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Agenda item 5: Parallel CORMON Sessions (Pollution and Marine Litter, and Biodiversity and Fisheries)

Update of Monitoring Protocols on Benthic Habitats

UNEP/MAP
Athens, 2020

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 2019[1] and Rome, Italy, 21 May 2019[2]), 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 protocols provide guidance to national managers and decision makers (e.g., environmental authority representatives, researchers, Marine Protected Area (MPA)'s representatives) with field methodologies for long-term monitoring of biodiversity common indicators, 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 protocols provide information on the monitoring of the agreed biodiversity-reltaed 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 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 and MAP Components to bring them to the attention of respective CORMONs in 2020.

The present document is an update of the monitoring protocols on bethic 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. 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 also consider habitat extent as a relevant attribute and Common Indicator 2: Condition of the habitat's typical species and communities.

The present updated monitoring protocols of marine vegetation and coralligenous and other calcareous bioconstructions is based on the 2019 document (UNEP/MED WG.467/16 Monitoring Protocols for IMAP Common Indicators related to Biodiversity and Non-Indigenous species) and the

[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

recommendations highlighted during the 7th Ecosystem Approach Coordination Group Meeting. The document is organized along two (2) monitoring guidelines:

1. Guidelines for monitoring marine vegetation; and
2. Guidelines for monitoring coralligenous and other calcareous bioconstructions.

Updates are edited in track mode in the text and are related to:

- the use of appropriate monitoring scales (i.e. optimisation of the data collection, sampling sizes and monitoring resolution grid) of marine vegetation taking into consideration national specificities (paragraph 62);
- the consideration of sub-regional specificities in monitoring benthic habitats, particularly regarding the upper and lower limit (Table 4);
- the use of appropriate non-invasive methods for monitoring and assessing the coralligenous habitats (paragraph 26 Page 59; Table 1); and
- the relevance to select the representative species depending to national waters (paragraph 46).

Moreover, the Contracting Parties adopted in 2019 the Updated Classification of Benthic Marine Habitat Types for the Mediterranean Region and the Updated Reference List of Marine Habitat Types for the Selection of Sites to be included in National Inventories of Natural Sites of Conservation Interest in the Mediterranean (Decision IG.24/07).

The adopted lists are aligned with the updated structure of the revised marine component of EUNIS habitats classification. This will enable a coherent use of the proposed lists in national inventories and monitoring programmes as well as a homogenous and adequate assessment of the IMAP Ecological objective 1 (EO1) and respective Common indicators in the whole Mediterranean. An interpretation manual of the Updated Reference List of Marine Habitat Types is under preparation and will complement the present Monitoring Guidelines/Protocols.

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

1. Guidelines for monitoring marine vegetation in Mediterranean

Introduction

1. Seagrass meadows are widely recognized as key habitats in tropical and temperate shallow coastal waters of the world (UNEP-MAP-Blue Plan, 2009). They form some of the most productive ecosystems on earth (McRoy and McMillan, 1977), shaping coastal seascapes and providing essential ecological and economic services (Green and Short, 2003; Vassallo et al., 2013). They support high biodiverse associated communities, primary production and nutrient cycling, sediment stabilization and protection of the littoral, and globally significant sequestration of carbon (Waycott et al., 2009 and references therein). A major 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, are marine flowering plants of terrestrial origin which returned to the marine environment approx. 120 to 100 million of years. The global species diversity of seagrass is low when compared to any other marine Phylum or Division, with less than sixty species throughout the world. However, they form extensive meadows that extend for thousands of kilometres of coastline between the surfaces to about 50 m depth in very clear marine waters or transitional waters (e.g., estuaries and lagoons). In the Mediterranean region five seagrass species occur: *Cymodocea nodosa*, *Halophila stipulacea* (an invasive Lessepsian species), *Posidonia oceanica*, *Zostera marina*, and *Zostera noltei*. The endemic *Posidonia oceanica* is doubtless the dominant and the most import seagrass species (Green and Short, 2003), and the only one able to build a ‘‘matte’’, a monumental construction resulting from horizontal and vertical growth of rhizomes with entangled roots and entrapped sediment (Boudouresque et al., 2006).

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

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

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 sustainable use of marine coastal areas. First step in the prior assessment of the status of any benthic habitat is thus the definition of its geographical distribution and bathymetrical ranges. Seagrass

distribution maps are a fundamental prerequisite to any conservation action on these habitats. The available information on the exact geographical distribution of seagrass meadows is still fragmentary on a regional level (UNEP/MAP-RAC/SPA, 2015) and a few extent of the coastline has been mapped, as only 5 States out of the 21 have a mapped inventory covering at least half of their coasts (UNEP/MAP-Blue Plan, 2009). Within the framework of the Action Plan for the Conservation of Marine Vegetation in the Mediterranean, adopted in 1999 by the Contracting Parties to the Barcelona Convention (UNEP/MAP-RAC/SPA, 1999) and during the implementation evaluation of this Action Plan in 2005 (UNEP/MAP-RAC/SPA, 2005), emerged that very few countries were able to set up adequate and standardized monitoring and mapping programs. As a consequence, and following explicit request by managers on the need of 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 scientific round tables 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 standardization and a clear consensus in the mapping methodology have been reached
- All the methods proposed are usable in all the Mediterranean regions, but some of them are more suitable for a given species (e.g., large-sized species) or particular assemblages (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 most of the Mediterranean monitoring systems are mainly dedicated to *Posidonia oceanica*, there are some programs (e.g., SeagrassNet) that can be used for almost all seagrass species
- Although the existing monitoring methods are similar, the descriptors used to provide information on the state of the system are quite diverse and cover a vast array of ecological complexity levels (i.e., from the plant to the seascape)
- Some descriptors are used by all the Mediterranean scientific communities (e.g., seagrass shoot density, lower limit depth), but the measuring techniques are often very different, and still require a larger effort to reach precise standardization
- The different monitoring methods available in the Mediterranean countries seem all feasible when appropriate training is undertaken.

9. Based on recommendations from the previous CPs group meeting, SPA/RAC has been requested to develop an updated version of the “Guidelines for monitoring marine vegetation in Mediterranean” (UNEP/MAP-RAC/SPA, 2015), in the context of the IMAP common indicators and in order to ease the task of the MPA managers when implementing their monitoring programs. A reviewing process on the scientific literature, taking into account the latest techniques and the recent works carried out by the scientific community at the international level, has 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 MESH (Mapping European Seabed Habitats) project, ended in 2008. The mapping procedure includes different actions (Fig. 1), that can be synthesised into three main steps:

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

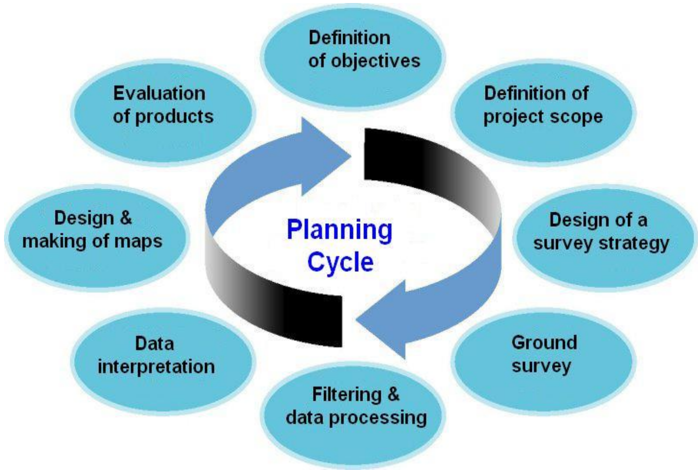

Figure 1: Planning cycle for a habitats' mapping programme (according to the MESH project, 2008).

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

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

13. Processing and data interpretationare 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 precision used and the surface area to be mapped (Mc Kenzie et al., 2001; Fig. 2).

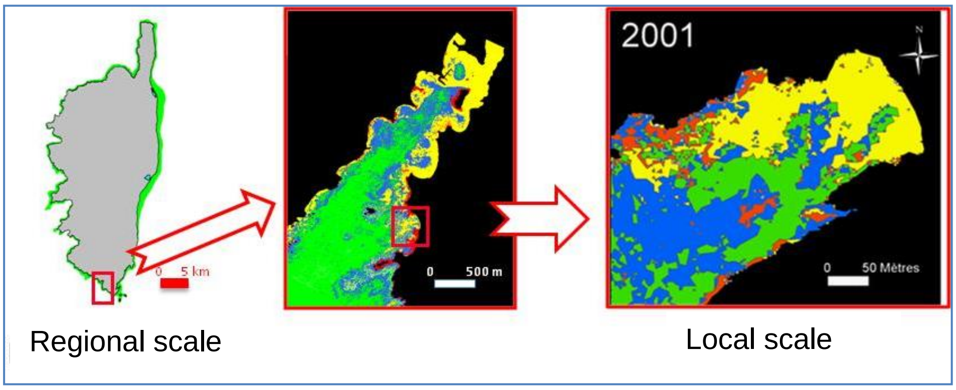

Figure 2: Resolution of a map from regional study to local study (from UNEP/MAP-RAC/SPA, 2015).

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

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

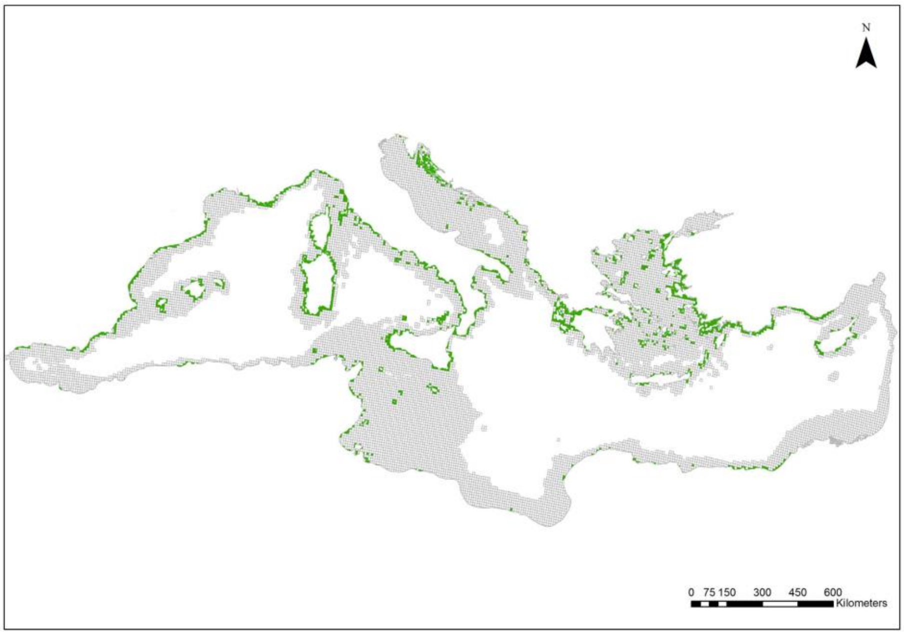

Figure 3: Distribution of *Posidonia oceanica* meadows in the Mediterranean Sea (green areas) (from Giakoumi et al., 2013).

Methods

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

18. The use of remote sensing allows characterising extensive coastal areas for assessment of the spatial patterns of seagrass meadows, and simultaneously can be used to reveal temporal patterns due to the high frequency of the observation. Remote sensing covers a variety of technologies from satellite telemetry, aerial photography, and vessel acoustic systems. The power of remote sensing techniques has been highlighted by Mumby et al. (2004), who highlighted that 20 s of airborne acquisition time would equal 6 days of field surveys. However, all indirect mapping techniques are intrinsically affected by uncertainties due to manual classification of spectral or acoustic signatures of seagrass meadows on the images and sonograms, respectively. Errors in images or sonograms interpretation may arise when two habitat types are not easily distinguished by the observer (e.g., shallow seagrass meadows or dense patch of canopy-forming macroalgae).

Interpretation of remote sensing data requires extensive field calibration and the ground-truthing process remains essential (Pergent et al., 2017). As the interpretation of images/sonograms is also time-requiring, several image processing techniques were proposed in order to rapidly automate the interpretation of sonograms and make this interpretation more reliable (Montefalcone et al., 2013 and references therein). These methods allow a good discrimination between soft sediments and seagrass meadows, between continuous and patchy seagrass, between a dense seagrass meadow and one exhibiting only limited bottom cover. Human eye, however, always remains the final judge.

