. 1) and rhodoliths_seabeds (Fig. 2) are the two most typical and 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 <u>dominated by the gorgonian Paramuricea clavata (upper panel © by Simone Musumeci), and facies with Corallium rubrum in enclave in the coralligenous (lower panel © Monica Montefalcone).</u>



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 of 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 higher than seagrass meadows (Cánovas-Molina et al., 2014). Construction of coralligenous reefs started during the post-Würm transgression, about 15000 years ago, and developeds on rocky and biodetritic bottoms in relatively constant-stable conditions of temperature, currents, and salinity.
- Two main eCoralligenous reefs are distributed both on rocky and soft bottoms, developing different morphologiestypologies can be defined:, i) coralligenous developing on the upper circalittoral rocks and at the entrance of caves with (cliffs, or-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 structure 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 are able tocan 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 createing a biogenic, unstable, three-dimensional habitat typically exposed to bottom currents, which harbours greater biodiversity in comparison compared to surrounding habitats bottoms, and thus are viewed as an indicator of biodiversity hotspots. Rhodoliths beds They mostly (mainly?) occur on coastal detritic bottoms in the upper mesophotic 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 includes maërl and calcareous Peyssonnelia beds, but the opposite is not true (Basso et al., 2016). Rhodoliths bed is recommended as a generic name to indicate those sedimentary bottoms characterised by any morphology and species of unattached nongeniculate 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. but_They are vulnerable to either_global ander local impactspressures. 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, either-by forming dense carpets or by increasing sedimentation rate.
- Despite the occurrence of many species with high ecological value (some of which are also legally protected, e.g., Savalia savaglia, Spongia (Spongia) officinalis 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 thise Directive, and appear also in the Bern Convention. This implies that the most important Mediterranean bioconstruction still remains remains without formal protection as it is not included within the list of Special ites of Areas of Community Interest Conservation (SACICs). Few years after the adoption of the Habitat Directive, coralligenous reefs were listed among the "special habitats types" needing rigorous protection by the Pprotocol concerning the Special Protected Areas and Biological <u>dDiversity</u> (SPA/BD <u>Protocol</u>) 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-threated", and the circalittoral coralligenous bioconcretions (code A5.6y) as it is classified as "data deficient" (Gubbay et al., 2016), thus demonstrating the urgent need for thorough investigations and accurate monitoring plans. In the same year, the Marine Strategy Framework Directive (MSFD, 2008/56/EC) included "seafloor integrity" as one of the descriptors to be evaluated for assessing the Good Environmental Statuse of the marine environment. Biogenic structures, such as coralligenous reefs, have thus been recognized as important biological indicators of environmental quality.

- 7. Similarly, rhodoliths_seabeds are expected to be damaged by dredging, heavy anchors and mooring chains, and trawling and are adversely affected by rising temperatures and, and ocean acidification and trawling. 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). Rhodoliths_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 <u>on</u> the composition (list of species) of these habitats;
 - (ii) Set up a standardized <u>spatiospatial</u>-temporal monitoring protocol for coralligenous and rhodoliths habitats.
- Detailed information on habitat geographical distribution and bathymetrical ranges is a prerequisite knowledge for a the sustainable use of marine coastal areas. Coralligenous and rhodoliths distribution maps are thus a fundamental prerequisite to any conservation action on these habitats and on-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) has been is currently increasing. However, but it is still far away from the knowledge we have from 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 spatiospatial-temporal studies on their geographical and depth distribution both at regional level and basin-wide scale. This information is essential in order to know the real extent of these habitats in the Mediterranean Sea and to implement appropriate management measures to guarantee their conservation (UNEP/MAP-SPA/RAC,_2017). Inventory and monitoring of coralligenous and rhodoliths raise several problems, due to their large bathymetric distribution and the consequent sampling constraints, and the often limited often-limited accessibility, their 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 do-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 monitoring 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 of 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 base 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 <u>underwater</u> scuba diving is <u>recommended often used</u> for mapping <u>and monitoring at</u> small <u>areasspatial scales and at shallower depths</u>, it becomes unsuitable when the study area and/or the depth increase (usually at depths >40 m);

- The use of aAcoustic survey methods (side scan sonar or multibeam echosounder) or underwater visual observation systems (ROV, towed camera) becomes then necessary indispensable at depths greater than 40 m. However, acoustic techniques must be always integrated and verified by visual inspections with ground-truth dataa large number of "field" underwater data.
- 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 relies 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 it-they allows monitoring with less precision but on larger areas and at greater depths:
 - Although the use of underwater photography or videovideorecording may be relevant, the use presence of specialists in taxonomy with a good experience in scuba diving surveying methods is often essential given the complexity of these habitats. If it is possible to estimate the aAbundance or coverage of specific taxa can be visually estimated underwater by standardized indices on defined surfaces or along transects through standardized indices, detailed characterisations often require the use of square frames (quadrates), transects_, or even the removal of all organisms on a given surface. The presences of broken individuals and of areas of necrosis are other factors to be considered;
 - Monitoring of coralligenous habitat starts with the realisation of micro-mapping and then the application of applying descriptors and/or ecological indices. However, these descriptors vary widely from one team to another, as well as their measurement protocols:
 - Monitoring of rhodoliths habitats can be done by <u>underwater</u> scuba diving, <u>but as well</u> <u>as the byand visual observation inspection</u> using ROVs or towed cameras and <u>with the collection of collecting</u> samples using dredges, grabs, <u>or and</u> box corers, <u>are privileged because of the greater homogeneity of these populations. However At present</u>, there is not <u>yet</u> any standardized method <u>yet that has been</u> widely accepted <u>to date</u> for monitoring rhodoliths, also because the action of <u>hydrodynamics water movement</u> 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 of the Mmeeting 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), in order to ease the task for the countries when implementing their monitoring programmes by The two considering the previous work guidelines elaborated published by SPA/RAC, the 'Standard methods for inventorying and monitoring coralligenous and rhodoliths assemblages' Guidelines for monitoring coralligenous and other bioconstructed habitats in Mediterranean (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. to be updated in the context of the IMAP common indicators in order to ease the task for the contries when implementing their monitoring programmes.—A reviewing process on the available scientific literature, taking into account considering the latest techniques and the recent works carried out by the scientific community at the international level, has also been also carried out. If standardized protocols for seagrass mapping and monitoring exist and are well-implemented, and a number of 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 a number of some "minimal of the most adopted "descriptors to be taken into account for inventorying and monitoring the coralligenous and rhodoliths populations in the Mediterranean are described. The main methods adopted for their monitoring, with the relative advantages, restrictions, and conditions for their of use, are presented.

Some of the existing monitoring methods for coralligenous have already been compared or cross-calibrated and <u>results</u> are <u>here briefly introduced reported briefly reported here.</u> and, finally, a standardized method procedure recently proposed for coralligenous monitoring is also described.

