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Agenda Item 8: Monitoring Protocols for IMAP Common Indicators related to Pollution and Guidance on monitoring concerning IMAP Common Indicators related to Biodiversity and Non-Indigenous Species

Monitoring Protocols for IMAP Common Indicators related to Biodiversity and Non-Indigenous species

UNEP/MAP
Athens, 2019

Note by the Secretariat

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

The agreed common indicators related to biodiversity and non-indigenous species cluster include:

1. common indicator 1: Habitat distributional range (EO1) to also consider habitat extent as a relevant attribute;
2. common indicator 2: Condition of the habitat's typical species and communities (EO1);
3. common indicator 3: Species distributional range (EO1 related to marine mammals, seabirds, marine reptiles);
4. common indicator 4: Population abundance of selected species (EO1, related to marine mammals, seabirds, marine reptiles);
5. common indicator 5: Population demographic characteristics (EO1, e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates related to marine mammals, seabirds, marine reptiles);
6. common indicator 6: Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species, particularly invasive, non-indigenous species, notably in risk areas (EO2, in relation to the main vectors and pathways of spreading of such species).

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

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

(i) harmonization and standardization of monitoring and assessment methods;

(ii) improvement of availability and ensuring of long time series of quality assured data to monitor the trends in the status of the marine environment;

(iii) improvement of availability of the synchronized datasets for marine environment state assessment, including use of data stored in other databases where some of the Mediterranean countries regularly contribute; and

(iv) improvement of data accessibility with the view to improving knowledge on the Mediterranean marine environment and ensuring that Info-MAP System is operational and continuously upgraded, to accommodate data submissions for all the IMAP Common Indicators.

The present document outlines the monitoring guidelines of the agreed common indicators 1 and 2 related to marine habitats, common indicators 3, 4 and 5 related to marine mammals, marine turtles and seabirds, and common indicator 6 related to non-indigenous species.

These guidelines were discussed and reviewed by the Meetings of the Ecosystem Approach Correspondence Group on Monitoring (CORMON), Biodiversity and Fisheries (Marseille, France, 12-13 February 2019 and Rome, Italy, 21 May 2019) and the 14th meeting of the SPA/BD thematic Focal points (Portoroz, Slovenia, 18-21 June 2019). All the comments and suggestions received from the Contracting Parties were considered and included in this version of the document.

This document is submitted to the 7th Meeting of the Ecosystem Approach Coordination Group (Athens, Greece, 9 September 2019) for information and final approval.

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A. Guidelines for monitoring Cetaceans in the Mediterranean Sea

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1. Introduction

1.1. Background

1. The Contracting Parties to the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention) have adopted the Ecosystem Approach (EcAp) in January 2008. This strategy allows all aspects of marine ecosystem to be taken into account. It includes management of coast, sea and living resources that promotes conservation and sustainable use in an equitable way, in order to respect interactions in the ecosystems. Indeed, it recognizes ecological systems as a rich mix of elements that interact with each other continuously. This process aims to achieve the good environmental status (GES) through informed management decisions, based on integrated quantitative assessment and monitoring of the marine and coastal environment of the Mediterranean. EcAp is also a way of making decisions in order to manage human activities sustainably. It recognizes that human's activities both affect the ecosystem and depend on it.

2. In February 2016, the Contracting Parties to the Barcelona Convention have also adopted an Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP). This text describes the strategy, themes and products to deliver by Contracting Parties over the second period of the implementation of the EcAp (2016-2021). The main goal of IMAP is to build and implement a regional monitoring system gathering reliable and up-to-date data and information on the marine and coastal Mediterranean environment. Mediterranean countries committed to monitor and report on 23 common indicators, articulated on 11 ecological objectives and covering topics related to pollution, marine litter, biodiversity, non-indigenous species, coast and hydrography.

3. One of eleven ecological objectives is “Biodiversity is maintained or enhanced” (EO1). Three determining factors are used to quantify the conservation:

- no further loss of the diversity within species, between species and of habitats/communities and ecosystems at ecologically relevant scales;
- any deteriorated attributes of biological diversity are restored to and maintained at or above target levels, where intrinsic conditions allow;
- where the use of the marine environment is sustainable.

1.2. Aim

4. These guidelines aim at helping managers and decision makers to understand and implement a strategy of long-term monitoring for cetaceans, in deciding what kind of method to choose at regional and national level to answer the indicators 3, 4 and 5. This document aims at presenting a global overview of methods, with the main advantages and disadvantages, the human resources and material requested in order to better estimate the investment needed and other practical points. For more details on one specific method, please follow the bibliographic references.

5. A lot of scientific papers, or guidelines exist on the subject and on all those methods that are recognised as standard. Some explain in detail the steps of implementation, the scientific background, highlight also pro and cons, advantages and disadvantages. A list of some of these documents are listed at the end and should be considered for further details.