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

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

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

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Satellite images	From 0 to 10-15 m	From few km^2 to large areas (over 100 km^2)	From 0.5 m	Over 100 km^2/hour	• A global and large-scale coverage of virtually all coastal areas • Availability of free digital images, usable without authorization, from the web (e.g., Google Earth) • High geometric resolution	• Limited to shallow waters characterization • Good weather conditions required (no clouds and no wind) • Possible errors in image interpretation among distinct habitats • Possible errors in image interpretation due to bathymetric variations	Kenny et al. (2003)
Multispectral and/or hyperspectral images	From 0 to 25 m, with an optimum up to 15 m	From 50 km^2 to 5000 km^2	From 1 m		• High resolution allowing 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	• 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	Frederiksen et al. (2004); Kenny et al. (2003); Diaz et al. (2004)
Side scan sonar	Below 8 m	From large to medium areas (50-100 km²)	From 0.1 m	0.8 to 3.5 km²/hour	• Very high resolution • Realistic representation of the seafloor • Good identification of boundaries between populations • Good identification between meadows of different density • Quick execution	• Small patches (smaller than 1 m²) or low-density meadows cannot be distinguished • Loss of definition at image edge, requiring adjustments between adjacent profiles • Possible errors in image interpretation due to large signal amplitude variations (levels of grey)	Paillard et al. (1993); Kenny et al. (2003); Clabaut et al. (2006)
Single-beam acoustic sonar	Below 10 m		From 0.5 m	1.5 km²/hour	• Good geo-referencing • Quick execution	• Low discrimination between habitats • Lower reliability compared to satellite techniques	Kenny et al. (2003); Riegl and Purkis (2005)

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Multi-beam acoustic sonar	Below 2-8 m	From large (50-100 km²) to small areas (a few hundred square meters)	From 50 cm	0.2 km²/hour	• Possibility to obtain 3 D image of meadows • 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 m² to 100 m² for permanent square	From 0.1 m	0.01 km²/hour	• Very high resolution and detail in the information collected • Possibility to identify small structures (patches) and to localize population boundaries • Ground-truthing of the remote sensing data • 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 km²	From 0.1 m	0.2 km²/hour	• Very high resolution • Easy to use • Possibility to record seafloor images for later interpretation	• Long time to gain and process data • Positioning errors due to gap between the vessel position and the camera when towed	Kenny et al. (2003); Diaz et al. (2004)

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

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

1) *Optical data*

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

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

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

Satellite	Resolution	References
LandSat 8	30 m	Dattola et al. (2018)
Sentinel 2A - 2B	10 m	Traganos and Reinartz (2018)
SPOT 5	2.5 m	Pasqualini et al. (2005)
IKONOS (HR)	1.0 m	Fornes et al. (2006)
QuickBird	0.7 m	Lyons et al. (2007)
Geoeyes	0.5 m	Amran (2017)

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

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

25. Although the spatial resolution of satellite imagery has significantlyimproved in the last decade, the data collected is still not sufficient for medium to small coastaldynamics. In particular, resolution of the LandSat 8 satellite is not adequate to have high resolution mappings of seagrass meadows. However, the image LandSat 8 OLI represents a valid 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. 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.

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

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

2) Acoustic data

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

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

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

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

3) Samplings and visual surveys

[1]CASI: Compact Airborne Spectrographic Imager

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

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

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

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

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

Data interpretation

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

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

39. During the processing analysis and classification stage, the updated list of benthic marine habitat types for the Mediterranean region[1] should be consulted (UNEP/MAP-SPA/RAC, 2019) to recognize any specific habitat type (i.e., seagrass species). As seagrass assemblages are often small in size, they can only be identified with high (metric) precision mapping. The updated list identifies the specific “seagrass meadow” habitats that are also listed in the annex of the Habitats Directive (Directive 92/43/EEC), and which must be taken into consideration within the framework of the NATURA 2000 programs. A complete description of these habitats and the criteria for their identification are available in Bellan-Santini et al. (2002). Habitats that must be represented on maps are the following (UNEP/MAP-SPA/RAC, 2019):

LITTORAL

MA3.5 Littoral coarse sediment

MA3.52 Mediolittoral coarse sediment

MA3.521 Association with indigenous marine angiosperms

MA3.522 Association with *Halophila stipulacea*

MA4.5 Littoral mixed sediment

MA4.52 Mediolittoral mixed sediment

MA4.521 Association with indigenous marine angiosperms

MA4.522 Association with *Halophila stipulacea*

MA5.5 Littoral sand

MA5.52 Mediolittoral sands

MA5.521 Association with indigenous marine angiosperms

MA5.522 Association with *Halophila stipulacea*

MA6.5 Littoral mud

MA6.52 Mediolittoral mud

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

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

[1]The 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.

angiosperms (e.g. *Zostera noltei*)

INFRALITTORAL

MB1.5 Infralittoral rock

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

MB1.541 Association with marine angiosperms or other halophyta

MB2.5 Infralittoral biogenic habitat

MB2.54 *Posidonia oceanica* meadows

MB2.541 *Posidonia oceanica* meadow on rock

MB2.542 *Posidonia oceanica* meadow on matte

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

MB2.544 Dead matte of *Posidonia oceanica*

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

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

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

MB5.5 Infralittoral sand

MB5.52 Well sorted fine sand

MB5.521 Association with indigenous marine angiosperms

MB5.522 Association with *Halophila stipulacea*

MB5.53 Fine sand in sheltered waters

MB5.531 Association with indigenous marine angiosperms

MB5.532 Association with *Halophila stipulacea*

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

MB5.541 Association with marine angiosperms or other halophyta

MB6.5 Infralittoral mud sediment

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

MB6.511 Association with marine angiosperms or other halophyta

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

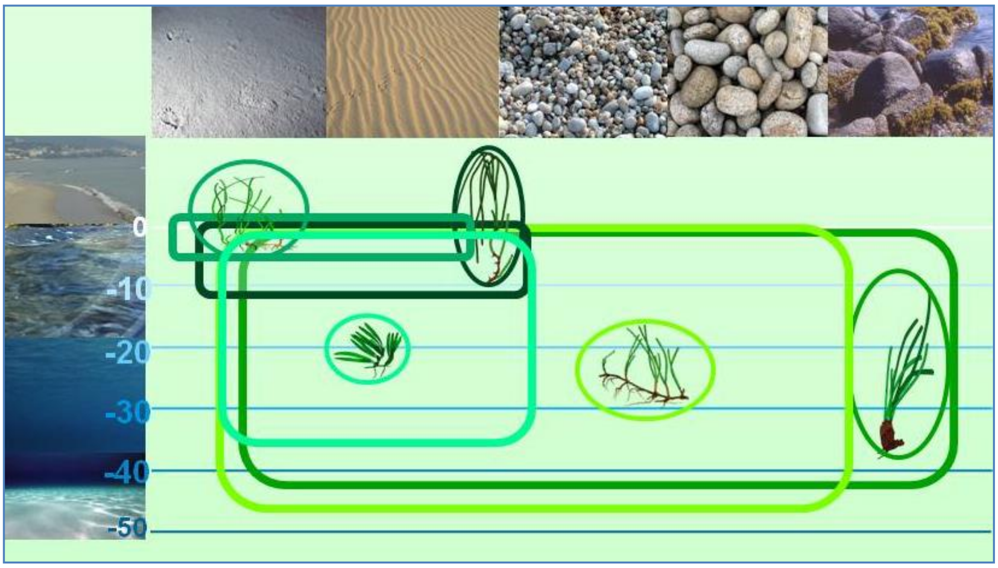

Figure 5: Distribution of seagrass species depending on the nature of the substrate and the depth in the Mediterranean (from UNEP/MAP-RAC/SPA, 2015).

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

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

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

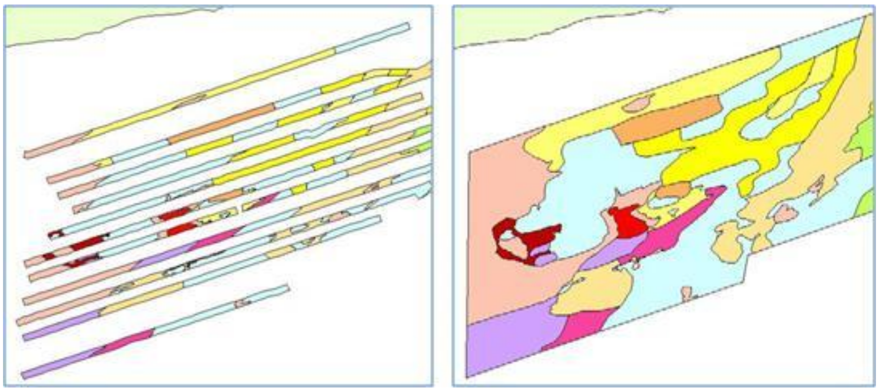

Figure 6: Example of partial coverage survey (left) and the output of the final map produced through interpolation (right). The area surveyed is about 20 km wide (from UNEP/MAP-RAC/SPA, 2015).

44. 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). When the cartographical detail is good enough, it is possible to indicate also the discontinuous meadows that are characterised by a cover below 50% or the two main species that constitute a mixed meadow (the colour of the patches allows identification of the species concerned). To represent some typical forms of *Posidonia oceanica* meadows (e.g., striped, atolls) no specific symbols are available being these forms (bands and circular structures, respectively) easily identifiable on map.

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

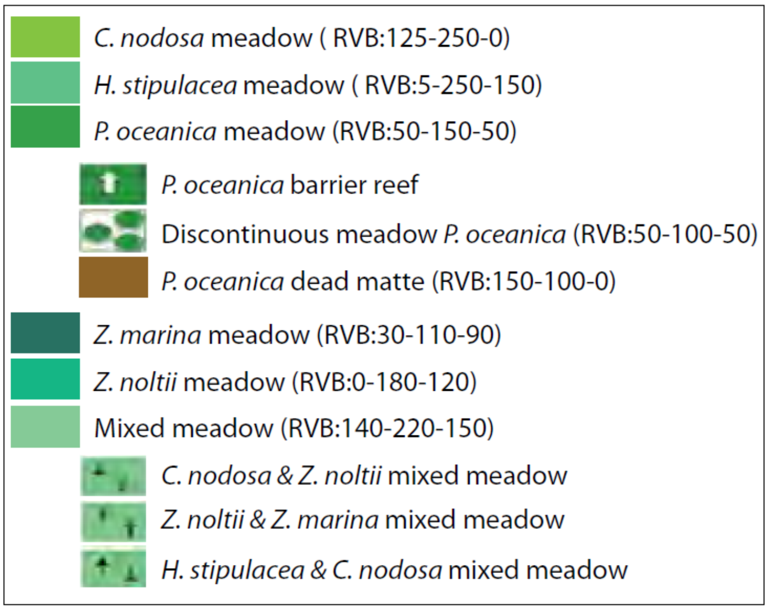

Figure 7: symbols and colours used for the graphic representation of the main seagrass assemblages. RVB: values in red, green and blue for each type of meadow (from UNEP/MAP-RAC/SPA, 2015).

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

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

48. 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

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

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

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

51. 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).

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

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

53. 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.

54. 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 most expensive, including costs for going out to sea during field activities, equipment for sampling, and human resources, especially under difficult weather conditions. Field activities must thus be planned during a favourable season, also because some of the parameters chosen for monitoring purposes must be collected during the same period. This phase might be quite long especially if numerous sites have to be monitored.

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

- Interpret the acquired data

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

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

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

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

58. 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 limits • Descriptors of the state of health of meadow (e.g., cover, shoot density)	• Medium and long term (min. 10 years) • Data acquisition at least annually for non-persistent species and 2-3 years for perennial species
Monitoring environmental quality	Identify the main anthropogenic pressures likely to affect the quality of the environment and initiate monitoring in at least 3 sites, 2 reference/control sites and 1 impacted site, all representative of the coastal area	• Descriptors of the quality of the environment (e.g., turbidity, depth of lower limit, enhancement in nutrients, nitrogen content of leaves, chemical contamination, trace metals in plant)	• Medium term (5 to 8 years) • Data acquisition is variable depending on the species concerned (1-3 years)
Environmental impact assessment	The site subject to coastal development or interventions. The selection of 2 reference/control sites might be also useful	• Specific descriptors to be defined depending on the possible consequences of human activities	• Short term (generally 1-2 years) • Initiate before the impact (“zero” time), it can be continued during, or just after the conclusion. A further control can be made one year after the conclusion

Methods

59. 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 detailed descriptions of sampling tools and methodologies can be found.

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

61. 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 observation and mesure 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 during the initial planning phase (Buia et al., 2004). Not-destructive procedures should be always preferred, especially in the case of protected species (e.g., *Posidonia oceanica*) and when the monitoring is carried out within MPAs. An effective monitoring should be done at intervals over a period of time, even if it could mean a reduced number of sites and a reduced number of descriptors being monitored. Number of adopted descriptors should be adequate enough to avoid errors of interpretation, but sufficiently reduced to ensure permanent monitoring. Simultaneous application of various descriptors working at different ecological complexity levels is the best choice to understand most of the possible responses of the system to environmental alterations (Montefalcone, 2009). The nature of the descriptors is less important than reproducibility, reliability and the precision of the method used for its acquisition.

62. In situ observation 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 (the plant), physiological or cellular, and most of the analyses associated at the community level (the associate organisms of leaves and rhizomes) require collection of shoots. For *P. oceanica*, the mean number of sampled and measured shoots ranges between a minimum of 10 to a maximum of 20 shoots collected at each sampling station (Pergent-Martini et al., 2005). For measuring *P. oceanica* shoot density, a standardized surface area is settled at 40 cm × 40 cm with a minimum of 3 replicated counts per station. To speed the count of shoot density in very dense *P. oceanica* meadows (as usually occur in correspondence of the upper limits), as well as in very sparse meadows (in correspondence of the lower limits), the use of a smaller surface can be adequate, e.g. 20 cm × 20 cm. 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*). An adequate number of sampling stations must be localised randomly within the meadow, and usually in correspondence of the meadow upper limit, the meadow lower limit and at intermediate depth. At each depth, 2 to 3 sampling stations are selected, tens of meters apart. The 3 replicated quadrates in each station must be randomly located when the meadow appears homogeneously covered. On the contrary, in the case of a patchy meadow, quadrates must be positioned randomly using a stratified sampling procedure on the vegetated patches. To assess the overall ecological condition of the meadow, samples of shoots can be performed only at the intermediate meadow depth, which is usually at about 15 m depth, where the meadow is expected to find the optimal conditions for its development (Buia et al., 2004) and during late spring or early summer season (Gobert et al., 2009).