Monitoring methods

a) COMMON INDICATOR 1: Habitat distributional range and extent

Approach

- 14. The CI1 is aimed at providingaims 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" in this document for major details):
 - 1) Initial planning, which includes the definition of the objectives in order to select the minimum surface to be mapped and the necessary resolution, tools and equipments:
 - 2) Ground survey is the practical phase for data collection, 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 a-critical stage in the initial planning phase (Mc Kenzie et al., 2001). When large surface areas have to be mapped and global investigations carried out, an average precision and a lower detail level can be accepted, which means that the habitat distribution and the definition of its extension limits boundaries are often only indicative. When smaller areas have to be mapped, a-much higher precision and resolution level isare 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 an accurate localisation of the habitat distribution and a precise definition of its extension limits boundaries and total habitat extent, all features necessary for future control and monitoring purposes over a period of time. These high-resolution scales are also used to select remarkable (great?) 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 m², 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 distribution 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 overall-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).
- Two global maps showing the distribution of coralligenous (Giakoumi et al., 2013) (Fig. 3) and maërl habitats (Martin et al., 2014) (Fig. 4) in the Mediterranean have been were produced based on the review of available information. Coralligenous habitats cover a surface area of about 2763 km² in 16 Mediterranean countries, i.e. Albania, Algeria, Croatia, Cyprus, France, Greece, Italy, Israel, Lebanon, Libya, Malta, Monaco, Morocco, Spain, Tunisia, and Turkey. All other ecoregions presented lower coverage, with the Alboran Sea having the lowest. Very limited data were found for 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 southeastern 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-Maërl-habitats cover a surface area of about 1654 km². Only sporadic and punctual information are available, mainly from the North Adriatic, the Aegean Seas, and the Tyrrhenian Sea. Datasets are available for Greece, France (Corsica), Cyprus, Turkey, Spain, Lebanon, and Italy. Malta and Corsica, in particular, have have significant datasets for-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 the mapping and monitoring efforts across the Mediterranean basin; further mapping is thus required to determine the full extent of these highly variable habitats at the Mediterranean spatial scale. However, these global mapsy 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.

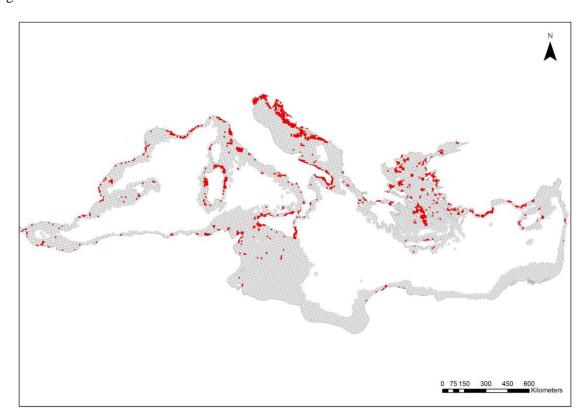


Figure 3: Global scale Ddistribution of coralligenous habitats in the Mediterranean Sea (red areas) (from Giakoumi et al., 2013).

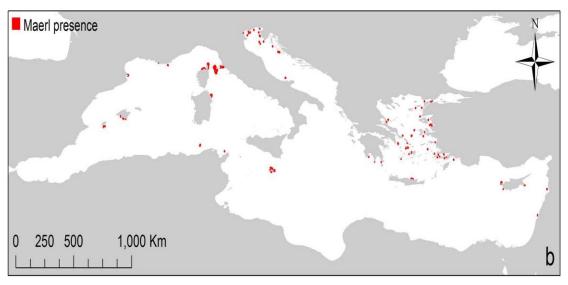


Figure 4: <u>Global scale Dd</u>istribution of <u>rhodoliths/maërl habitats</u> in the Mediterranean Sea (red areas) (from Martin et al., 2014).

Methods

21. Definition of distributional <u>range boundaries</u> and extent of coralligenous and rhodoliths habitats requires "traditional" habitat mapping techniques, <u>similar tolike</u> those used for seagrass meadows in deep waters (Tab. 1). <u>Indirect instrumental Remote sensing</u> -mapping techniques and/or <u>direct underwater field</u> visual surveys <u>ean bemust be</u> used and are often integrated. The simultaneous use of two or more <u>mapping</u> methods makes it possible to optimise the results

being the information obtained complementary. The strategy to be adopted will thus depend on the aim of the study's aim and the area concerned, means, and time available (The strategy to be adopted will depend on the study's aim and the area concerned, means, and time available?).

Underwater observations and sampling methods

- Although underwater direct observation by scuba diving (e.g., visual assessments along transects using transects, permanent square frames) is often used for mapping small areas, this method of investigation quickly shows its limits when the study area of study and the depth increase significantly, even if the etechnique assessment can be optimised improved for a general description of the site through the integration with a towed diver or video transects (Cinelli, 2009). 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 square framesquadrates) positioned on the seafloor and located to follow the limits of the habitat. The 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 with respect to the coastline (Bianchi et al., 2004a). Any changes in the habitat and in the substrate typology, within a belt at both sides of the line (considering a surface area of about 1-2 m per side), are is recorded on underwater slates. The information registered allows precise and detailed mapping of the sector studied (Tab. 1).
- 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, whilst to describe while describing the belowground community samples on the bottom become necessary. The surface of a living rhodoliths bed is naturally composed of (of) a variable amount of live thalli and their fragments, lying on a variable varying thickness of dead material and finer sediment. There are 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 substrateum 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, the mean percentage cover of live thalli, live/dead rhodoliths ratio, dominant morphologies of rhodoliths (see Fig. 5), and identification of the most common and volumetrically important species of calcareous algae. In this first description, the need for specialized taxonomists and the time-consuming laboratory analyses are kept to a minimum.
- 25. Recently an innovative tool, namely the BioCube, which is a 1 m high device that enables the acquisition of 80 cm × 80 cm frame photo-quadrates, has been implemented for the characterisation to charecterise of 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 <u>landsea</u>scape for complementary information on demersal fish and extent of assemblages.

26. Sampling methods from vessels involving blind grabs, dredges, and box corers in a number of randomly selected points within a study area can be used to check for the occurrence of deep rhodoliths beds (to ground-truth of 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 m² is recommended because it has the advantage of preserving the original substratetum 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.

26.27. The potential contribution of citizen science networks for mapping and monitoring coralligenous habitat should be mentioned (Gerovasileiou et al., 2017), especially for the assessmentassessing 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://tmednet.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; Cinar et al., 2020).

Remote sensing surveys

27.28. Being the bioconstructed biogenic coralligenous and rhodoliths habitats mainly distributed in deep waters (down to 20-30 m depth), the remote sensing acoustic techniques (e.g., side scan sonar, and multi-beam echosounder) or and the underwater video recordings (through ROVs and, towed cameras) are usually recommended (Georgiadis et al., 2009). The use of remote sensing allows characterising extensive coastal areas for assessment of the 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 distributional bathymetrical ranges, its boundaries, and the total habitat extent can be easily obtained. Acoustic methods are presently the most convenient technique for mapping rhodoliths beds, associated with ground-truthing by ROV and/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 geo-location system, allows monitoring change in the extent of rhodoliths habitat over time (Bonacorsi et al., 2010).

28.29. Visual Oobservations 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), whilest the ROVs have their own propulsion system and are remotely controlled from the surface. The use of towed video cameras (or ROVs) during surveys makes it possible to see the images on the screen in real time, to-identify specific features of the habitat, and to-evaluate any changes in the habitat or in any other characteristic elements of the seafloor. , and T-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 of the areas surveyed. To facilitate and to-improve the results obtained with the camera, joint acquisition modules integrating the depth, images of the seafloor, and geographical positioning have been developed (UNEP/MAP-RAC/SPA, 2015).