6. This document focuses more on the techniques at sea than on the consequent and associated analyses. It has to bear in mind that analyses need expert’s time and skills and has a certain cost related in order to be properly done. A lot of models and types of analyses exist and are well described in many scientific papers. What should be stressed is that powerful analyses can be led only with reliable data that have been collected in a standardised and recognised manner. So, to be sure data will be useful, comparable and used, the decision

and implementation of rigorous methods should be the first step, following standard monitoring methods here highlighted.

1.3. Indicators 3, 4, 5

7. In the context of the Barcelona Convention, a common indicator is an indicator that summarizes data into a simple, standardized, and communicable figure. It is able to give an indication of the degree of threat or change in the marine ecosystem and can deliver valuable information to decision makers.

8. Among five common indicators related to biodiversity (EO1) fixed by IMAP, three are about marine mammals:

- Indicator 3 - Species distributional range

This indicator is aimed at providing information about the geographical area in which marine mammal species occur. It is intended to reflect the species distributional range of cetaceans that are present in Mediterranean waters, with a special focus on the species selected by the Parties. The main outputs of the monitoring under this indicator will be maps of species presence, distribution and occurrence. Resulting analysis can lead also to identification of important habitat and core areas for the species. The aim is to detect any important changes in the distributional pattern of the cetaceans.

- Indicator 4 - Population abundance of selected species

As cetaceans are highly mobile and distributed mainly over vast areas, this indicator refers preferably also to an area-defined abundance of selected species (in a specified area in a given timeframe). Resulting analysis led to absolute abundance, density maps or indices of abundance. The aim is to detect any important changes in those numbers. Methods for estimating density and abundance are generally species-specific and ecological characteristics of a target species should be considered carefully when planning a research campaign. The main limitation of some implementation of monitoring method is relates to how representative the results are in terms of the relevant population. So, it needs first to define which population is targeted.

- Indicator 5 - Population demographic characteristics (e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates)

This indicator required to demographic parameters as the age structure, age at sexual maturity, sex ratio and rates of birth (fecundity) and of death (mortality). These data are particularly difficult to obtain for marine mammals and to monitor but are important to understand and collect. Monitoring effort should be directed to collect long-term data series covering the various life stages of the selected species. This would involve the participation of several teams using standard methodologies and covering sites of particular importance for the key life stages of the target species. Results are in terms of numbers or rates. The aim is to detect any important changes in those numbers or ratio. One of the main limitations of some implementation of monitoring method is relates to how representative the results are in terms of the relevant population. So, it needs first to define which population is targeted.

2. Species concerned

9. IMAP fixes a reference list of species and habitats to be monitored. All cetacean species occuring the Mediterranean Sea are considered in the IMAP. Particular attention is given to the eight resident cetacean species, divided into three different functional groups:

- Baleen whales: fin whale (*Balaenoptera physalus*)

- Deep-diving cetaceans: sperm whale (*Physeter macrocephalus*), Cuvier's beaked whale (*Ziphius cavirostris*)), long-finned pilot whale (*Globicephala melas*) and Risso's dolphin (*Grampus griseus*).
- Other toothed species: short-beaked common dolphin (*Delphinus delphis*), striped dolphin (*Stenella coeruleoalba*), common bottlenose dolphin (*Tursiops truncatus*).

IMAP recommends monitoring and assessing common indicators for this selection of representative species for cetacean. However, three other rare species of cetaceans occur also in the Mediterranean Sea: harbour porpoise (*Phocoena phocoena*), rough-toothed dolphin (*Steno bredanensis*), and killer whale (*Orcinus orca*).

10. The decision to monitor additional species among these should not hinder the monitoring of the standard species set, as these are being monitored at wider scale (e.g., whole Mediterranean region), and the data that will be obtained at national or local scale would add a very high value.

11. Monitoring is needed on a consistent scale for each population studied. The Contracting Parties, while updating their national monitoring programmes, shall make every effort to identify the list of species and if possible, population to be considered. The choice will have to take into account on the specificity of their marine environment and biodiversity, and also on the number of animals occurring in the Contracting Parties' waters and how many there are in relation to total populations size to warrant investigating one or more of the indicators.

3. Monitoring methods

12. Before embarking upon a monitoring programme, the most important is to identify the objective, determine the appropriate indicator(s) in principle, then determine precisely what information can be gained and what are the limitations. Then a cost-benefit analysis of the various options available should be conducted. The type of platform, level of sophistication of survey, and detection method should be considered in each case, and the most appropriate ones identified, relying upon if the indicator can be monitored to be able to robustly detect changes should they occur given certain levels of effort (sample size).