63. 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 in the European countries, the existing absolute scale 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). This scale provides a tool to classify the ecological status of the meadow that can be used in the frame of the IMAP under the EcAp. Evaluating depth and typology of both the upper and the lower limits of the meadow and monitoring over time their positions with permanent marks (i.e., *balises*) are commonly adopted procedures to assess the evolution of the meadow in term of stability, improvement or regression that is linked to water transparency, hydrodynamic regimes, sedimentary balance and human activities along the coastline (Fig. 8). The classification scale of the lower limit depth (Annex 1) is another valid tool to assess the meadow ecological status. Although all the existing absolute scales for *P. oceanica* represent important standardized tools to classify the status of meadows and for the following comparisons among regions, they could require some adaptations according to the specific geographical area and the morphodynamics setting of the site. Can thus be possible that the thereshold values between classes are not valid at the whole Mediterranean scale: subregional and

even local scales can be used (Montefalcone et al., 2007), providing the same methodologies and intercalibration procedures. For instance, in many *P. oceanica* meadows in the Ligurian Sea (NW Mediterranean) the lower limit rarely reaches depths greater than 20-25 m, due to natural constrains (e.g., substrate typology, seafloor topography). Adopting the absolute scale for the lower limit depth, all Ligurian meadows would be classified from moderate to bad ecological status, even in the case of low human pressure.

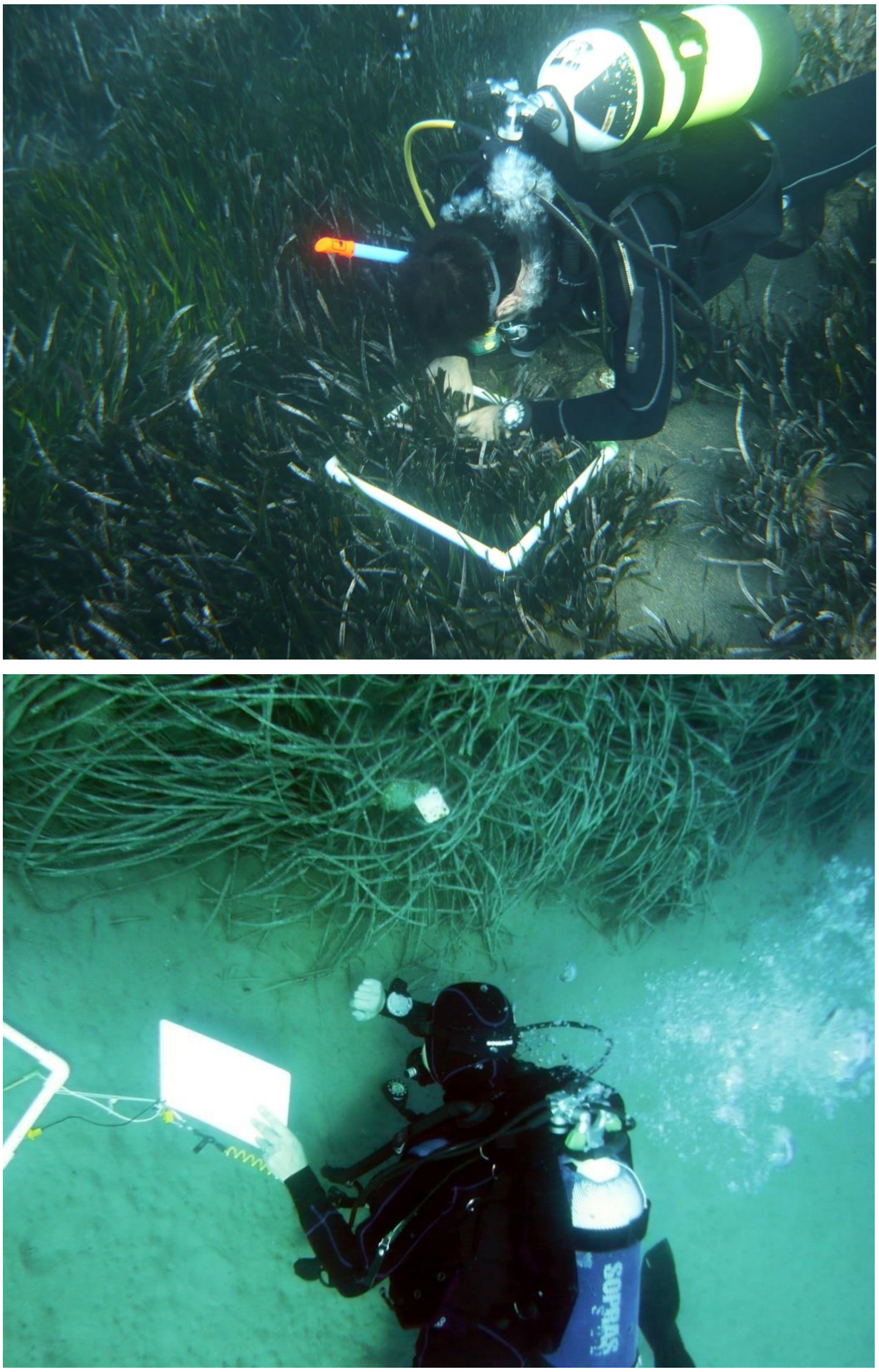

Figure 8: In situ measurement of *Posidonia oceanica* shoot density using the standard square frame of 40 cm × 40 cm (upper image) and monitoring over time of the meadow lower limit position with permanent marks (lower image).

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

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

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

Descriptor	Method	Expected response/factors	Destr.	Target species	Advantages	Limits	References
Density (shoots · m^{-2})	No. of shoots counted within a square frame (a quadrate of fixed dimension) by divers. The square size depends on the seagrass species and on the meadow density. For *P. oceanica* the most adopted sizes are 40 cm × 40 cm and 20 cm × 20 cm	• Reduction • Turbidity, mechanical impacts (e.g., anchoring)	No	All	• Easily measured • Low-cost • Can be measured at all depths • Classification scale available for Po	• Strong variability with depth • Long acquisition time for densities over 800 shoots • Many replicates necessary to evaluate meadow heterogeneity • Considerable risk of error if: a) surveyor is inexperienced; b) high density; c) small sized species. In this latter case in situ counting can be replaced by sampling over a given area and the counting can be done in the lab. (destructive technique)	Duarte and Kirkman (2001); Pergent-Martini et al. (2005); Pergent et al. (2008); Annex 1
Cover (in %)	Average percentage of the surface area occupied (in vertical projection) by meadow in relation to the surface area observed. Various methods to measure the cover in situ by divers or in lab. (photos or video, visual estimation). Variable observation surface area (0.16 to 625 m²), visualised by a quadrate or transparent plate	• Reduction • Turbidity	No	All	• Rapid • On photos, possibility of comparison over time and less errors due to subjectivity • All depths • Estimated also from aerial images or sonograms at large scale	• Strong seasonal and bathymetric variability • Comparison of data obtained using different methods and different observation surface areas is not always reliable due to the fractal nature of cover • Sampling strategy and design must include proper spatial variability • High subjectivity of in situ estimations	Buia et al. (2004); Pergent-Martini et al. (2005); Boudouresque et al. (2006); Romero et al. (2007); Montefalcone (2009)

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

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

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

Descriptor	Method	Expected response/factors	Destr.	Target species	Advantages	Limits	References
Sulphur isotopic relationship ($d^{34}S$ in ‰)	Dosage through mass spectrometer in different plant tissues (e.g., rhizomes of Po)	• Reduction • Human impacts	Yes	Po	• Short response time to environmental changes	• Very expensive • Analytical equipment and specific competence necessary	Romero et al. (2007)
Community							
Epiphytes biomass (in mg dry weight · $shoots^{-1}$ or % dry weight · $shoots^{-1}$) and epiphytes cover (in %) of leaves	Measure of biomass (µg · $shoots^{-1}$) after scraping, drying and weighing. Measure of nitrogen content (in % dry weight). Measure using simple CHN analyser. Estimate the epiphytes cover on leaves under a binocular. Indirect estimation of biomass from epiphytes cover.	• Increase • Nutriments enhancement from rivers, high touristic frequentation	Yes	All	• Easily measured • 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)

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

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

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

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

68. As a final remark, the IMAP should also consider the long-term organic carbon stored in seagrass sediment from both in situ production and sedimentation of particulate carbon from the water column, known as “Blue Carbon” (Nellemann et al., 2009). 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.

69. 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.

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

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

Data processing and interpretation

70. Measurements made in situ must be analyzed and archived. Samples collected during field activities must be properly stored for following laboratory analyses. Data interpretation needs

expert judgment and evaluation and can be made by comparing the measured data with the data available in the literature, either directly or through scales. Checking that the results obtained respond to the monitoring objectives (reliability and reproducibility of the results, valid interpretations and coherence with the observations made) is another important step to validate monitoring effectiveness.

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

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

Ecological indices

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

74. 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 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 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), thus showing little usage in the *P. oceanica* monitoring programs (Pergent-Martini et al., 2005). The Valencian CS index integrates 9 descriptors from individual to community level. The PREI index is based on 5 descriptors working at the population, individual and community levels. The BiPo index is based only on 4 non-

destructive descriptors at the population and individual levels and is particularly well suited for the monitoring of protected species or within MPAs.

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

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

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

78. At the present state of knowledge, it is difficult to prefer one or another of these synthetic indices, as it has not yet been possible to compare all of them over a number of sites and to start 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).

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

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

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

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Annex 1.1

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

<u>Meadow (population level)</u>

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

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

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

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

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

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

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

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

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

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

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

Plant (individual level)

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

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

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

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

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

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

Cell (physiological 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 level): environment contamination

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

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

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

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

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

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

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

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

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

Introduction

1. The calcareous formations of biogenic origin in the Mediterranean Sea are represented by coralligenous reefs, vermetid reefs, cold water corals reefs, *Lithophyllum byssoides* concrations/trottoirs, banks formed by the corals *Cladocora caespitosa*, *Astroides calycularis*, *Phyllangia americana mouchezii*, *Polycyathus muellerae*, reefs formed by the stylasteridae *Errina aspera*, 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 circalittoral zone, built-up by coralline algal frameworks that grow in dim light conditions, for which inventorying and mapping methods, as well as monitoring protocols, still lack of homogeneity and standardization.

Figure 1: Coralligenous habitat. Photos by Simone Musumeci (above) and Monica Montefalcone (below).

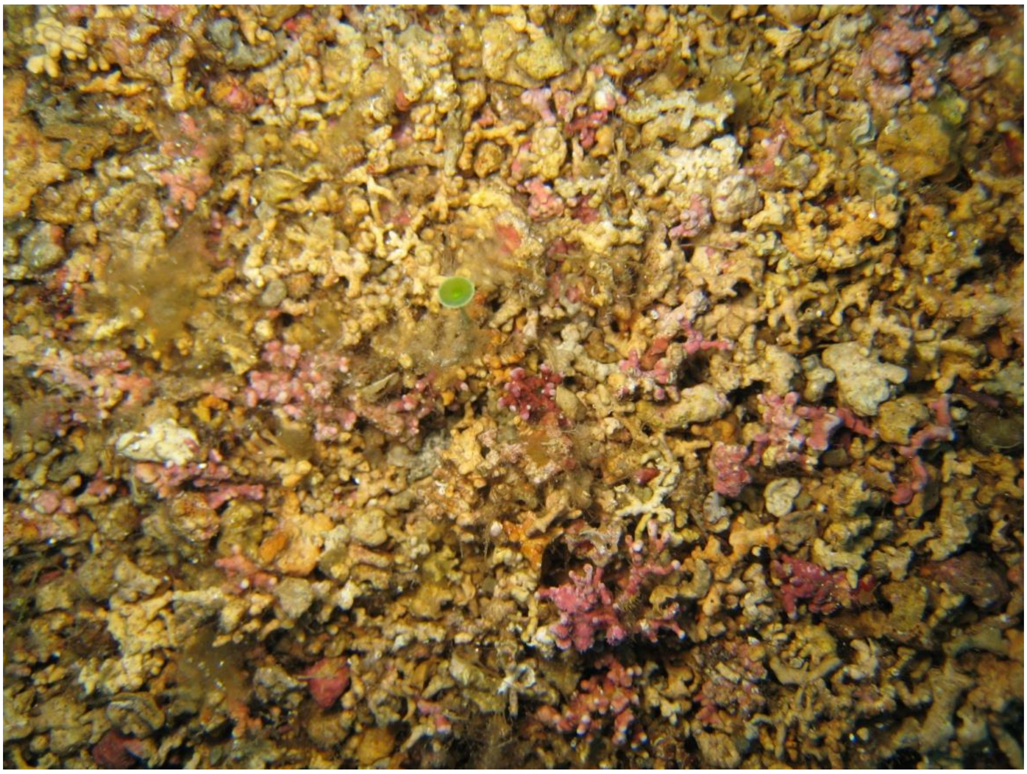

Figure 2: Rhodoliths habitat (photo from UNEP/MAP-RAC/SPA, 2015).