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 multi-beam echosounder are usually employed in mapping coralligenous and rhodoliths habitats. All the acoustic mapping techniques are intrinsically affected by uncertainties due to manual classification of the different acoustic signatures of associated towith substrate types on sonograms. Errors in sonograms interpretation may arise when two substrate types are not easily distinguished by the observer. Interpretation of remote

sensing data requires extensive field calibration and the ground-truthing process remains essential. As the interpretation of sonograms is time-requiring, several <u>automatic supervised</u> processing techniques <u>were-have been recently</u> proposed <u>in order</u> to rapidly automate the interpretation <u>and the classification</u> of <u>acoustic signatures sonograms</u> and <u>to make this interpretation more reliable</u> (Montefalcone et al., 2013 and references therein; <u>Viala et al., 2021</u>), also considering that current technology provides systems of neural networks and artificial intelligence to support these operations. These <u>classification</u> methods allow <u>for a-good</u> discrimination between soft sediments and rocky reefs. Human eye, however, always remains the final judge.

Modelling

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 the regional and global scales, in contrast to data on species and habitats data. A recent study showeds the correlation between wind-wave energy at the sea-bottom and the rhodoliths bed presence (Agnesi et al., 2020)., iIt also provideds the confidence interval of this environmental variable associated with the probability to the of rhodoliths beds to occur, probability therefore informing on the wave energy values required for the modelling in the off-shore continents therefore informing on wave energy values reuired for the modelling in the offshore continental shelf (Agnesi et al., 2020). 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 reported defined in the North African coast, for which where there are no available <u>cartographic</u> 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 paucity-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.

30.32. A recent application of predictive spatial modelling was done starting from a complete acoustic coverage of the seafloor together combined with a comparatively low number of seatruthings 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 habitats within the MPA and showed that the distribution of the habitats was mainly driven by the distance from coast, the depth, and the lithotypes. AnoOther examples of habitat predictions can be found in Zapata-Ramírez et al. (2016) and Rossi et al. (2021).

Table 1: Synthesis of the main-survey tools used for defining the Common Indicator 1_Habitat distributional range and extent for coralligenous and rhodoliths habitats. When available, the depth range, the surface area mapped, the spatial resolution, the efficiency (expressed as area mapped in km² per hour), the main advantages or the limits of each tool are indicated, with some bibliographical references.

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Underwater diving 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 km²/hour	 Very great precision for 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)
Transects by towed divers	0 m up to 40 m, according to local rules on scientific diving	Intermediate areas (less than 1 km²)	From 1 to 10 m	0.025 to 0.01 km²/hour	 Easy to implement and possibility of taking pictures Good identification of populations Non destructive and low cost 	 Time-consuming Limited operational depth Highly qualified divers required (safety constraints) Variable geo referencing of the diver route Water transparency 	Cinelli (2009)
Sampling from vessels with blind grabs, dredges, or box corers	0 m to about 50 m (until the lower limit of the rhodoliths habitatbed)	Intermediate areas (a few km ²)	From 1 to 10 m	0.025 to 0.01 km²/hour	 Very great precision for the identification (taxonomy) and distribution of species (micro-mapping) All species taken into accountidentified Possibility of a posteriori identification Low cost, easy to involved. 	 Destructive method Small area inventoried Need of Ssampling materials needed Analyses on samples Work takes a lot of time very timeconsuming Limited operational depth 	UNEP/MAP- RAC/SPA (2015)
Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References

Side scan sonar	8 m to over 120 m (until the lower limit of the coralligenous habitat)	From intermediate to large areas (50-100 km²)	<u><1 m</u>	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)
Side scan sonar	8 m to over 120 m (until the lower limit of the coralligenous habitat)	From intermediate to large areas (50- 100 km²)	From ≤1 m	1 to 4 km²/hour	Wide bathymetric range Realistic representation of the seafloor Good identification of the nature of the bottom and of assemblages (rhodoliths) with location of edges Quick execution Very big mass of data Non destructive	 Flat (2 D) picture to represent 3-D complex habitat Possible errors in sonograms interpretation Acquisition of field data necessary to validate sonograms High cost Not very used for mapping vertical slopes 	CánovasMolina et al. (2016b)
Survey tool	Depth range	Surface area	Resolution	Efficiency	<u>Advantages</u>	<u>Limits</u>	References

Multi-beam 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 centimeteres	0.5 to 6 km²/hour	 Possibility of to obtaining 3-D picturerepresentation 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 imaging recognition of the(nature of the seabed) than side scan sonar Acquisition of field data necessary to validate the sonograms 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 of taking to collect pictures Good identification of habitat and conspicuous species Wide bathymetric range 	• High cost	Cánovas- Molina et al. (2016a); Enrichetti et al. (2019)
Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References

Towed camera	2 m to over	Intermediate_	From 1 m to	0.025 to 1	• Easy to implement and	Limited to homogeneous and	UNEP/MAP-
	120 m (until	<u>large</u> areas (a	10 m	km²/hour	possibility to collect of	horizontal bottoms	RAC/SPA
	the lower limit	fewsome km ²)			taking pictures	• Slow recording and processing	(2015)
	of the				 Good identification of 	of information	I
	coralligenous				habitat and conspicuous	• Variable positioning (geo-	
	habitat)				species	referencing)	I
					Non-destructive	Water transparency	
					Large area covered	• Hard to handle in the case of	
						heavy nautical heavy surface	

Data interpretation

- 31.33. Once the surveying is completed, data collected need to be organized so that they can be used in the future by everyone and can be appropriately archived and easily consulted. A clear definition of all metadata must be provided with the dataset in order toto ensure future integration with similar data from other sources. To produce a habitat map, Ffour important steps for the production of a habitat map must be followed:
 - a. Processing, analysis and classification of the 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 <u>punctual</u> information;
 - d. The map produced must then be evaluated for its accuracy, i.e. its capacity to represent reality, and therefore its reliability.
- 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.racspa.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):

the updated list of benthic marine habitat types for the Mediterranean region¹ should be consulted (UNEP/MAP SPA/RAC, 2019) to recognize any specific habitat type (i.e., coralligenous or rhodoliths) and its main characteristic associations and facies. A description of these habitats and the criteria for their identification are also available in Bellan Santini et al. (2002). Habitats that must be reported on maps are the following (UNEP/MAP SPA/RAC, 2019):

INFRALITTORAL

MB1.5 Infralittoral rock

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

CIRCALITTORAL

MC1.5 Circalittoral rock

¹The updated list of benthic marine habitat types for the Mediterranean region is in a draft stage. It was endorsed by the Meeting of Experts on the finalization of the Classification of benthic marine habitat types for the Mediterranean region and the Reference List of Marine and Coastal Habitat Types in the Mediterranean (Roma, Italy 22–23 January 2019). The draft updated list will be examined by the 14th Meeting of SPA/BD Focal Points (Portoroz, Slovenia, 18–21 June 2019) and submitted to the MAP Focal Points meeting and to the 21st Ordinary Meeting of the Contracting Parties, for adoption.