13. Thus, when being in the process to decide which monitoring method to be implemented, it is important to consider several issues, that will be synthetized in different tables to get a global first overview. General consideration will give some advices considering on unifying data collection protocols and the statistical requirements on data and samples, and also the complementarity of methods at different spatial and temporal scales, as no single method will be enough to monitor all parameters and all species. The other chapters will present more in details the different methodologies.

14. Methods for estimating density and abundance are generally species-specific and ecological characteristics of a target species should be considered carefully when planning a research campaign. Furthermore, as cetaceans have no frontiers and their conservation should be thought at the Mediterranean level, it is recommended to promote the implementation of transnational and coordinated monitoring on a standard way.

3.1. Synthesis tables

15. Four tables synthetized the main information needed to take the decision on what method(s) to implement to elucidate indicator 3, 4 and 5 of the EO1 of the IMAP process:

- which method will give useful data to answer which indicator, depending on the target specie(s) and its characteristics. This is presented in a synthetic way in Table 1 for an overview;

- according to the method chosen, indications are presented concerning the time delay to get results, the cost associated, the difficulty in implementing the method, the constraints and limits associated and finally the compatibility with other method(s) (in order to optimize time and resources, as several methods can be used in parallel on the same platform during the same campaigns). Also, a column presents the metrics that can be obtain by the method.

- according to the method chosen, what will be the investment needed, in terms of material and human resources. Also, some indications are presented concerning the data storage volume and the time dedicated to process the analysis.

- according to the level at which there are designed for, population or individuals, and at which spatial scale they correspond the best (small or large area). In Table 4 each method has been designed to collect data to answer question at one of the levels and spatial scales, whereas some adaptation can be made to other level and spatial scale. Additionally, some methods are designed for large areas and the platform will have to move within the large areas. Whereas some methods, especially the one based on individuals, will be implemented in small areas and can give information on large areas in two ways: if the implementation is done in several places and built in a frame of a network (e.g., strandings, photo-ID), or by the nature of the parameter studied which can be extrapolate in a wider area if enough samples are available (reproductive status, genetic, telemetry).

16. Finally, as working at sea can be expensive and as marine environment and IMAP process deal also with other marine species,

17. Tableau 5 presents the monitoring methods for cetaceans and their compatibility with other marine species monitoring.

Table 1 - Synthesis listing different cetacean's monitoring methods recommended answering to indicators of IMAP process by cetacean species (legend: bold type = best suitable method; in bracket (less suitable method but can give interesting information) and in bracket and italic (*indication of limits*)). For the definition of the methods, see other chapters of the document.

	Baleen whales	Deep-diving cetaceans				Other toothed species		
	fin whale (*Balaenoptera physalus*)	**sperm whale (*Physeter macrocephalus*)**	**Cuvier's beaked whale (*Ziphius cavirostris*)**	**long-finned pilot whale (*Globicephala melas*)**	**Risso's dolphin (*Grampus griseus*)** also applies to killer whale (*Orcinus orca*)	**common bottlenose dolphin (*Tursiops truncatus*)** also applies to rough-toothed dolphin (*Steno bredanensis*),	**striped dolphin (*Stenella coeruleoalba*)** also applies to harbour porpoise (*Phocoena phocoena*),	**short-beaked common dolphin (*Delphinus delphis*)** also applies to harbour porpoise (*Phocoena phocoena*),
INDICATOR 3, species distributional range	**Visual Line transect "distance sampling" boat or aerial** Telemetry Acoustic line transect (or fixed point) (*presence/absence*) Land based method (*locally*)	**Visual Line transect "distance sampling" boat coupled to acoustic line transect** Photo-Identification Telemetry (Visual Line transect aerial)	**Visual Line transect "distance sampling" boat coupled to acoustic line transect** Telemetry and acoustic fixed point Photo-Identification (Visual Line transect aerial)	**Visual Line transect "distance sampling" boat or aerial** Acoustic line transect (or fixed point) (*presence/absence*)	**Visual Line transect "distance sampling" boat or aerial** Photo-Identification Acoustic line transect (or fixed point) (*presence/absence*)	**Visual Line transect "distance sampling" boat or aerial** Photo-Identification Acoustic line transect (or fixed point) (*presence/absence*) Land based method (*locally*)	**Visual Line transect "distance sampling" boat or aerial** Acoustic line transect (or fixed point) (*presence/absence*)	**Visual Line transect "distance sampling" boat or aerial** Acoustic line transect (or fixed point) (*presence/absence*)