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 biocenosis of the circalittoral zone (Pérès and Picard, 1964). Coralligenous is characterised by high species richness, biomass and carbonate deposition values comparable to tropical coral reefs (Bianchi, 2001), and economic values higher than seagrass meadows (Cánovas-Molina et al., 2014). Construction of coralligenous reefs started during the post-Würm transgression, about 15000 years ago, and develops on rocky and biodetritic bottoms in relatively constant conditions of temperature, currents and salinity.

3. Two main coralligenous typologies can be defined, coralligenous developing on the circalittoral rocks (cliffs or outcrops), and coralligenous developing over circalittoral soft/detritic bottoms creating biogenic platforms (Piazzi et al., 2019b). Coralligenous structure results from the dynamic equilibrium between bioconstruction, mainly made by encrusting calcified Rhodophyta belonging to Corallinales and Peyssonneliales (such as the genera *Lithophyllum, Lithothamnion, Mesophyllum*, *Neogoniolithon*, and *Peyssonnelia*), with an accessory contribution by serpulid polychaetes, bryozoans and scleractinian corals, and destruction processes (by borers and physical abrasion), which create a morphologically complex habitat where highly diverse benthic assemblages develop (Ballesteros, 2006). Light represents the main factor limiting bioconstruction, and coralligenous reefs are able to develop in dim light conditions (<3% of the surface irradiance), from about 20 m down to 120 m depth. Also, the upper mesophotic zone (where the light is still present, from 40 m to 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, creating a biogenic, unstable, three-dimensional habitat typically exposed to bottom currents, which harbours greater biodiversity in comparison to surrounding habitats, and thus viewed as an indicator of biodiversity hotspot. They mostly occur on coastal detritic bottoms in the upper mesophotic zone, between 40-60 m depth (Basso et al., 2016). Rhodoliths are made by slow growing organisms and can be long-lived (>100 years) (Riosmena-Rodríguez and Nelson, 2017). These algae

can display a branching or a laminar appearance, can sometimes grow as nodules that cover all the seafloor, or accumulate within ripple marks. In the literature, the terms rhodoliths and maërl are often used as synonyms (UNEP/MAP-RAC/SPA, 2009). Maërl is the original Atlantic term to identify deposits of calcified non-nucleated algae mostly composed of *Phymatolithon calcareum* and *Lithothamnion corallioides*. Rhodoliths are intended as unattached nodules formed by calcareous red algae and their growths, showing a continuous spectrum of forms with size spanning from 2 to 250 mm of mean diameter. Thus, rhodoliths beds also includes maërl and calcareous *Peyssonnelia* beds, but the opposite is not true (Basso et al., 2016). Rhodoliths bed is recommended as a generic name to indicate those sedimentary bottoms characterised by any morphology and species of unattached non-geniculate calcareous red algae with >10% of live cover (Basso et al., 2016). The name maërl should be restricted to those rhodoliths bed 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), but are vulnerable to either global or local impacts. 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 (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.

6. Despite the occurrence of many species with high ecological value (some of which are also legally protected, e.g. *Savalia savaglia*, *Spongia officinalis*), coralligenous reefs were not listed among the priority habitats defined by the EU Habitat Directive (92/43/EEC), even if they can be included under the habitat “1170 Reefs” of the Directive, and appear also in the Bern Convention. This implies that the most important Mediterranean bioconstruction still remains without formal protection as it is not included within the list of Sites of Community Interest (SCIs). Few years after the adoption of the Habitat Directive, coralligenous reefs were listed among the “special habitats types” needing rigorous protection by the Protocol concerning the special protected areas and biological diversity (SPA/BD) 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 monitoringemphasized. Coralligenous has also been included in the European Red List of marine habitats by IUCN, where it is classified as “data deficient” (Gubbay et al., 2016), thus demonstrating the urgent need for thorough investigations and accurate monitoring plans. In the same year, the Marine Strategy Framework Directive (MSFD, 2008/56/EC) included “seafloor integrity” as one of the descriptors to be evaluated for assessing the Good Environmental State of the marine environment. Biogenic structures, such as 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 adversely affected by rising temperatures and ocean acidification. Two maërl forming species, *Phymatolithon calcareum* and *Lithothamnion corallioides*, are protected under the EU Habitats Directive (92/43/EEC) in the Annex V and, in some locations, maërl is also a key habitat within the Annex I list of habitats of the Directive and therefore is given protection through the designation of Special Areas of Conservation. Moreover, a special plan for the legal protection of Mediterranean rhodoliths has been adopted within the framework of the “Action Plan for the Conservation of Coralligenous and other Mediterranean bio-constructions” (UNEP/MAP-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 the composition (list of species) of these habitats

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

9. Detailed information on habitat geographical distribution and bathymetrical ranges is a prerequisite knowledge for a sustainable use of marine coastal areas. Coralligenous and rhodoliths distribution maps are thus a fundamental prerequisite to any conservation action on these habitats. The scientific knowledge concerning several aspects of biogenic concretions (e.g., taxonomy, processes, functioning, biotic relationships, and dynamics) has been currently increasing, but it is still far away from the knowledge we have from other coastal ecosystems, such as seagrass meadows, shallow coastal rocky reefs, etc. One of the major gaps concerning the current state of knowledge on coralligenous and rhodoliths habitats is the limited spatio-temporal studies on their geographical and depth distribution at regional level and basin-wide scale. This information is essential in order to know the real extent of these habitats in the Mediterranean Sea and to implement appropriate management measures to guarantee their conservation (UNEP/MAP-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 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 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 coralligenous 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 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 scuba diving is often used for mapping small areas, it becomes unsuitable when the study area and/or the depth increase (usually at depths >40 m)
- The use of acoustic survey methods (side scan sonar or multibeam) or underwater observation systems (ROV, towed camera) becomes then necessary. However, acoustic techniques must be always integrated and verified by a large number of "field" underwater data.

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 mainly on scuba diving but given the above listed constraints, using other tools of investigation (e.g., ROV, towed camera) should be also considered because it allows monitoring with less precision but on larger areas
- Although the use of underwater photograph or video may be relevant, the use of specialists in taxonomy with a good experience in scuba diving is often essential given the complexity of these habitats. If it is possible to estimate the abundance or coverage by 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 necrosis are other factors to be considered

- Monitoring of coralligenous habitat starts with the realisation of micro-mapping and then the application of descriptors and/or ecological indices. However, these descriptors vary widely from one team to another, as well as their measurement protocol
- Monitoring of rhodoliths habitats can be done by scuba diving, but the observation using ROVs or towed cameras and the collection of samples using dredges, grabs or box corers are privileged because of the greater homogeneity of these populations. However, there is not yet any standardized method widely accepted to date for monitoring rhodoliths, also because the action of hydrodynamics 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 implementation and based on the recommendations of the Meeting of the Ecosystem Approach Correspondence Group on Monitoring (CORMON), Biodiversity and Fisheries (Madrid, Spain, 28 February – 1 March 2017), the CPs requested SPA/RAC to develop standardized monitoring protocols by considering the previous work elaborated in the Guidelines for monitoring coralligenous and other bioconstructed habitats in Mediterranean (UNEP/MAP-RAC/SPA, 2015), 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 scientific literature, taking into account the latest techniques and the recent works carried out by the scientific community at the international level, has also been carried out. If standardized protocols for seagrass mapping and monitoring exist and are well-implemented, and a number of ecological indices have already been validated and inter-calibrated among different regions, this is not the case for coralligenous and rhodoliths habitats. In this document a number of “minimal” descriptors to be taken into account for inventorying and monitoring the coralligenous and rhodoliths populations in the Mediterranean are described. The main methods adopted for their monitoring, with the relative advantages, restrictions and conditions of use, are presented. Some of the existing monitoring methods for coralligenous have already been compared or cross-calibrated and are here briefly introduced and, finally, a standardized method recently proposed for coralligenous monitoring is described.

Monitoring methods

a) COMMON INDICATOR 1: Habitat distributional range and extent

Approach

14. The CI1 is aimed at providing 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 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 require 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 are often only indicative. When smaller areas have to be mapped, a much higher precision and resolution level is required and is easily achievable, thanks to the high-resolution mapping techniques available to date. However, obtaining detailed maps is costly, thus practically impossible when time or resources are limited (Giakoumi et al., 2013). These detailed maps provide an accurate localisation of the habitat distribution and a precise definition of its extension limits and total habitat extent, all features necessary for future control and monitoring purposes over a period of time. These high-resolution scales are also used to select remarkable sites where monitoring actions must be concentrated.

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

17. Although we have an overall knowledge about the composition and distribution 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 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 years in some countries thanks to the new monitoring activities afferent to the MSFD, and this information will become available in the coming years.

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

19. Knowledge on maërl seabeds was somewhat limited compared to what is available for coralligenous. Maërl habitats cover a surface area of about 1654 km^2. Only sporadic and punctual information are available, mainly from the North Adriatic, the Aegean Seas and the Tyrrhenian Sea. Datasets are available for Greece, France (Corsica), Cyprus, Turkey, Spain, and Italy. Malta and

Corsica, in particular, have significant datasets for this habitat as highlighted by fine-scale surveys in targeted areas (Martin et al., 2014).

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

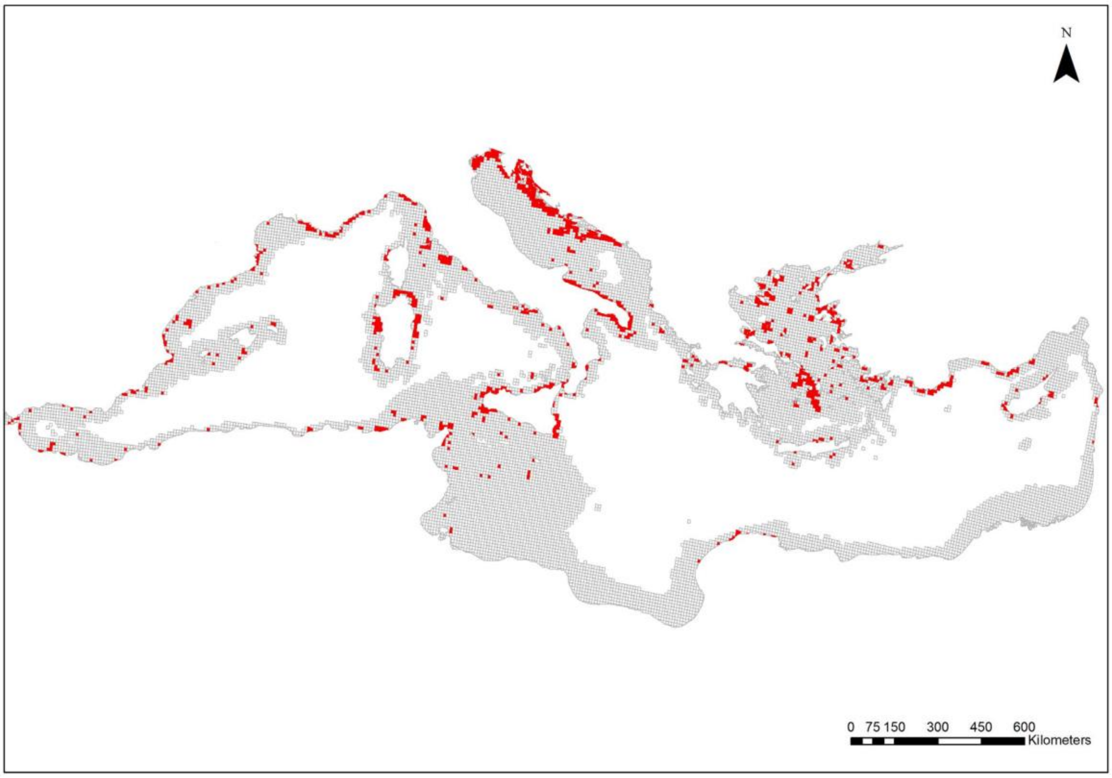

Figure 3: Distribution of coralligenous habitats in the Mediterranean Sea (red areas) (from Giakoumi et al., 2013).

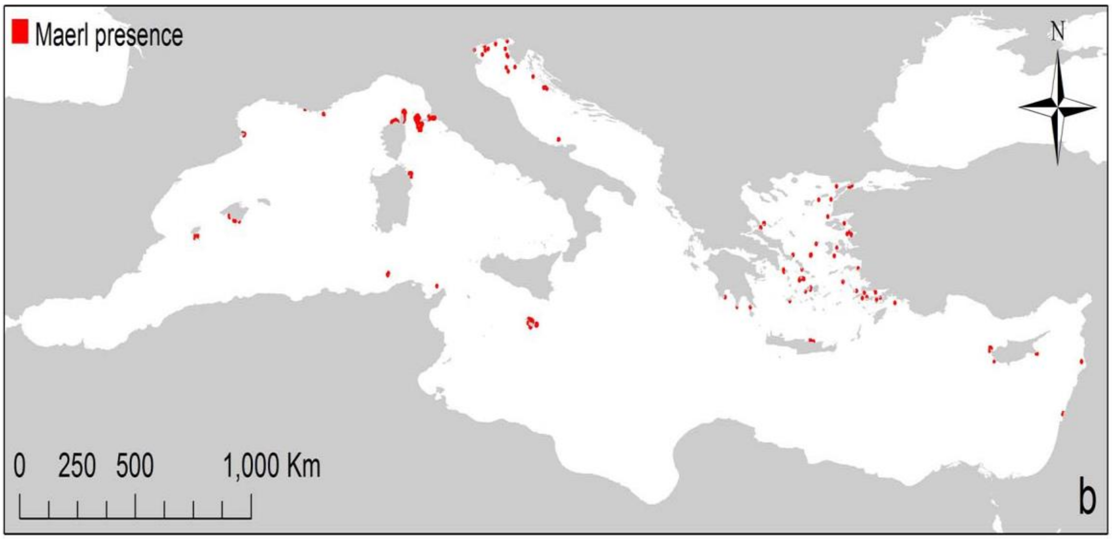

Figure 4: Distribution of maërl habitats in the Mediterranean Sea (red areas) (from Martin et al., 2014).