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 <u>sciaphilic</u> 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 MC1.51b Invertebrate dominated coralligenous

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 MC1.511b Facies with small sponges (sponge ground, e.g. <i>Ircinia</i> spp.)
MC1.512b Facies with large and erect sponges (e.g. Spongia lamella, Sarcotragus foetidus, Axinella spp.)
MC1.513b Facies with Hydrozoa
MC1.514b Facies with Alcyonacea (e.g. Eunicella spp., Leptogorgia spp., Paramuricea spp., Corallium rubrum)
MC1.515b Facies with Ceriantharia (e.g. <i>Cerianthus</i> spp.)
MC1.516b Facies with Zoantharia (e.g. <i>Parazoanthus axinellae</i> , <i>Savalia savaglia</i>)
MC1.517b Facies with Scleractinia (e.g. <i>Dendrophyllia</i> spp., <i>Leptopsammia</i> pruvoti, Madracis pharensis)
MC1.518b Facies with Vermetidae and/or Serpulidae
MC1.519b Facies with Bryozoa (e.g. Reteporella grimaldii, Pentapora fascialis)
MC1.51Ab Facies with Ascidiacea
MC1.51c Invertebrate-dominated coralligenous covered by sediment
See MC1.51b for examples of facies
MC1.52 Shelf edge rock
MC1.52a Coralligenous outcrops
MC1.521a Facies with small sponges (sponge ground)
MC1.522a Facies with Hydrozoa
MC1.523a Facies with Alcyonacea (e.g. Alcyonium spp., Eunicella spp., Leptogorgia spp., Paramuricea spp., Corallium rubrum)
MC1.524a Facies with Antipatharia (e.g. Antipathella
subpinnata)
MC1.525a Facies with Scleractinia (e.g. Dendrophyllia spp., Madracis pharensis)
MC1.526a Facies with Bryozoa (e.g. Reteporella grimaldii, Pentapora fascialis)
MC1.527a Facies with Polychaeta
MC1.528a Facies with Bivalvia
MC1.529a Facies with Brachiopoda
MC1.52b Coralligenous outcrops covered by sediment
See MC1.52a for examples of facies

MC1.521c Facies with Antipatharia (e.g. Antipathella subpinnata) MC1.522c Facies with Alcyonacea (e.g. Nidalia studeri) 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-MC1.523c Facies with Scleractinia (e.g. Dendrophyllia spp.) MC1.531d Facies with Heteroscleromorpha sponges MC2.5 Circalittoral biogenic habitat MC2.51 Coralligenous platforms MC2.511 Association with encrusting Corallinales MC2.512 Association with Fucales MC2.513 Association with non-indigenous Mediterranean Caulerpa spp. MC2.514 Facies with small sponges (sponge ground, e.g. Ircinia spp.) MC2.515 Facies with large and erect sponges (e.g. Spongia lamella, Sarcotragusfoetidus, Axinella spp.) MC2.516 Facies with Hydrozoa MC2.517 Facies with Alcyonacea (e.g. Alcyonium spp., Eunicella spp., Leptogorgia spp., Paramuricea spp., Corallium rubrum) MC2.518 Facies with Zoantharia (e.g. Parazoanthusaxinellae, Savaliasavaglia) MC2.519 Facies with Scleractinia (e.g. Dendrophyllia spp., Madracispharensis, Phyllangiamouchezii) MC2.51A Facies with Vermetidae and/or Serpulidae MC2.51B Facies with Bryozoa (e.g. Reteporella grimaldii, Pentapora fascialis) MC2.51C Facies with Ascidiacea

MC3.5 Circalittoral coarse sediment MC3.5 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

MC3.52 Coastal detritic bottoms with rhodoliths
MC3.521 Association with maërl (e.g. Lithothamnion spp., Neogoniolithon spp., Lithophyllum spp., Spongitesfruticulosa)
MC3.522 Association with Peyssonnelia spp.
MC3.523 Association with Laminariales
MC3.524 Facies with large and erect sponges (e.g. Spongia lamella, Sarcotragusfoetidus, Axinella spp.)
MC3.525 Facies with Hydrozoa
MC3.526 Facies with Alcyonacea (e.g. Alcyonium spp., Paralcyoniumspinulosum)
MC3.527 Facies with Pennatulacea (e.g. Veretillumcynomorium)
MC3.528 Facies with Zoantharia (e.g. Epizoanthus spp.)

MC3.529 Facies with Ascidiacea

- 32.35. The <u>selection of physical layers</u> 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 <u>rhodolith</u> habitats, <u>reducing</u>, <u>as it would reduce</u> 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, <u>and</u> nutrient input <u>for coralligenous</u> and phosphate concentration <u>for coralligenous</u>, geostrophic velocity of sea surface current, silicate concentration, and bathymetry for rhodoliths (Martin et al., 2014).
- 33.36. The data integration and modelling is are often anecessary step because indirect visual or remote sensing surveys from vessels 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 obtain get high resolution maps and therefore interpolation procedures have tomust 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 actual data have been collectedion locations. For elaborating the final distribution map of benthic habitats on a GIS platform, different spatial interpolation tools (e.g., Inverse Distance Weighted, Kriging) can be used and are provided by the GIS software. Even though this is rarely mentioned, it is important to provide information on the number and the percentage of data acquired on field and the percentage of interpolations run.
- 34.37. The processing and digital analysis of acoustic data on GIS allows creating charts where each tonality of grey is associated to-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 3-D distribution and complexity of the coralligenous seascape developing over hard substrates, high quality bathymetric data often constitutes an indispensable and appreciated element.
- 35.38. 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 maps the habitat distributional range (its boundaries and bathymetric limits) and its total extent (expressed in square meters or hectares) can be defined. This ese maps could also be also compared with previous historical available data from literature to evaluate any changes experienced by benthic habitats over a period of time (Giakoumi et al., 2013). Using the overlay vector methods on GIS, a diachronic analysis can be done, where temporal changes are measured in terms of percentage gain or loss of the habitat extension, through the creation of concordance and discordance maps (Canessa et al., 2017).
- 36.39. 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 Guidelines on marine vegetation" in this document for further details). These scales usually take into account 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

- <u>37.40.</u> Monitoring <u>are is</u> necessary for conservation purposes, which require efficient management measures to ensure that marine benthic habitats, their constituent <u>speciesspecies</u>, and their associated communities are and remain in <u>a satisfactorya good</u> 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).
- 38.41. 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 rhodoliths_seabeds are listed among the "special habitatshabitat 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.
- 39.42. 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 rhodoliths habitats, as they being represent two of the main hotspots of biodiversity in the Mediterranean (UNEP/MAP, 2008). The MSFD (2008/56/EC) included both "biological diversity" (D1) and "seafloor integrity" (D6) as descriptors to be evaluated for assessing the GES of the marine environment. In this regard, biogenic structures, such as coralligenous reefs and rhodolith_seabeds, have been recognized as important biological indicators of environmental quality.
- 40.43. A defined and standardized procedure for monitoring the status of coralligenous and rhodoliths 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. <u>MM</u>onitoring over time and <u>data</u> analysis. <u>During these activities</u>, <u>is a step where clear robust</u> scientific competences are needed because the acquired data must be interpreted. Duration of the monitoring, <u>in order</u> to be useful, must be medium time at least.
- 41.44. The objectives of the monitoring are primarily linked with the conservation of bio-constructed genic habitats, but they also answer to the necessity of using them as ecological indicators of the marine environment quality. The main aims of the monitoring programs are generally:
 - Preserve and conserve the heritage of bioconstructions, with the aim of ensuring thatto ensure that coralligenous and rhodoliths habitats are in a satisfactorya good ecological status (GES), and also and identify as early as possible any degradation of these habitats or any changes in their distributional range and extent. Assessment of the ecological status of these habitats allows 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 Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP) during the implementation of the EcAp in the framework of the Mediterranean Action Plan (UNEP/MAP, 2008). The main goal of IMAP is to gather reliable quantitative and updated data on the status of marine and coastal Mediterranean environment.

42. Evaluate effects of any coastal activity and construction likely to impact coralligenous and rhodoliths 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. This type of monitoring aims to establish the condition of the habitat at the time "zero" before the beginning of activities, then monitor the state of health of the habitat during the development works phase or at the end of the phase, to check for any impacts.

45.

43.46. The objective(s) of the monitoring system chosen will influence the choices of the monitoring criteria 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 objectives. The interval of data acquisition could be annual, as most of the typical species belonging to coralligenous assemblages and to rhodoliths beds display slow grow rates and long generation times. In general, and irrespective of the objective advocated, it is judicious to focus initially on a small number of sites that are easily accessible and that can be regularly monitored after short intervals of time. The sites chosen must be: i) representative of the portion of the coastal area investigated, ii) cover most of the possible range of environmental situations (e.g., depth range, slope, substrate type), and iii) include sensitive zones, stable zones, or reference zones with low anthropogenic pressures (i.e., MPAs) 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 control 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.

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

45.48. Following the preliminary definition of the distributional range and extent of coralligenous and rhodoliths habitats (the previous CI1), the assessment of the condition of the two habitats starts with an overall descriptive characterisation of the typical species and assemblages occurring within each habitat. Monitoring of these two habitats basically 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 : it can must only 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). Adoption of Adopting new 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 video recordings, or sampling procedures with dredges, grabs or box corers in the case of rhodoliths seabeds. The acoustic methods that were described above are totally inoperative for detailed characterisations of 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 ies of these techniques must be taken into account 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 species list is not exhaustive but includes species/taxa frequently reported from coralligenous habitat and rhodolithes beds at the Mediterranean scale. Each Contracting Party can regularly improve these lists and chose the most appropriate species/taxa according to its waters geographical situation.

46.49. 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 rhodoliths seabeds that developing 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 cm²). Videos and photographs can then be archived to create temporal datasets.

47.50. 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 (3-D distribution of species 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 to 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.

48.51. For a rough and rapid characterisation of coralligenous assemblages, semi-quantitative evaluations often give sufficient information (Bianchi et al., 2004b); thus, it is possible to estimate the abundance (usually expressed as % cover) by 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 the use of square frames (quadrates) of defined surface or transects (with or without photographs; Piazzi et al., 2018) to collect quantitative data on the assemblages composition. or even tThe sampling by scraping of all the organisms present over a given area for and further laboratory analyses (Bianchi et al., 2004b) represents an alternative. Delestructive procedure by scraping which however should must be avoided beacause of the importance of to preserve coralligenous ation of this habitat. are not usually recommended on coralligenous being a time consuming technique and due to the limited available time underwater. In situ observation and samples must be done over defined and, possibly, 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.

49.52. 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 the 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 substrateum (silted up, roughness, interstices, exposure, slope), the temperature of the water, the vagile fauna associated, the coverage by epibiontaepibiont, 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 km² 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 km² (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/taxaNeed of specialists in taxonomy 	Cánovas-Molina et al. (2016a); Enrichetti et al. (2019); Piazzi et al. (2019b)
Underwater diving 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 Need of specialists in taxonomy 	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 Low cost, 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 diving 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 for 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 	 Need of specialists in taxonomy Small area inventoried Photographs or and video analysis very time-consuming Limited operational depths Highly qualified scientific divers required Tools to collect photos/video necessary Limited number of species/taxa observed Quali-quantitative assessments only on conspicuous species/taxa Only 2-D observation 	Gatti et al. (2015b); Montefalcone et al. (2017); Piazzi et al. (2017a, 2019a); Çinar et al. (2020)
Methods Sampling from vessels with blind grabs, dredges, or box corers	Depth range 0 m to about 120 m (until the lower limit of the rhodoliths habitat)	Intermediate areas (a few km²)	Resolution From 1 to 10 m	Efficiency 0.025 to 0.01 km²/hour	Very good precision for in the identification (taxonomy) and characterisation of the habitat All species-identified taken into account A posteriori identification ELow cost, easy to	Limits Destructive method, usually not recommended Small area inventoried Sampling material needed Samples analysis in laboratory very time-consuming and costly	References UNEP/MAP- RAC/SPA (2015a)

50.53. EAn effective monitoring should be done at defined intervals over a period of time, even if it could mean a reduced number of fewer sites being monitored. The reference "zero-state" will be then—contrasted with data coming from subsequent monitoring periods, always assuring reproducibility of data over time. Thus, the experimental design and protocol has ahave capital importance. The gGeographical position of surveys and sampling stations must be located with precision (using buoys on the surface and recording their coordinates with a dGPS), and it often requires the use of marksing underwater (with fixed pickets into the rock) for positioning the square frames 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 rhodoliths habitats, their long generation time enables sampling to be done at long intervals of time (> 1 year) to monitor them in the long term (Garrabou et al., 2002).

51.54. Although destructive methods (total 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 monitoringsmonitoring (UNEP/MAP-RAC/SPA, 2008), and especially within MPAs. Moreover, identification of all organisms needs great taxonomic expertiseexpertise and a long time to analyse samples, making it difficult to process the large number of replicates required for ecological studies and monitoring surveys. It is more suitable to favour favor non-destructive methods, like photographic sampling. ROV survey, or direct underwater observation in given areas (using square frames quadrates or transects) to collect quali-quantitative data. These methods do not require sampling of organisms and are therefore absolutely appropriateappropriate for long-term monitoring. The Ddifferent methods can be used either separately or together, according to the aims objective of the study, the area inventoried, and means available (Tab. 3). Non-destructive methods are have been increasingly used and, —mainly for video and photographic sampling,—enjoy significant technological advances.

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

In situ samp	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 pho	otography						
Advantages	Objective evaluation, can be reproduced, reference samples, can be automated, speedy diving work, big_large area inventor ied, non-destructive method, no depth_limitations						
Limits	Low taxonomical precision, problem of <i>a posteriori</i> 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						

<u>55.</u> Differently from seagrass, the descriptors used to <u>evaluate the status of</u> 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 programmes, which solved only partially the issues about comparability among data (Fig. 5). However, methods and descriptors

taken into accountconsidered 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).

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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 habitatreefs

52.56. 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 used adopted by in the different ecological indices that have been developed adopted to date on coralligenous reefs, through a single sampling

effort and data analysis. <u>The CIGESMED protocol</u>, <u>applied in different parts of the Mediterranean</u> (David et al., 2014; Cinar et al., 2020), should also be mentioned.

- 53.57. 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.
- 54.58. 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 that than once per year.
- 55.59. 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. In order to 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.
- 56.60. 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.
- 57.61. 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 has tomust be larger than that utilized for shallow Mediterranean rocky habitats (i.e., ≥400 cm²; 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 m². 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

biocoenotic 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 m² located tens of metres apart should be sampled, and a minimum of 10 replicated photographic samples of 0.2 m² each should be collected in each area by scientific divers, for a total sampling surface area of 6 m². This design can be repeated depending on the size of the study site and allows for the analysis of the data through both seascape and biocoenotic approaches (see the 'Ecological Indices' paragraph below).

58.62. Sampling techniques: coralligenous assemblages have been usually studied by destructive methods employing the total scraping of the substrate, by photographic methods associated with determination of taxa and/or morphological groups and by visual census techniques. The best results can be obtained integrating photographic sampling and *in situ* visual observations. The former is the most cost-effective method that requires less time spent underwater and allows collecting the large number of samples required for community analysis in a habitat with high spatial variability at small spatial scales. The latter method, using square frames rames enclosing a standard area of the substrate, has been shown equally effective, but requires longer working time underwater (Parravicini et al., 2010), which may represent a limiting factor at the depths where coralligenous assemblages thrive. A rapid visual assessment (RVA) method has been proposed for a seascape approach (Gatti et al., 2012, 2015a). RVA allows capturing additional information compared with 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.

59.63. Photographic samples analysis: 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 softwares may be useful in order toto reduce the subjectivity of the operator's estimate.