Methods

21. Definition of distributional range and extent of coralligenous and rhodoliths habitats requires “traditional” habitat mapping techniques, similar to those used for seagrass meadows in deep waters (Tab. 1). Indirect instrumental mapping techniques and/or direct field visual surveys can be usedand are often integrated. The simultaneous use of two or more 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 and the area concerned, means and time available.

Underwater observations and sampling methods

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

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 scientific diving (Tab. 1). Surveys can be done along lines (transects), or over small surface areas (permanent square frames) positioned on the seafloor and located to follow the limits of the habitat. The transect consists of 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 recorded on underwater slates. The information registered allows precise and detailed mapping of the sector studied (Tab. 1).

24. Scuba diving is also suggested as a safe and cost-effective tool to obtain a visual description and sampling of shallow rhodoliths beds up to 30-40 m depth, according to local rules for scientific diving (Tab. 1). Underwater observations are effective for a first characterisation of the aboveground facies of this habitat, whilst to describe the belowground community samples on the bottom become necessary. The surface of a living rhodoliths bed is naturally composed of a variable amount of live thalli and their fragments, lying on a variable thickness of dead material and finer sediment. There are no literature data about the required minimum spatial extent for a portion of the seafloor to be defined as a rhodoliths bed. A rhodoliths bed is defined as a habitat that is distinguished from the surrounding seafloor by having >10% of the mobile substratum covered by live calcareous coralline algae as unattached branches and/or nodules (Basso et al., 2016). Live rhodoliths beds are naturally accompanied by a variable quantity of dead rhodoliths and their fragments; thus, a threshold of >50% of the surface 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 range, areal extent, occurrence of sedimentary structures of the seafloor (such as ripples, mega-ripples, and underwater dunes), thickness of live layer, the mean percentage cover of live thalli, live/dead rhodoliths ratio, dominant morphologies of rhodoliths (see Fig. 5), and identification of the most common and volumetrically important species of calcareous algae. In this first description, the need for specialized taxonomists and 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 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 landscape 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 (ground-truth of acoustic data) and for a complete description of the habitat (Tab. 1). The thickness of the live cover could be measured through the transparent or removable side of a box-corer. Alternatively, a sub-sample could be taken from the recovered box-core using a Plexiglas core of about 10 cm in diameter and at least 20 cm long. Box-coring with a cross-section ≥0.16 m^2 is recommended because it has the advantage of preserving the original substratum stratification. The use of desctructive sampling methods from vessels for characterizing rhodoliths beds should be, however, as much as possible discouraged, in order to minimize the impact of the investigation.

Remote sensing surveys

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

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

29. Sonar provides images of the seafloor through the emission and reception of ultrasounds. Amongst the main acoustic mapping techniques available (Kenny et al., 2003), wide acoustic beam systems like the side scan sonar (SSS) and multi-beam echosounder are usually employed in mapping coralligenous and rhodolithshabitats. All the acoustic mapping techniques are intrinsically affected by uncertainties due to manual classification of the different acoustic signatures of substrate types on sonograms. Errors in sonograms interpretation may arise when two substrate types are not easily distinguished by the observer. Interpretation of remote sensing data requires extensive field calibration and the ground-truthing process remains essential. As the interpretation of sonograms is time-requiring, several processing techniques were proposed in order to rapidly automate the interpretation of sonograms and make this interpretation more reliable (Montefalcone et al., 2013 and references therein), also considering that current technology provides systems of neural networks and artificial intelligence to support these operations. These methods allow a good

discrimination between soft sediments and rocky reefs. Human eye, however, always remains the final judge.

Modelling

30. Modelling techniques can be used to fill the gaps in the knowledge of the spatial distribution of habitats by predicting the areas that are likely to be suitable for a community to live. Models are usually based on physical and environmental variables (e.g., water temperature, salinity, depth, nutrient concentrations, seabed types), which are typically easier to record and map at the regional and global scales, in contrast to species and habitat data. Despite inherent limitations and associated uncertainties, predictive modelling is a cost-effective alternative to field surveys as it can help identifying and mapping areas where sensitive marine ecosystems may occur. Based on the spatial datasets available for coralligenous and rhodolith 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 in the North African coast, for which there are no available 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 of occurrence data for this habitat across the Mediterranean, and especially in the North African coast, the model output is relatively informative in highlighting several suitable areas where no data are available to date.

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

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

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Underwater diving	0 m up to 40 m, according to local rules on scientific diving	Small areas, less than 250 m^2	From 0.1 m	0.0001 to 0.001 km²/hour	• Very great precision for the identification (taxonomy) and distribution of species (micro-mapping) • Non-destructive • Low cost, easy to implement	• Small area inventoried • Very time-consuming • Limited operational depth • Highly qualified 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 km^2)	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 scientific divers required (safety constraints) • Variable geo-referencing of the diver route • Water transparency	Cinelli (2009)
Sampling from vessels with blind grabs, dredges or box corers	0 m to about 50 m (until the lower limit of the rhodoliths habitat)	Intermediate areas (a few km^2)	From 1 to 10 m	0.025 to 0.01 km²/hour	• Very great precision for the identification (taxonomy) and distribution of species (micro-mapping) • All species taken into account • Possibility of a posteriori identification • Low cost, easy to implement	• Destructive method, usually not recommended • Small area inventoried • Sampling material needed • Work takes a lot of time • Limited operational depth	UNEP/MAP-RAC/SPA (2015)

Survey tool	**Depth range**	**Surface area**	**Resolution**	**Efficiency**	**Advantages**	**Limits**	**References**
Side scan sonar	8 m to over 120 m (until the lower limit of the coralligenous habitat)	From intermediate to large areas (50-100 km²)	From 1 m	1 to 4 km²/hour	• Wide bathymetric range • Realistic representation of the seafloor • Good identification of the nature of the bottom and of assemblages (rhodoliths) with location of edges • Quick execution • 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	Cánovas-Molina et al. (2016b)
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 centimeters	0.5 to 6 km²/hour	• Possibility of obtaining 3-D picture • Double information collected (bathymetry and seafloor image) • Very precise and wide bathymetric range • Quick execution • Very big mass of data • Non-destructive	• Less precise imaging (nature of bed) than side scan sonar • Acquisition of field data necessary to validate sonograms • High cost	Cánovas-Molina et al. (2016b)
Remote Operating Vehicle (ROV)	2 m to over 120 m (until the lower limit of the coralligenous habitat)	Small-intermediate areas (a few km²)	From 1 m to 10 m	0.025 to 0.01 km²/hour	• Non-destructive • Possibility of taking pictures • Good identification of habitat and species • Wide bathymetric range	• 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 120 m (until the lower limit of the coralligenous habitat)	Intermediate areas (a few km^2)	From 1 m to 10 m	0.025 to 1 km^2/hour	• Easy to implement and possibility of taking pictures • Good identification of habitat and species • Non-destructive • Large area covered	• Limited to homogeneous and horizontal bottom • Slow recording and processing of information • Variable positioning (geo-referencing) • Water transparency • Hard to handle in heavy surface traffic	UNEP/MAP-RAC/SPA (2015)

Data interpretation

32. 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 to ensure future integration with similar data from other sources. Four important steps for the production of a habitat map must be followed:

a. Processing, analysis and classification of the biological data, 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 information
d. The map produced must then be evaluated for its accuracy, i.e. its capacity to represent reality, and therefore its reliability.

33. During the processing analysis and classification step, the updated list of benthic marine habitat types for the Mediterranean region[1] 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

MC1.51 Coralligenous

MC1.51a Algal-dominated coralligenous

MC1.511a Association with encrusting Corallinales

MC1.512a Association with Fucales or Laminariales

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

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

MC1.51b Invertebrate-dominated coralligenous

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

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

MC1.513b Facies with Hydrozoa

[1] The 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.

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

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

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

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

MC1.518b Facies with Vermetidae and/or Serpulidae

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

MC1.51Ab Facies with Ascidiacea

MC1.51c Invertebrate-dominated coralligenous covered by sediment

See MC1.51b for examples of facies

MC1.52 Shelf edge rock

MC1.52a Coralligenous outcrops

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

MC1.522a Facies with Hydrozoa

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

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

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

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

MC1.527a Facies with Polychaeta

MC1.528a Facies with Bivalvia

MC1.529a Facies with Brachiopoda

MC1.52b Coralligenous outcrops covered by sediment

See MC1.52a for examples of facies

MC1.52c Deep banks

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

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

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

MC1.531d Facies with 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*, *Sarcotragus foetidus*, *Axinella* spp.)

MC2.516 Facies with Hydrozoa

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

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

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

MC2.51A Facies with Vermetidae and/or Serpulidae

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

MC2.51C Facies with Ascidiacea

MC3.5 Circalittoral coarse sediment

MC3.52 Coastal detritic bottoms with rhodoliths

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

MC3.522 Association with *Peyssonnelia* spp.

MC3.523 Association with Laminariales

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

MC3.525 Facies with Hydrozoa

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

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

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

MC3.529 Facies with Ascidiacea

34. The selection of physical layers to be shown on maps and to be used for following predictive statistical analyses may be an interesting approach within the general framework of mapping coralligenous and rhodolith habitats, as it would reduce 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, and nutrient input for coralligenous and phosphate concentration, geostrophic velocity of sea surface current, silicate concentration, and bathymetryfor rhodoliths (Martin et al., 2014).

35. The data integration and modelling is often a necessary 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 high resolution maps and therefore interpolation procedures have to be used, so that from partial surveys a lower resolution map can be obtained. Spatial interpolation is a statistical procedure for estimating data values at unsampled sites between actual data collection

locations. For elaborating the final distribution map of benthic habitats on a GIS platform, different spatial interpolation tools (e.g., Inverse Distance Weighted, Kriging) can be used and are provided by the GIS software. Even though this is rarely mentioned, it is important to provide information on the number and the percentage of data acquired on field and the percentage of interpolations run.

36. The processing and digital analysis of acoustic data on GIS allows creating charts where each tonality of grey is associated to a specific texture representing a type of habitat or substrate, also on the basis of the *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.

37. 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 and its total extent (expressed in square meters or hectares) can be defined. These maps could be also compared with previous historical available data from literature to evaluate any changes experienced by benthic habitats over a period of time (Giakoumi et al., 2013). Using the overlay vector methods on GIS, a diachronic analysis can be done, where temporal changes are measured in term of percentage gain or loss of the habitat extension, through the creation of concordance and discordance maps (Canessa et al., 2017).

38. 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 on marine vegetation in this document for further details). These scales usually take into account the processing of sonograms, the scale of data acquisition and restitution, the methods adopted, and the positioning system.

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

Approach

39. Monitoring are necessary for conservation purposes, which require efficient management measures to ensure that marine benthic habitats, their constituent species and their associated communities are and remain in a satisfactory ecological status. The good state of health of both coralligenous and 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).

40. 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 habitats types" needing rigorous protection by the Protocol concerning the Specially Protected Areas and Biological Diversity in the Mediterranean (SPA/BD) of the Barcelona Convention

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

42. 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 monitor during the same season
c. Monitoring over time and analysis is a step where clear scientific competences are needed because the acquired data must be interpreted. Duration of the monitoring, in order to be useful, must be mediumtime at least.

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

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

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

44. The objective(s) chosen will influence the choices of the monitoring criteria in the following steps (e.g., duration, sites to be monitored, descriptors, and sampling methods). The duration of the monitoring should be at least medium-long term (minimum 5-10 years long) for heritage conservation and monitoring environmental quality objectives. The interval of data acquisition could be 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 areas with high pressure related to human activities. Then, with the experience gained by the surveyors and the means (funds) available, this network could be extended to a larger number of sites. For environmental impact assessment, short term monitoring (generally 1-2 years) is recommended and should be initiated before the interventions ("zero" time), and possibly continued during, or just after the conclusion of the works. A further control can be made one year after the conclusion. The ecological status of the site subjected to coastal interventions (i.e. the impact site) must be contrasted with the status of at least 2 reference/control sites.

45. 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).

Methods

46. 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, although this technique gives rise to many operational constraints due to the conditions of the environment in which these habitats develop (great depths, weak luminosity, low temperatures, presence of currents, etc.): it can only be done by confirmed and expert scientific divers (for safety), within a limited range of depths (from the surface down to maximum depths of 30-40 m, according to local rules on scientific diving), and over a limited underwater time (Bianchi et al., 2004b; Tetzaff

and Thorsen, 2005). Adoption of new investigation tools (e.g., ROVs) allows for a less precise assessment but over larger spatial scales. A first characterisation of the habitat (species present, abundance, vitality, etc.) can be done by direct visual underwater inspections, indirect ROVs or towed camera video recordings, or sampling procedure with dredges, grabs or box corers in the case of rhodoliths seabeds. The acoustic methods that were described above are totally inoperative for detailed characterisations of these habitats, especially for coralligenous. The survey method depends greatly on the scale of the work and the spatial resolution requested (Tab. 2). The complementarities of these techniques must be taken into account when planning an operational strategy (Cánovas-Molina et al., 2016b). A list of the main conspicuous species/taxa or morphological groups recognisable underwater, or on images, is presented in the Annex 1. This species list is not exhaustive but includes species frequently reported from coralligenous habitat and rhodolites beds at the Mediterranean scale. Each Contracting Party can regularly improve these lists and chose the most appropriate species according to its waters.

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

48. 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 and high biodiversity), divers must be specialists in taxonomy of the main coralligenous species to ensure the validity of the information recorded underwater. Photographs or video collected with underwater cameras can be usefully integrated to visual survey to speed the work (Gatti et al., 2015a). The use of operational taxonomical units (OTUs), or taxonomic surrogates such as morphological groups (lumping species, genera or higher taxa displaying similar morphological features; Parravicini et al., 2010), may represent a useful compromise when a consistent species distinction is not possible (either underwater or on photographs) or to reduce the surveying/analysis time.

49. 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 quality and fine characterisation of the assemblages often requires the use of square frames (quadrates) or transects (with or without photographs; Piazzi et al., 2018) to collect quantitative data on assemblages composition, or even the sampling by scraping of all the organisms present over a given area for further laboratory analyses (Bianchi et al., 2004b). Destructive procedures by scraping are not usually recommended on coralligenous being a time-consuming technique and due to the limited available time underwater. In situ observation and samples must be done over defined and, possibly, 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.

50. 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, gorgonians) and signs of necrosis are important elements to be taken into consideration (Garrabou et al., 1998, 2001; Gatti et al., 2012). Finally, the nature of the substratum (silted up, roughness, interstices, exposure, slope), the temperature of the water, the vagile fauna associated, the coverage

by epibionta and the presence of invasive species must also be considered to give a clear characterisation of bioconstructed habitats (Harmelin, 1990; Gatti et al., 2012).

Methods	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Remote Operating Vehicle (ROV)	From 2 m to over 120 m	Small-Intermediate areas of about 1 km^2	From 1 m to 10 m	0.025 to 0.01 km^2/hour	• Non-destructive method • Possibility of taking pictures • Wide bathymetric range • Good identification of facies and associations • Possibility of semi-quantitative/quantitative evaluation	• Need of specialists in taxonomy • High cost, major means out at sea • Difficulty of observation and access according to the complexity of the habitat (multilayer assemblages) • Quali-quantitative assessments only on conspicuous species/taxa	Cánovas-Molina et al. (2016a); Enrichetti et al. (2019); Piazzi et al. (2019b)
Underwater diving 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 km^2/hour	• Non-destructive • Very good precision for the identification (taxonomy) and characterisation of the habitat (also its 3-D) • Low cost, easy to implement • Possibility to collect samples • Data already available after dive	• Need of specialists in taxonomy • Small area inventoried • Very time-consuming underwater • Limited operational depths • Highly qualified scientific divers required • Subjectivity of the observer • Quali-quantitative assessments only on conspicuous species/taxa	Gatti et al. (2012, 2015a); Piazzi et al. (2019a)
Methods	**Depth range**	**Surface area**	**Resolution**	**Efficiency**	**Advantages**	**Limits**	**References**
Underwater diving sampling by	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 km^2/hour	• Very good precision in the identification (taxonomy) and characterisation of the habitat	• Destructive method, usually not recommended • Very small area inventoried • Sampling material needed • Limited operational depths	Bianchi et al. (2004b)

scraping or collection					• All species taken into account • A posteriori identification • Low cost, easy to implement	• Highly qualified scientific divers required • Very time-consuming underwater • Analysis of samples in laboratory very time-consuming	
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 km^2/hour	• Non-destructive • Good precision for 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 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)
Methods	**Depth range**	**Surface area**	**Resolution**	**Efficiency**	**Advantages**	**Limits**	**References**
Sampling from vessels with blind	0 m to about 120 m (until the lower limit of	Intermediate areas (a few km^2)	From 1 to 10 m	0.025 to 0.01 km^2/hour	• Very good precision for the identification (taxonomy) and characterisation of the habitat	• Destructive method, usually not recommended • Small area inventoried • Sampling material needed	UNEP/MAP-RAC/SPA (2015)

grabs, dredges or box corers	the rhodoliths habitat)				• All species taken into account • A posteriori identification • Low cost, easy to implement	• Samples analysis in laboratory very time-consuming	

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

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

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

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

In situ sampling	
Advantages	Taxonomical precision, objective evaluation, reference samples
Limits	High cost, slow laborious work, intervention of specialists, limited area inventoried, destructive method, depth-limitations when done by divers
Use	Studies integrating a strong taxonomical element
Video or photography	
Advantages	Objective evaluation, can be reproduced, reference samples, can be automated, speedy diving work, big area inventoried, non-destructive method, no depth-limitations
Limits	Low taxonomical precision, problem of *a posteriori* interpretation of pictures
Use	Studies on the biological cycle or over-time monitoring, large depth-range investigated
Underwater visual observation	
Advantages	Low cost, results immediately available, large area inventoried, can be reproduced, non-destructive method
Limits	Risk of taxonomic subjectivity, slow diving work, depth-limitations
Use	Exploratory studies, monitoring of populations, bionomic studies

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

Natura 2000 - Fiche Coralligène – ANTONIOLI 2010 – GIS Posidonie

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

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

- **Gorgone :**

Non → Oui

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

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

- **Aspect général :**

Non → Oui

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

Filet ☐
Ancrage ☐
Fil ☐
Déchet ☐

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

- **Inventaire :**

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

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

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

- **Observation :**

Photos quadrats et paysagères à réaliser

Figure 5: Example of a 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 habitat

~~53.~~54. The protocol STAR (STAndaRdized coralligenous evaluation procedure) (Piazzi et al., 2019a) has been proposed for monitoring the ecological status of coralligenous reefs to obtain information about most of the descriptors used by the different ecological indices adopted to date on coralligenous reefs, through a single sampling effort and data analysis.

~~54.~~55. Monitoring plans should first distinguish between the two major bathymetrical ranges where coralligenous reefs develop, i.e. the shallow and the deep reefs, within and deeper than about 40 m depth respectively (UNEP/MAP-RAC/SPA, 2008). In fact, shallow and deep coralligenous habitats can show different structure of assemblages, and they are usually subject to different types of anthropogenic pressures. Shallow reefs can be effectively surveyed by scuba diving, allowing obtaining information about descriptors that cannot be evaluated or measured through any other instrumental methods (Gatti et al., 2012, 2015a).

~~55.~~56. 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 once per year.

~~56.~~57. Depth and slope: the depth range where coralligenous reefs can develop changes with latitude and characteristics of the water. Moreover, different kind of assemblages may develop within the depth range of shallow coralligenous reefs. The slope of the rocky substrate is also important to determine the structure of coralligenous assemblages. In order to define a 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.

~~57.~~58. 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.

~~58.~~59. The sampling surface is related to the number of replicates and represents an important factor to be considered. A minimum surface suitable to sample coralligenous assemblages has never been established unambiguously, so different replicated sampling surfaces have been proposed depending on the methods adopted (Piazzi et al., 2018 and references therein). Researchers agree that the replicated sampling surface has to be larger than that utilized for shallow Mediterranean rocky habitats (i.e., ≥400 cm^2; Boudouresque, 1971), since the abundance of large colonial animals that characterise coralligenous assemblages could be underestimated when using small sampling areas (Bianchi et al., 2004b). Independent of the number of replicates, most of the proposed approaches suggest a total sampling area ranging between 5.6 and 9 m^2. Parravicini et al. (2009) reported that a sufficiently large sampling surface is more important than the specific method (e.g., visual quadrates or photography) to measure human impacts on Mediterranean rocky reef communities. Larger sampling areas with a lower number of replicates are used for seascape approaches (Gatti et al., 2012). On the contrary, most of the proposed sampling techniques for biocenotic approaches consider a greater number of replicates with a comparatively smaller sampling area, usually disposed along horizontal transects (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;). A comparison between the two sampling designs tested in the field showed no significant differences (Piazzi et al., 2019a), suggesting that both approaches can be usefully employed. Thus, three areas of 4 m^2 located tens of metres apart should be sampled, and a minimum of 10 replicated photographic samples of 0.2 m^2 each should be collected in each area by scientific divers, for a total sampling surface area of

6 m^2. This design can be repeated depending on the size of the study site and allows analysis of the data through both seascape and biocenotic approaches (see the *Ecological Indices* paragraph below).

~~59.~~60. 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 enclosing a standard area of the substrate, has been shown equally effective, but requires longer working time underwater (Parravicini et al., 2010), which may represent a limiting factor at the depths where coralligenous assemblages thrive. A rapid visual assessment (RVA) method has been proposed for a seascape approach (Gatti et al., 2012, 2015a). RVA allows capturing additional information compared with the photographic technique, such as the size of colonies of erect species and the thickness and consistency of the calcareous accretion (see *Descriptors* below). A combination of photographic and visual approaches, using photographic sampling to assess the structure of assemblages and integrating information by collecting a reduced amount of data with the RVA method (i.e., the size of colonies of erect species and the thickness and consistency of the calcareous accretion) is thus suggested.

~~60.~~61. 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 manual contouring techniques through appropriate softwares may be useful in order to reduce the subjectivity of the operator’s estimate.

~~61.~~62. 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 valuable information, together with biotic descriptors, to assess the ecological quality of a study area. The amount of sediment may be indirectly evaluated as percentage cover in photographic samples, as this method showed consistent results with those obtained through techniques measuring directly sediment deposition (i.e., by a suction pump).

- *Calcareous accretion*. The calcareous accretion of coralligenous reefs may be impaired by human-induced impacts. The growth of the calcareous organisms that deposit calcium carbonate on coralligenous reefs is a slow process that can be easily disrupted by environmental alterations. Thus, the thickness and consistency of the calcareous deposit can be considered an effective indicator of the occurrence of a positive balance in the bioconstruction process (Gatti et al., 2012, 2015a). The thickness and consistency of the calcareous deposit can be measured underwater through a hand-held penetrometer, with six replicated measures in each of the three areas of about 4 m^2 and located tens of metres apart. For each measure, the hand-held penetrometer marked with a millimetric scale must be pushed into the carbonate layer, allowing the direct measurement of the calcareous thickness. By definition, a penetrometer measures the penetration of a device (a thin blade in this case) into a substrate, and the penetration will depend on the force exerted and on the strength of the material. In the case of a hand-held penetrometer, the force is that of the diver, and thus cannot be measured properly and provides a semi-quantitative estimate only. Supposing that the diver always exerts approximately the same force, the measure of the penetration will provide a rough estimate of the thickness of the material penetrated. A null penetration is indicative of a hard rock and suggests that the biogenic substrate is absent or the bioconstructional process is no longer active; a millimetric penetration indicates the presence of active bioconstruction resulting in a calcareous biogenic substrate; and a centimetric penetration reveals a still unconsolidated bioconstruction.

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

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

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

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

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

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

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

~~62.~~63. 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.

~~63.~~64. Three replicated video-transects, each at least 200 m long, should be collected in each area investigated (Enrichetti et al., 2019). Footages can be obtained by means of a ROV, equipped with a high definition digital camera, a strobe, a high definition video camera, lights, and a 3-jaw grabber. The ROV should also host an underwater acoustic positioning system, a depth sensor, and a compass to obtain georeferenced tracks to be overlapped to multi-beam maps when available. Two parallel laser beams (90° angle) can provide a scale for size reference. In order to guarantee the best quality of video footages, ROV 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 end time of the transect track as reference. Visual census of megabenthic species is carried out along the complete extent of each 200 m-long transect and within a 50 cm-wide visual field, for a total of 100 m^2 of bottom surface covered per transect.

~~64.~~65. From each transect the following parameters are measured on videos:

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

~~65.~~66. Within each transect, 20 random high definition photographs targeting hard bottom must be obtained, and for each of them four parameters are estimated, following an ordinal scale. Modal values for each transect are calculated. Evaluated parameters on photos include:

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

Protocol for monitoring rhodoliths habitat

~~66.~~67. 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 more diverse species assemblages of coralline and peyssonneliacean algae than their Atlantic counterparts, and to be structured by a suite of combinations of rhodolith shapes and coralline compositions: from monospecific branched growth-forms, to multispecific rhodoliths (Basso et al., 2016). Therefore, the monitoring protocols available for sampling and monitoring rhodoliths in shallow subtidal waters cannot be applied as such and require calibrating to the Mediterranean specificities.