60.64. Descriptors:

- 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 oin photographic samples, as this method showed consistent results with those obtained through underwater techniques—measurements ofing directly—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 m² 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 bioconstructional 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 substratesa 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 m² 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 on the basis of 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 <u>for on</u> each photographic sample. The cover values (in %) of each taxon/morphological group are then classified in eight classes of abundance (Boudouresque, 1971): (1) $0 \text{ to } \le 0.01\%$; (2) $0.01 \text{ to } \le 0.1\%$; (3) $0.1 \text{ to } \le 1\%$; (4) $0 \text{ to } \le 1\%$; (5) $0 \text{ to } \le 1\%$; (6) $0 \text{ to } \le 1\%$; (7) $0 \text{ to } \le 1\%$; (8) $0 \text{ to } \le 100\%$. 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 damicomis, 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 cervicomis	10

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

• Spatial heterogeneity. Coralligenous assemblages are also characterised by a high variability at small spatial scale, and consequently by high values of β -diversity, which is linked to the patchy distribution of the organisms. Under stressed conditions, the importance of biotic factors in regulating an the distribution of organism's distribution 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 on the basis of as the -distance from centroids (Piazzi et al., 2017a) through permutational analysis of multivariate dispersion (PERMDISP). Thus, any changes in the compositional variability displayed by PERMDISP may be directly interpretable as changes of in the β -diversity.

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

61.65. 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.

62.66. 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 high-definition digital camera, a strobe, a high-definition video cameravideo camera, lights, and a 3-jaw grabber. The ROV should also host an underwater acoustic positioning system, a depth sensor, and a compass to obtain georeferenced tracks to be overlapped to multi-beam maps when available. Two parallel laser beams (90° angle) can provide a scale for size reference. In order to 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 m² of bottom surface covered per transect.

63.67. 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 substrateum (rocky reefs and biogenic reefs) and subsequently expressed in m²;
- 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 enidarians, can occasionally invade soft bottoms by settling on small hard debris dispersed in the sedimentary environment. For this reason, typical hard_bottom species (e.g., *Eunicella_verrucosa*) encountered on_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 m²).

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

Protocol for monitoring rhodoliths habitatbeds

65.69. A standardized and common sampling method for monitoring rhodoliths_seabeds is not available to date (UNEP/MAP-RAC/SPA, 2008). Mediterranean rhodoliths_seabeds appear to possess-seem to display more diverse species-assemblages of coralline and peyssonneliacean algale 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 calibrationing to the Mediterranean specificities.

A recent proposal of protocol for monitoring rhodoliths beds can be found in Basso et al. (2016). Monitoring of the rhodoliths 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). However, Ssurveys 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. sampling from vessels using blind grabs, dredges or box corers (Tab. 4). Monitoring should address all the variables already described for the first descriptive characterisation of the habitat, with the addition of the a full quantitative description of the rhodoliths community composition, through periodical surveys, including number of typical or indicator species. A decrease in rhodoliths 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 rhodoliths beds. All possible variations in growth form, shape, and internal structure of rhodoliths have been simplified in a scheme with three major categories as focal points along a continuum: 1) compact and nodular pralines; 2) larger and vacuolar box work rhodoliths; and 3) unattached branches (Fig. 75). 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 substratume instability (mainly due to hydrodynamicswater movement) and sedimentation rate are the most obvious. Thus, the indication of the percentage cover (in %) by the three live rhodoliths categories at the surface of each rhodoliths beds is a proxy of the rhodoliths habitat structural and ecological complexity. The high species diversity hosted by rhodoliths beds requires time-consuming and expensive laboratory analysis for species identification. Videos and photos provideallow for a less fine assessment on the composition information of rhodoliths community composition owing due to the absence of conspicuous, easy-to-detect species. Moreover, since most coralline algal species belong to a few genera only, the use of taxonomic ranks higher than species is not useful.

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

Underwater	visual observation
Advantages	Low cost, results immediately available, non-destructive method, reference samples,
C	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 sampl	ing (dredges, grabs, or and box corers)
Advantages	<u>ELow cost</u> , easy to implement, taxonomical precision, reference samples, analysis of
_	on the substratume (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 to	wed camera
Advantages	Objective evaluation, reference samples (images), large area inventoried, non-
	destructive method, information on the distribution of <u>conspicuous</u> species, large
	depth-range investigated
Limits	High cost, low taxonomical precision, problem of a posteriori interpretation of
	images, observation only of the superficial layers, little information on the
	substrateum and on the basal layer
Use	Studies on distribution and temporal monitoringchange, validation of acoustic
	methods
Acoustic me	thods
Advantages	Very large areas inventoried, information on hydrodynamics water movement
	(sedimentary figures), can be reproduced, non-destructive method, large depth-range
	investigated
Limits	High cost, <u>uncertainties in the interpreting of sonograms interpretations</u> , additional
	validation (inter-calibration), observation only of the superficial layers, no
	taxonomical information
Use	Studies over large spatial scales, monitoring of populations, bionomic studies

67.71. When necessary, for a detailed characterization of rhodolith communities, a minimum of three box-cores with opening ≥0.16 m² should be collected in each rhodoliths 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 rhodoliths area with the highest percentage of live cover (on the basis of 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 rhodoliths bed extension. In many instances grab samples could be useful, but attention must be paid to seafloor surface disruption and mixing, and the possible loss of material during recovery. In those extreme cases of very coarse material preventing box-core penetration and closure, a grab could be used instead, although it cannot preserve stratification. Once the box-core is recovered a colour photograph of the whole surface of the box-core, at a high enough resolution to recognise the morphology of single live rhodoliths and other conspicuous organisms, must be collected. In

addition, the possible occurrence of heavy overgrowths of fleshy algae that may affect rhodoliths growth rate must be reported. The following descriptors must then be assessed: 1) visual estimation of the percentage cover of live red calcareous algae; 2) visual estimation of the live/dead rhodoliths ratio calculated for the surface of the box-core; 3) visual assessment of the rhodoliths morphologies characterising the sample (Fig. 75); 4) measurement of the thickness of the live rhodoliths layer. According to the specific objective of investigation, Tthe sediment sample is-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 should-could be identified and quantified, in order toto allow for the detection of detect variability in space and time, and for any changes after possible impacts. Algal species must be evaluated using a semi-quantitative approach (classes of abundance of algal coverage: absent, 1-20%, 21-40%, 41-60%, 61-80%, >81%). For molecular investigations, samples from voucher rhodoliths morphotypes should be air-dried, and then preserved in silica gel. The sediment sample should be analysed for grain-size (mandatory), and carbonate content.

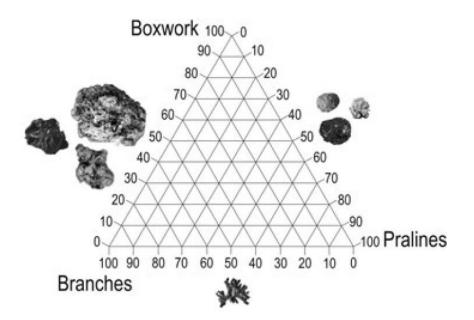


Figure 75: Ternary diagram for the description of the rhodoliths bed tridimensionality. The percentage cover of each rhodoliths morphotype, relative to the total rhodoliths cover, can be plotted on the correspondent axis. The three main rhodoliths morphotypes (box_work_rhodoliths, pralines, and unattached branches) are intended as focal points of a continuum, to which any possible rhodoliths morphology can be approximately assigned. From Basso et al. (2016).