~~67.~~68. A recent proposal for monitoring rhodoliths beds can be found in Basso et al. (2016). Monitoring the rhodoliths habitat can be done by underwater diving and direct visual observation, with sampling and following taxa identification in laboratory. However, surveys using ROVs and towed cameras are often favoured because of the greater homogeneity of this habitat, and when strictly necessary sampling from vessels using blind grabs, dredges or box corers can be also performed (Tab. 4). Monitoring should address all the variables already described for the first descriptive characterisation of the habitat, with the addition of the full quantitative description of the rhodoliths community 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 change in the composition of the macrobenthic community (calcareous algal engineers and associated taxa) may reveal potential negative impacts acting on rhodoliths beds. All possible variations in growth form, shape, and internal structure of rhodoliths have been simplified in a scheme with three major categories as focal points along a continuum: compact and nodular pralines, larger and vacuolar box work rhodoliths, and unattached branches (Fig. 5). Each of the three end-members within rhodoliths morphological variability corresponds to a typical (but not exclusive) group of composing coralline species and associated biota and is possibly correlated with environmental variables, among which substratum instability (mainly due to hydrodynamics) and sedimentation rate are the most obvious. Thus, the indication of the percentage cover by the three live rhodoliths categories at the surface of each rhodoliths beds is a proxy of rhodoliths habitat structural and ecological complexity. The high species diversity hosted by rhodoliths beds requires time-consuming and expensive laboratory analysis for species identification. Videos and photos provide little information on rhodoliths community composition owing to the absence of conspicuous, easy-to-detect species. Moreover, since most coralline species belong to a few genera only, the use of taxonomic ranks higher than species is not useful.

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

Underwater visual observation

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

~~68.~~69. When necessary for a detailed characterization of rhodoliths communities, a minimum of three box-cores with opening ≥0.16 m^2 should be collected in each rhodoliths bed at the same depth, and to a depth of about 20 cm of sediment. One box-corer must be collected within the rhodoliths area with the highest percentage of live cover (on the basis of preliminary ROV surveys), and the others as far as possible from it, following the depth gradient in opposite directions of the maximum rhodoliths bed extension. In many instances grab samples could be useful, but attention must be paid to seafloor surface disruption and mixing, and the possible loss of material during recovery. In those extreme cases of very coarse material preventing box-core penetration and closure, a grab could be used instead, although it cannot preserve stratification. Once the box-core is recovered a colour photograph of the whole surface of the box-core, at a high enough resolution to recognise the morphology of single live rhodoliths and other conspicuous organisms, must be collected. In addition, the possible occurrence of heavy overgrowths of fleshy algae that may affect rhodoliths growth rate must be reported. The following descriptors must then be assessed: 1) visual estimation of the percentage cover of live red calcareous algae; 2) visual estimation of the live/dead rhodoliths ratio calculated for the surface of the box-core; 3) visual assessment of the rhodoliths morphologies characterising the sample (Fig. 5); 4) measurement of the thickness of the live rhodoliths layer. The sediment sample is then washed through a sieve (e.g., 0.5 mm mesh) and the sample treated with Rose Bengal to stain living material before being preserved for sorting under a microscope for taxa identification. All live calcareous algae and accompanying phytobenthos and zoobenthos should be identified and quantified, in order to allow for detection of variability in space and time, and any changes after possible impacts. Algal species must be evaluated using a semi-quantitative approach (classes of abundance of algal coverage: absent, 1-20%, 21-40%, 41-60%, 61-80%, >81%). For molecular investigations, samples from voucher rhodoliths morphotypes should be air-dried, and preserved in silica gel. The sediment sample should be analysed for grain-size (mandatory), and carbonate content.

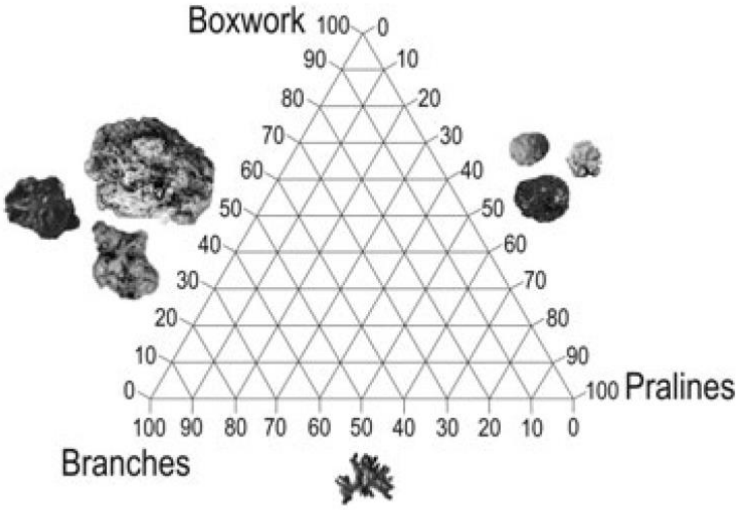

Figure 5: ternary diagram for the description of the rhodoliths bed tridimensionality. The percentage cover of each rhodoliths morphotype, relative to the total rhodoliths cover, can be plotted on the correspondent axis. The three main rhodoliths morphotypes (box_work_rhodoliths, pralines and unattached 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 Indices

~~69.~~70. To assess the ecological status of coralligenous reefs, several ecological indices have been developed based on different approaches (Kipson et al., 2011, 2014; Teixidó et al., 2013; Zapata-Ramírez et al., 2013; David et al., 2014; Féral et al., 2014; Piazzi et al., 2019), which are summarised in Table 5. Most of the ecological indices available for monitoring shallow coralligenous reefs require underwater surveys by scuba diving. These indices have been developed following different approaches and adopt distinct descriptors and sampling techniques, thus hampering the comparison of data and results, and requiring inter-calibration procedures. Detailed descriptions of the sampling tools and the methodologies adopted for each index listed in Table 5 can be found in the relative bibliographic references.

~~70.~~71. ESCA (Ecological Status of Coralligenous Assemblages; Cecchi et al., 2014; Piazzi et al., 2015, 2017a), ISLA (Integrated Sensitivity Level of coralligenous Assemblages; Montefalcone et al., 2017), and CAI (Coralligenous Assessment Index; Deter et al., 2012) indices are based on a biocenotic approach where coralligenous assemblages are investigated in terms of composition and abundance of all species for ESCA and ISLA, and percentage cover of mud and builder organisms (i.e., Corallinales, bryozoans, scleractinians) for CAI.

~~71.~~72. 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.

~~72.~~73. COARSE (COralligenous Assessment by ReefScape Estimate; Gatti et al., 2012, 2015a) uses a seascape approach to provide information about the structure of coralligenous reefs in order to assess the seafloor integrity. Since the coralligenous is characterised by high heterogeneity, extreme patchiness and coexistence of several biotic assemblages, a seascape approach seems to be the most reasonable solution for its characterisation.

~~73.~~74. OCI (Overall Complexity Index; Paoli et al., 2016) combines measures of structural and functional complexity, while the INDEX-COR (Sartoretto et al., 2017) integrates three descriptors (the sensitivity of taxa to organic matter and sediment deposition, the observable taxonomic richness, and the structural complexity of assemblages) to assess the health status of coralligenous assemblages.

~~74.~~75. Inter-calibrations among some of the above listed ecological indices have already been carried out. Comparison between ESCA and COARSE (Montefalcone et al., 2014; Piazzi et al., 2014, 2017a, 2017b), which are the two indices with the greatest number of successful applications to date (Piazzi et al., 2017b) in 24 sites of the NW Mediterranean Sea showed that the two indices provided different but complementary information to determine the intrinsic quality of coralligenous reefs and to detect the effects of human pressures on the associated assemblages. The concurrent use of ESCA and COARSE can thus be effective in providing information about the alteration of ecological quality of coralligenous reefs. A recent comparison among ESCA, ISLA, and COARSE has also been carried out (Piazzi et al., 2018), which proved that main differences among indices are linked to the different approaches used, and that ESCA and ISLA showed highly consistent results being based on a biocenotic approach. Finally, CAI, ESCA, COARSE, and INDEX-COR have been compared in 21 sites along the southern coasts of France (Gatti et al., 2016). Results showed that the four indices are not always concordant in indicating the ecological quality of coralligenous habitats, some metrics being more sensitive than others to the increasing pressure levels.

~~75.~~76. Few efforts have been made to define indices for mesophotic environments based on ROV footages, resulting in three seascape indices (Tab. 6), namely MAES (Mesophotic Assemblages Ecological Status; Cánovas-Molina et al., 2016a), CBQI (Coralligenous Bioconstructions Quality Index; Ferrigno et al., 2017), and MACS (Mesophotic Assemblages Conservation Status; Enrichetti et al., 2019). MACS is a new multi-parametric index that is composed by two independent units, the Index of Status (*Is*) and the Index of Impact (*Ii*) following a DPSIR (Driving forces – Pressures – Status – Impacts – Response) approach. The index integrates three descriptors included in the MSFD and listed by the Barcelona Convention to define the environmental status of seas, namely biological diversity, seafloor integrity, and marine litter. The *Is* depicts the biocoenotic complexity of the investigated ecosystem, whereas the *Ii* describes the impacts affecting it. Environmental status is the outcome of the status of benthic communities plus the amount of impacts upon them: the integrated MACS index measures the resulting environmental status of deep coralligenous habitats reflecting the combination of the two units and their ecological significance. The MACS index has been effectively calibrated on 14 temperate mesophotic reefs of the Ligurian and Tyrrhenian seas, all characterised by the occurrence of temperate reefs but subjected to different environmental conditions and levels of human pressures.

Final remarks

~~76.~~77. Inventorying and monitoring the condition of coralligenous reefs and rhodoliths seabeds in the Mediterranean constitute 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.

~~77.~~78. 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 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 (up to 40 m depth) coralligenous habitat and based on different approaches.

Index	Method	Image analysis	Descriptors
Biocenotic			
ESCA	Photographic samples: 30 photographic quadrates (50 cm × 37.5 cm) in two areas hundreds of metres apart	Software Image J' for the estimation of the % cover of the main taxa and/or morphological groups of sessile macro-invertebrates and macroalgae	3 descriptors: Sensitivity Level of all species (SL); α diversity (diversity of assemblages); β diversity (heterogeneity of assemblages)
ISLA	Photographic samples: 30 photographic quadrates (50 cm × 37.5 cm) in two areas hundreds of metres apart	Software Image J' for the estimation of the % cover of the main taxa and/or morphological groups of sessile macro-invertebrates and macroalgae	2 descriptors: Integrated Sensitivity Level of all species (ISL), i.e. Sensitivity Level to stress (SSL) and Sensitivity Level to disturbance (DSL)
CAI	Photographic samples: 30 photographic quadrates (50 cm×50 cm) along a 40 m long transect	Software CPCe 3.6 for the estimation of the % cover by each species	3 descriptors: % cover of mud; % cover of builders; % cover of bryozoans
Ecosystem			
EBQI	Direct *in situ* observations and samples. A simplified conceptual model of the functioning of the ecosystem with 10 functional compartments		11 descriptors: % cover of builders; % cover of non-calcareous species; abundance of filter and suspension feeders; occurrence of bioeroders and density of sea urchins; abundance of browsers and grazers; biomass of planktivorous fish; biomass of predatory fish; biomass of piscivorous fish; Specific Relative Diversity Index for fish; % cover of benthic detritus matter; density of detritus feeders
Seascape			
COARSE	Direct *in situ* observations with Rapid Visual Assessment (RVA): 3 replicated visual estimations over an area of about 2 m^2 each		9 descriptors, 3 per each layer: Basal layer: % cover of encrusting calcified rhodophyta, non-calcified encrusting algae, encrusting animals, turf-forming algae and sediment; amount of boring species marks; thickness and consistency of calcareous layer with a hand held penetrometer (5 replicates) Intermediate layer: specific richness; n° of erect calcified organisms; sensitivity of bryozoans Upper layer: total % cover of species; % of necrosis of each population; maximum height of the tallest specimen

Integrated			
INDEX-COR	Photographic samples and direct observations: 30 photographic quadrates (60 cm × 40 cm) along two 15 m long transects (15 photos per transect); visual census of marine litter, conspicuous benthic sessile and mobile species (echinoderms, crustacean decapods and nudibranchs), estimation of the % cover of gorgonians and sponges, % of necrotic gorgonian colonies	Free software photoQuad, using the uniform point count technique	3 descriptors: Taxa Sensitivity level (TS) to organic matter and sediment input; taxonomic richness of conspicuous taxa that were recognizable visually on photo-quadrates and *in situ*; structural complexity of the habitat, defined from the % cover of the taxa belonging to basal and intermediate layers estimated from the photo-quadrates and the % cover of gorgonians and large sponges observed in situ along the transects for the upper layer
OCI	Available detailed maps of benthic habitats		Surface area covered by coralligenous obtained from maps; list of the main taxonomic groups found in the habitat; biomass per unit area of each taxonomic group obtained from the literature. These descriptors are used to compute exergy and specific exergy as a measure of structural complexity, whilst throughput and information as a measure of functional complexity

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

Index	Method	Image analysis	Descriptors
Seascape			
MAES	ROV survey: 500 m long video transects per area and 20 random high-resolution photographs frontally on the seafloor	VLC program for video and Image J' software for photos	6 descriptors: n° of megabenthic taxa, % biotic cover in the basal layer; density of erect species; average height and % cover of the dominant erect species; % of colonies with epibiosis/necrosis; density of marine litter
CBQI	ROV survey and photographs	VisualSoft software for video and DVDVideoSoft software to obtain random frames every 10 s for quantitative analysis	9 descriptors: % cover of coralligenous on the bottom; n° of morphological groups; density of fan corals; % of colonies with epibiosis/necrosis; % of colonies with covered/entangled signs; % of fishing gear; depth; slope; 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 waters.

Coralligenous
Builders
Algal builders
Lithophyllum cabiochae (Boudouresque & Verlaque) Athanasiadis, 1999
Lithophyllum stictaeforme (J.E. Areschoug) Hauck, 1877
Lithothamnion sonderi Hauck, 1883
Lithothamnion philippii Foslie, 1897
Mesophyllum alternans (Foslie) Cabioch & M.L. Mendoza, 1998
Mesophyllum expansum (Philippi) Cabioch & M.L. Mendoza, 2003
Mesophyllum macedonis Athanasiadis, 1999
Mesophyllum macroblastum (Foslie) W.H. Adey, 1970
Neogoniolithon mamillosum (Hauck) Setchell & L.R. Mason, 1943
Peyssonnelia rosa-marina Boudouresque & Denizot, 1973
Peyssonnelia polymorpha (Zanardini) F. Schmitz, 1879
Sporolithon ptychoides Heydrich, 1897

Animal builders
Foraminifera
Miniacina miniacea Pallas, 1766

Bryozoans
Myriapora truncata Pallas, 1766
Schizomavella spp.
Turbicellepora spp.
Adeonella calveti Canu & Bassler, 1930
Smittina cervicornis Pallas, 1766
Pentapora fascialis Pallas, 1766
Schizoretepora serratimargo (Hincks, 1886)
Rhynchozoon neapolitanum Gautier, 1962

Polychaeta
Serpula spp.
Spirorbis sp.
Spirobranchus polytrema Philippi, 1844

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

Bioeroders
Sponges
Clionidae (Cliona, Pione)

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

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

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

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

OTHER RELEVANT SPECIES (*invasive; **disturbed or stressed environments-usually, when abundant)
Algae
Green algae
Flabellia petiolata (Turra) Nizamuddin, 1987
Halimeda tuna (J. Ellis & Solander) J.V. Lamouroux, 1816
Palmophyllum crassum (Naccari) Rabenhorst, 1868
Caulerpa cylindracea Sonder, 1845
Caulerpa taxifolia (M. Vahl) C. Agardh, 1817*
Codium bursa (Olivi) C. Agardh, 1817**
Codium fragile (Suringar) Hariot, 1889*
Codium vermilara (Olivi) Chiaje, 1829**

Brown algae
Cystoseira zosteroides (Turner) C. Agardh, 1821

Cystoseira montagnei var. *compressa* (Ercegovic) M. Verlaque, A. Blanfuné, C.F. Boudouresque, T. Thibaut & L.N. Sellam, 2017
Laminaria rodriguezii Bornet, 1888
Halopteris filicina (Grateloup) Kützing, 1843
Phyllariopsis brevipes (C. Agardh) E.C. Henry & G.R. South, 1987
Dictyopteris lucida M.A. Ribera Siguán, A. Gómez Garreta, Pérez Ruzafa, Barceló Martí & Rull Lluch, 2005**
Dictyota spp.**
Stypopodium schimperi (Kützing) M. Verlaque & Boudouresque, 1991*
Acinetospora crinita (Carmichael) Sauvageau, 1899**
Stilophora tenella (Esper) P.C. Silva in P.C. Silva, Basson & Moe, 1996**
Stictyosiphon adriaticus Kützing, 1843**

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

<u>Red algae</u>
Osmundaria volubilis (Linnaeus) R.E. Norris, 1991
Rodriguezella spp.
Ptilophora mediterranea (H.Huvé) R.E. Norris, 1987
Kallymenia spp.
Halymenia spp.
Sebdenia spp.
Peyssonnelia spp. (non calcareous)
Phyllophora crispa (Hudson) P.S. Dixon, 1964
Gloiocladia spp.
Leptofauchea coralligena Rodríguez-Prieto & De Clerck, 2009
Acrothamnion preissii (Sonder) E.M. Wollaston, 1968*
Lophocladia lallemandii (Montagne) F. Schmitz, 1893*
Asparagopsis taxiformis (Delile) Trevisan de Saint-Léon, 1845*
Womersleyella setacea (Hollenberg) R.E. Norris, 1992*

Animals
<u>Sponges</u>
Acanthella acuta Schmidt, 1862
Agelas oroides Schmidt, 1864
*Aplysinaaerophoba*Nardo, 1843
Aplysina cavernicola Vacelet, 1959
Axinella spp.
Chondrosia reniformis Nardo, 1847
Clathrina clathrus Schmidt, 1864
Cliona viridis (Schmidt, 1862)
Dysidea spp.
Haliclona (Reniera) mediterranea Griessinger, 1971
Haliclona (Soestella) mucosa Griessinger, 1971
Hemimycale columella Bowerbank, 1874
Ircinia oros Schmidt, 1864
Ircinia variabilis Schmidt, 1862
Oscarella sp.
Petrosia (Petrosia) ficiformis (Poiret, 1789)
*Phorbas tenacior*Topsent, 1925
Sarcotragus fasciculatus (Pallas, 1766)
Spirastrella cunctatrix Schmidt, 1868
Spongia (Spongia) officinalis Linnaeus, 1759
Spongia (Spongia) lamella Schulze, 1879

<u>Cnidaria</u>
Alcyonium acaule Marion, 1878
Alcyonium palmatum Pallas, 1766
Corallium rubrum Linnaeus, 1758
Paramuricea clavata Risso, 1826
Eunicella spp.
Leptogorgia sarmentosa Esper, 1789
Ellisella paraplexauroides Stiasny, 1936
Antipathes spp.
Parazoanthus axinellae Schmidt ,1862
Savalia savaglia Bertoloni, 1819
Callogorgia verticillata Pallas, 1766

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

<u>Bryozoans</u>
Chartella tenella Hincks, 1887
Margaretta cereoides Ellis & Solander, 1786
Hornera frondiculata (Lamarck, 1816)

<u>Tunicates</u>
Pseudodistoma cyrnusense Pérès, 1952
Aplidium spp.
Microcosmus sabatieri Roule, 1885
Halocynthia papillosa Linnaeus, 1767

<u>Molluscs</u>
Charonia lampas Linnaeus, 1758
Charonia variegata Lamarck, 1816
Pinna rudis Linnaeus, 1758
Naria spurca (Linnaeus, 1758)
Luria lurida Linnaeus, 1758

<u>Decapoda</u>
Palinurus elephas Fabricius, 1787
Scyllarides latus Latreille, 1803
Maja squinado Herbst, 1788

<u>Echinodermata</u>
Antedon mediterranea Lamarck, 1816
Hacelia attenuata Gray, 1840
Centrostephanus longispinus Philippi, 1845
Holothuria (Panningothuria) forskali Delle Chiaje, 1823
Holothuria (Platyperona) sanctori Delle Chiaje, 1823

<u>Pisces</u>
Epinephelus spp.
Mycteroperca rubra Bloch, 1793
Sciaena umbra Linnaeus, 1758
Scorpaena scrofa Linnaeus, 1758
Raja spp.
Torpedo spp.
Mustelus spp.
Phycis phycis Linnaeus, 1766
Serranus cabrilla Linnaeus, 1758
Scyliorhinus canicula Linnaeus, 1758

Rhodoliths

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

Algae

Red algae (calcareous)

#*Lithophyllum racemus* (Lamarck) Foslie, 1901
#*Lithothamnion corallioides* (P.L. Crouan & H.M. Crouan) P.L. Crouan & H.M. Crouan, 1867
#*Lithothamnion valens* Foslie, 1909
#*Peyssonnelia 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 heteromorpha (Zanardini) Athanasiadis, 2016
Sporolithon ptychoides Heydrich, 1897

Red algae (non builders)

#*Osmundaria volubilis* (Linnaeus) R.E. Norris, 1991
#*Phyllophora crispa* (Hudson) P.S. Dixon, 1964
Peyssonnelia spp. (non calcareous)
Acrothamnion preissii (Sonder) E.M. Wollaston, 1968*
Alsidium corallinum C. Agardh, 1827
Cryptonemia spp.
Felicinia marginata (Roussel) Manghisi, Le Gall, Ribera, Gargiulo & M. Morabito, 2014
Gloiocladia microspora (Bornet ex Bornet ex Rodríguez y Femenías) N. Sánchez & C. Rodríguez-Prieto ex Berecibar, M.J. Wynne, Barbara & R. Santos, 2009
Gloiocladia repens (C. Agardh) Sánchez & Rodríguez-Prieto, 2007
Gracilaria spp.
Halymenia spp.
Kallymenia spp.
Leptofauchea coralligena Rodríguez-Prieto & De Clerck, 2009
Nitophyllum tristromaticum J.J. Rodríguez y Femenías ex Mazza, 1903
Osmundea pelagosae (Schiffner) K.W. Nam, 1994
Phyllophora heredia (Clemente) J. Agardh, 1842
Rhodophyllis divaricata (Stackhouse) Papenfuss, 1950
Rytiphlaea tinctoria (Clemente) C. Agardh, 1824
Sebdenia spp.
Vertebrata byssoides (Goodenough & Woodward) Kuntze, 1891
Vertebrata subulifera (C. Agardh) Kuntze, 1891
Womersleyella setacea (Hollenberg) R.E. Norris, 1992*

Green algae

Flabellia petiolata (Turra) Nizamuddin, 1987
Caulerpa cylindracea Sonder, 1845*

Caulerpa taxifolia (M. Vahl) C. Agardh, 1817*
Codium bursa (Olivi) C. Agardh, 1817
Microdictyon umbilicatum (Velley) Zanardini, 1862
Palmophyllum crassum (Naccari) Rabenhorst, 1868
Umbraulva dangeardii M.J. Wynne & G. Furnari, 2014

Brown algae
Arthrocladia villosa (Hudson) Duby, 1830
Laminaria rodriguezii Bornet, 1888
Sporochnus pedunculatus (Hudson) C. Agardh, 1817
Acinetospora crinita (Carmichael) Sauvageau, 1899**
Carpomitra costata (Stackhouse) Batters, 1902
Cystoseira abies-marina (S.G. Gmelin) C. Agardh, 1820
Cystoseira foeniculacea (Linnaeus) Greville, 1830
Cystoseira foeniculacea f. *latiramosa* (Ercegovic?) A. Gómez Garreta, M.C. Barceló, M.A. Ribera & J.R. Lluch, 2001
Cystoseira montagnei var. *compressa* (Ercegovic) M. Verlaque, A. Blanfuné, C.F. Boudouresque, T. Thibaut & L.N. Sellam, 2017
Cystoseira zosteroides (Turner) C. Agardh, 1821
Dictyopteris lucida M.A. Ribera Siguán, A. Gómez Garreta, Pérez Ruzafa, Barceló Martí & Rull Lluch, 2005
Dictyota spp.
Halopteris filicina (Grateloup) Kützing, 1843
Nereia filiformis (J. Agardh) Zanardini, 1846
Phyllariopsis brevipes (C. Agardh) E.C. Henry & G.R. South, 1987
Spermatochnus paradoxus (Roth) Kützing, 1843
Stictyosiphon adriaticus Kützing, 1843
Stilophora tenella (Esper) P.C. Silva, 1996
Zanardinia typus (Nardo) P.C. Silva, 2000

Animals
Sponges
Aplysina spp.
Axinella spp.
Cliona viridis Schmidt, 1862
Dysidea spp.
Haliclona spp.
Hemimycale columella Bowerbank, 1874
Oscarella spp.
Phorbas tenacior Topsent, 1925
Spongia (Spongia) officinalis Linnaeus, 1759
Spongia (Spongia) lamella Schulze, 1879

Cnidaria
Alcyonium palmatum Pallas, 1766
Eunicella verrucosa Pallas, 1766
Paramuricea macrospina Koch, 1882
Aglaophenia spp.
Adamsia palliata (Müller, 1776)
Calliactis parasitica Couch, 1838
Cereus pedunculatus Pennant 1777
Cerianthus membranaceus (Gmelin, 1791)
Funiculina quadrangularis Pallas, 1766
Leptogorgia sarmentosa Esper, 1789
Nemertesia antennina Linnaeus, 1758
Pennatula spp.

Veretillum cynomorium Pallas, 1766
Virgularia mirabilis Müller, 1776

Polychaetes
Aphrodita aculeata Linnaeus, 1758
Sabella pavonina Savigny, 1822
Sabella spallanzanii Gmelin, 1791

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

Tunicates
Aplidium spp.
Ascidia mentula Müller, 1776
Diazona violacea Savigny, 1816
Halocynthia papillosa Linnaeus, 1767
Microcosmus spp.
Phallusia mammillata Cuvier, 1815
Polycarpa spp.
Pseudodistoma crucigaster Gaill, 1972
Pyura dura Heller, 1877
Rhopalaea neapolitana Philippi, 1843
Synoicum blochmanni Heiden, 1894

Echinodermata
Astropecten irregularis Pennant, 1777
Chaetaster longipes (Bruzelius, 1805)
Echinaster (Echinaster) sepositus Retzius, 1783
Hacelia attenuata Gray, 1840
Holothuria (Panningothuria) forskali Delle Chiaje, 1823
Leptometra phalangium Müller, 1841
Luidia ciliaris Philippi, 1837
Ophiocomina nigra Abildgaard in O.F. Müller, 1789
Parastichopus regalis Cuvier, 1817
Spatangus purpureus O.F. Müller 1776
Sphaerechinus granularis Lamarck, 1816
Stylocidaris affinis Philippi, 1845

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