Ecological *i*Indices

68.72. At present, an ecological index to evaluate the status of rhodolith beds has not been proposed yet. On the contrary, Tto 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 have been developed following different approaches and adopt distinct descriptors and sampling techniques, thus hampering the comparison of data and results, and requiring intercalibration 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 adopted needed to apply for each ecological index listed in Table 5 can be found in the relative bibliographic references.

- 69.73. 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 biocoenotic 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.
- 70.74. 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.
- 71.75. COARSE (COralligenous Assessment by ReefScape Estimate; Gatti et al., 2012, 2015a) uses a seascape approach to provide information about the structure of coralligenous reefs in order toto 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.
- 72.76. 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 stateus of coralligenous assemblages.
- 73.77. 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., 20147b, 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 can—is thus be effective in providing information about the alteration of ecological quality of coralligenous reefs. A recent comparison among ESCA, ISLA, and COARSE has also been carried out (Piazzi et al., 2018), which proved that the main differences among indices are linked to the different approaches used, and that with ESCA and ISLA showing theed highest by consistencyt—results—being based on a biocoenotic 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.
- 74.78. Comparatively Ffewer efforts have been made to define-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 by 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 biocoenotic biocenotic complexity of the investigated ecosystem, whereas the Ii describes the its impacts_affecting it. Environmental status is the outcome of the status of benthic communities plus the effects amount (the number?) of impacts (effects) upon them: the integrated MACS index measures the resulting environmental status of deep coralligenous habitats reflecting the combination of the two units and their ecological significance. The MACS index has

been effectively calibrated on 14 temperate mesophotic reefs of the Ligurian and Tyrrhenian seas, all characterised by the occurrence of temperate reefs but and subjected to different environmental conditions and levels of human pressures.

Final remarks

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

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

- Knowledge on coralligenous reefs and rhodoliths_seabeds distribution should be continuously enhanced at the Mediterranean scale, especially in the its Eeastern 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 rhodoliths_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 habitat reefs and based on different approaches.

Index	Method	Image analysis	Descriptors		
<u>Biocoenotic</u> 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 macroinvertebrates 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 macroinvertebrates and macroalgae	2 descriptors: Integrated Sensitivity Level of all species (ISL), i.e. Sensitivity Level to setress (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 <i>in situ</i> 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 <i>in situ</i> 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: <u>Basal layer</u> : % 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 handheld penetrometer (5 replicates) <u>Intermediate layer</u> : specific richness; n° of erect calcified organisms; sensitivity of bryozoans		
			<u>Upper layer</u> : total % cover of species; % of necrosis of each population; maximum height of the tallest specimen		

<u>Index</u>	<u>Method</u>	<u>Image analysis</u>	<u>Descriptors</u>				
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		3 descriptors: Taxa Sensitivity level (TS) to organic matter and sediment input; taxonomic richness of conspicuous taxa that were-are recognizable visually on photo-quadrates and <i>in situ</i> ; 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 <i>in situ</i> 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 <u>water</u> (from <u>about</u> 40 m to about 120 m depth) coralligenous <u>habitat reefs</u> occurring in the <u>shallow</u> 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 10seconds 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 2.1

List of the main species to be considered in the inventorying and monitoring of coralligenous and rhodoliths 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 stictaeforme (J.E.

Areschoug) Hauck, 1877

Lithothamnion sonderi Hauck, 1883

Lithothamnion_philippii_Foslie, 1897

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

Mesophyllum_expansum (Philippi) Cabioch_& M.L. Mendoza, 2003

Mesophyllum_macedonis_Athanasiadis, 1999 Mesophyllum_macroblastum_(Foslie) W.H. Adey, 1970

Neogoniolithon_mamillosum (Hauck) Setchell_& L.R. Mason, 1943

Peyssonnelia rosa-marina Boudouresque & Denizot, 1973

Peyssonnelia polymorpha (Zanardini) F. Schmitz, 1879

Sporolithon_ptychoides Heydrich, 1897

Animal builders

Foraminifera

Miniacina miniacea Pallas, 1766

Bryozoans

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.

Adeonella calveti Canu & Bassler, 1930

Smittina cervicornis Pallas, 1766

Pentapora fascialis Pallas, 1766

Schizoretepora serratimargo (Hincks, 1886) Rhynchozoon neapolitanum Gautier, 1962 Turbicellepora spp.

Polychaeta

Serpula spp.

Protula tubularia (Montagu, 1803)

Spirobranchus polytrema Philippi, 1844

Serpula spp.

Spirorbis sp.

Spirobranchus polytrema Philippi, 1844

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

Hoplangia durotrix Gosse, 1860

Madracis pharensis (Heller, 1868)

Polycyathus muellerae Abel, 1959

Cladocora caespitosa Linnaeus, 1767

Phyllangia americana mouchezii Lacaze-Duthiers, 1897

Dendrophyllia ramea Linnaeus, 1758 Dendrophyllia cornigera Lamarck, 1816

Bioeroders

Sponges

Clionidae (Cliona, Pione)

Echinoids

Echinus melo Lamarck, 1816

Sphaerechinus granularis (Lamarck, 1816)

Molluscs

Rocellaria dubia (Pennant, 1777)

Hiatella arctica Linnaeus, 1767

Lithophaga lithophaga Linnaeus, 1758***

Petricola lithophaga (Retzius, 1788)

Rocellaria dubia (Pennant, 1777)

Polychaetes

Polydora spp.

Dipolydora spp.

Dodecaceria concharum Örsted, 1843 Polydora spp.

Sipunculids

Aspidosiphon (Aspidosiphon) muelleri muelleri Diesing, 1851

Phascolosoma (Phascolosoma) stephensoni Stephen, 1942

Oother relevant species

(*invasive; **disturbed or stressed environmentsusually, when abundant)

Algae

Green algae

Caulerpa cylindracea Sonder, 1845*

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

<u>Codium bursa</u> (Olivi) C. Agardh, 1817** <u>Codium fragile</u> (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

Caulerpa cylindracea Sonder, 1845

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

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

Codium fragile (Suringar) Hariot, 1889*

Codium vermilara (Olivi) Chiaje, 1829**

Brown algae

<u>Acinetospora crinita (Carmichael) Sauvageau, 1899**</u>

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***

<u>Dictyopteris lucida M.A. Ribera Siguán, A. Gómez</u>

<u>Garreta, Pérez Ruzafa, Barceló Martí & Rull Lluch,</u>

2005**

Dictyota spp.**

Halopteris filicina (Grateloup) Kützing, 1843

Cystoseira montagnei var. compressa (Ercegovie)

M. Verlaque, A. Blanfuné, C.F. Boudouresque, T. Thibaut & L.N. Sellam. 2017

Laminaria rodriguezii Bornet, 1888***

Halopteris filicina (Grateloup) Kützing, 1843

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

Stictyosiphon adriaticus Kützing, 1843**

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

Stictyosiphon adriaticus Kützing, 1843**

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

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

Dictyota spp.**

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

Acinetospora crinita (Carmichael) Sauvageau, 1899**

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

Stietyosiphon adriaticus Kützing, 1843**

"Yellow" algae (Pelagophyceae)

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

Red algae

<u>Acrothamnion preissii</u> (Sonder) E.M. Wollaston, 1968*

<u>Asparagopsis taxiformis</u> (Delile) Trevisan de Saint-Léon, 1845*

Cryptonemia lomation (Bertoloni) J. Agardh, 1851

Gloiocladia spp.

Halymenia spp.

Kallymenia spp.

Gloiocladia spp.

<u>Leptofauchea coralligena</u> Rodríguez-Prieto & De <u>Clerck, 2009</u>

<u>Lophocladia lallemandii</u> (Montagne) F. Schmitz, 1893*

Osmundaria volubilis (Linnaeus) R.E. Norris, 1991 Peyssonnelia spp. (non calcareous)

<u>Phyllophora crispa</u> (Hudson) P.S. Dixon, 1964 <u>Ptilophora mediterranea</u> (H.Huvé) R.E. Norris, 1987

Rodriguezella spp.

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

Kallymenia spp.

Halymenia spp.

Sebdenia spp.

Peyssonnelia spp. (non calcareous)

Phyllophora crispa (Hudson) P.S. Dixon, 1964 *Gloiocladia* spp.

Leptofauchea coralligena Rodríguez-Prieto & De Clerck, 2009

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

Lophocladialallemandii (Montagne) F. Schmitz, 1893*

Asparagopsistaxiformis (Delile) Trevisan de Saint-Léon, 1845*

Womersleyella_setacea (Hollenberg) R.E. Norris, 1992*

Animals

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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

<u>Haliclona (Halichoclona) fulva (Topsent, 1893)</u> Hemimycale columella Bowerbank, 1874

Ircinia oros Schmidt, 1864

Ircinia variabilis Schmidt, 1862

Oscarella spp.

Petrosia (Petrosia) ficiformis (Poiret, 1789)

Phorbas_tenaciorTopsent, 1925

Sarcotragus foetidus Schmidt,

1862 fasciculatus (Pallas, 1766)

-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

Eunicella spp.

Leptogorgia sarmentosa Esper, 1789

Madracis pharensis (Heller, 1868)

Ellisella paraplexauroides Stiasny, 1936

Antipathes spp.

Parazoanthus axinellae Schmidt ,1862

Savalia savaglia Bertoloni, 1819***

Callogorgia verticillata Pallas, 1766

Polychaeta

Filograna implexa Berkeley, 1835

Sabella spallanzanii Gmelin, 1791

Filograna implexa Berkeley, 1835

Salmacina dysteri Huxley, 1855

Protula spp.

Bryozoans

Chartella tenella Hincks, 1887

Hornera frondiculata (Lamarck, 1816)***

Margaretta cereoides Ellis & Solander, 1786

Hornera frondiculata (Lamarek, 1816)***

Tunicates

Aplidium spp.

Pseudodistoma cyrnusense Pérès, 1952

Aplidium spp. Cystodytes dellechiajei (Della Valle,

<u>1877)</u>

Halocynthia papillosa Linnaeus, 1767

Herdmania momus (Savigny, 1816)

Microcosmus sabatieri Roule, 1885

Pseudodistoma cyrnusense Pérès, 1952

Halocynthia papillosa Linnaeus, 1767

Molluscs

Cerithium scabridum Philippi, 1848*

Charonia lampas Linnaeus, 1758***

Charonia variegata Lamarck, 1816

Pinna rudis Linnaeus, 1758***

Naria spurca (Linnaeus, 1758)

Cerithium scabridum Philippi, 1848*

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***

Maja squinado Herbst, 1788***

Echinodermata

Antedon mediterranea Lamarck, 1816

Hacelia attenuata Gray, 1840

Centrostephanus longispinus Philippi, 1845***

Diadema setosum (Leske, 1778)*

Echinaster (Echinaster) sepositus (Retzius, 1783)

Hacelia attenuata Gray, 1840

 $Holothuria\ (Panning othuria)\ for skali\ {\tt Delle\ Chiaje},$

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

Algae

Red algae (calcareous)

Lithophyllum cabiochae (Boudouresque et Verlague) Athanasiadis

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

Lithothamnion minervae Basso, 1995

#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

#Peyssonnelia crispate Boudouresque & Denizot, 1975

#Peyssonnelia rosa-marina Boudouresque & Denizot, 1973

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

#Spongites fruticulosa Kützing, 1841

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

Lithophyllum cabiochae (Boudouresque et Verlaque) Athanasiadis

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

Lithothamnion minervae Basso, 1995

Mesophyllum alternans (Foslie) Cabioch & Mendoza, 1998

Mesophyllum expansum (Philippi) Cabioch & Mendoza, 2003

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

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

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

#Peyssonnelia 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

Peyssonnelia heteromorpha (Zanardini) Athanasiadis, 2016

Sporolithon ptychoides Heydrich, 1897

Red algae (non builders non-builders)

#Osmundaria volubilis (Linnacus) R.E. Norris, 1991

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

Peyssonnelia spp. (non calcareous)

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

Alsidium corallinum C. Agardh, 1827

Cryptonemia spp.

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

Gloiocladia microspora (Bornet ex Bornet ex Rodríguez y Femenías) N. Sánchez & C. Rodríguez-

Prieto ex Berecibar, M.J. Wynne, Barbara & R. Santos, 2009

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

Gracilaria spp.

Halymenia spp.

Kallymenia spp.

Leptofauchea coralligena Rodríguez-Prieto & De Clerck, 2009

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

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

#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

Flabellia petiolata (Turra) Nizamuddin, 1987

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

Laminaria rodriguezii Bornet, 1888

Sporochnus pedunculatus (Hudson) C. Agardh, 1817

Acinetospora crinita (Carmichael) Sauvageau, 1899**

Carpomitra costata (Stackhouse) Batters, 1902

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

Cystoseira foeniculacea (Linnaeus) Greville, 1830

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

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

Cystoseira_zosteroides (Turner) C. Agardh, 1821***

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

Dictyota spp.

Halopteris filicina (Grateloup) Kützing, 1843

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.***

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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

Eunicella verrucosa Pallas, 1766

Paramuricea macrospina Koch, 1882

Aglaophenia spp.

Adamsia palliata (Müller, 1776)

Calliactis parasitica Couch, 1838

Cereus pedunculatus Pennant 1777

Cerianthus membranaceus (Gmelin, 1791)

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

Polychaetes

Aphrodita aculeata Linnaeus, 1758

Sabella pavonina Savigny, 1822

Sabella spallanzanii Gmelin, 1791

Bryozoans

Cellaria fistulosa Linnaeus, 1758

Hornera frondiculata (Lamarck, 1816)

Pentapora fascialis Pallas, 1766

Turbicellepora spp.

Tunicates

Aplidium spp.

Ascidia mentula Müller, 1776

Diazona violacea Savigny, 1816

Halocynthia papillosa Linnaeus, 1767

Microcosmus spp.

Phallusia mammillata Cuvier, 1815

Polycarpa spp.

Pseudodistoma crucigaster Gaill, 1972

Pyura dura Heller, 1877

Rhopalaea neapolitana Philippi, 1843

Synoicum blochmanni Heiden, 1894

Echinodermata

Astropecten irregularis Pennant, 1777

Chaetaster longipes (Bruzelius, 1805)
Echinaster (Echinaster) sepositus Retzius, 1783
Hacelia attenuata Gray, 1840
Holothuria (Panningothuria) forskali Delle Chiaje, 1823
Leptometra phalangium Müller, 1841
Luidia ciliaris Philippi, 1837
Ophiocomina nigra Abildgaard in O.F. Müller, 1789
Parastichopus_regalis Cuvier, 1817
Spatangus_purpureus O.F. Müller 1776
Sphaerechinus_granularis Lamarck, 1816
Stylocidaris affinis Philippi, 1845

Pisces

Mustelus_spp.

Pagellus acarne (Risso, 1827)

Pagellus_erythrinus (Linnaeus, 1758)

Raja undulata Lacepède, 1802

Scyliorhinus canicula (Linnaeus, 1758)

Squatina spp.***

Trachinus_radiatus Cuvier, 1829