INDICATOR 4, species population abundance	**Visual Line transect “distance sampling” boat or aerial** Acoustic line transect (*indices of relative abundance*) Photo-identification	**Visual Line transect “distance sampling” boat coupled to acoustic line transect** **Photo-Identification**	**Visual Line transect “distance sampling” boat coupled to acoustic line transect** **Photo-Identification**	**Visual Line transect “distance sampling” boat or aerial** Acoustic line transect (*indices of relative abundance*)	**Visual Line transect “distance sampling” boat or aerial** **Photo-Identification** Acoustic line transect (*indices of relative abundance*)	**Visual Line transect “distance sampling” boat or aerial** **Photo-Identification** Acoustic line transect (*indices of relative abundance*)	**Visual Line transect “distance sampling” boat or aerial** Acoustic line transect (*indices of relative abundance*)	**Visual Line transect “distance sampling” boat or aerial** Acoustic line transect (*indices of relative abundance*)
INDICATOR 5, Population demographic characteristics	Biopsy Stranding By-catch	Biopsy Stranding By-catch Photo-identification	Biopsy Stranding By-catch Photo-identification	Biopsy Stranding By-catch	Biopsy Stranding By-catch Photo-identification	Biopsy Stranding By-catch Photo-identification	Biopsy Stranding By-catch	Biopsy Stranding By-catch

Table 2- Synthesis for the different cetacean's monitoring methods concerning which indicators of the IMAP process they may help with, the time delay to obtain results, the type of results, their cost, the level of constraints associated, their limits or bias and an indication concerning the compatibility among methods. + = low, +++ = high.

Method	Indicator	Type of results	Rapidity of results	Compatibility with other methods	Costs	Constraints	Limits
Visual Line transect "distance sampling" **boat**	3- distributional range 4- abundance	3- distributional range : *presence/absence, spatial and temporal distribution, relative density* 4- abundance *absolute and relative, density*	Short-term	**acoustic line transect** (sometimes photo-Identification if approaching mode)	++++	+++	Bias due to responsive movements of animals; detectability to be assessed,
Visual Line transect "distance sampling" **aerial**	3- distributional range 4- abundance	3- distributional range : *presence/absence, spatial and temporal distribution, relative density* 4- abundance: *absolute and relative, density*	Short-term		++++	++++	For deep diving species the number of sightings will be too low to give reliable results.
Photo-identification	3- distributional range 4- abundance 5- demographic characteristics	3- distributional range: *occurrence, spatial and temporal distribution* 4- abundance: *absolute* 5- demographic characteristics: *ranging behaviour, migration patterns, body size or age class structure, sex ratio, fecundity rates, survival/mortality rates*	Can be medium-term but is far more reliable on long-term	**biopsy and telemetry** (sometimes line transect boat, depending if approaching mode)	++	++	Only applicable for species with long-lasting individual identifiable natural marks.

Method	Indicator	Type of results	Rapidity of results	Compatibility with other methods	Costs	Constraints	Limits
Land based method	3- distributional range 4- abundance	- distributional range: *presence/absence, locally temporal distribution* 4- abundance: *indices of relative abundance*	Short-term and long-term	acoustic fixed point, (photo-Identification depending on conditions)	+	+	Limited to small detection area and suitable coastal landscape.
Acoustic line transect	3- distributional range 4- abundance	3- distributional range: *occurrence index* 4- abundance: *indices of relative abundance*	Short-term	**visual line transect**	+++	+++	Relies upon animals being vocal.
Acoustic fixed point	3- distributional range 4- abundance	*3- distributional range: occurrence index* 4- abundance: *indices of relative abundance*	Short-term	land based method (if near coast)	++	+	Relies upon animals being vocal. Low spatial resolution or need a network of several hydrophone, and logistical problems with deployment.
Telemetry	3- distributional range	3- distributional range: *spatial and temporal distribution*	Short term Long-term	**biopsy and photo-Identification**	+++	++++	Only allows small samples resulting in much inter-individual variation. Invasive.
Biopsy	5- demographic characteristics	5- demographic characteristics: *sex ratio, fecundity rates*	Long-term	**photo-Identification, telemetry**	++	+++	Invasive method. Requires large sample size.
Stranding	3- distributional range (4- abundance) 5- demographic characteristics	3- distributional range: *occurrence index* 4- abundance: *indices of relative abundance* 5- demographic characteristics: *body size or age class*	Short- and long-term		+	+	Efficient if networking is implemented.

Method	Indicator	Type of results	Rapidity of results	Compatibility with other methods	Costs	Constraints	Limits
		structure, sex ratio, survival/mortality rates					
By-catch	3- distributional range 5- demographic characteristics	3- distributional range: *occurrence index* 5- demographic characteristics: *body size or age class structure, sex ratio, survival/mortality rates*	Short- and long-term		+	+	Efficient if special observers are involved, or a reporting well established program is implemented by Fisheries Agency
Unmanned Autonomous vehicle (drone and submarine AUV)	3- distributional range 4- abundance	3- distributional range: *spatial and temporal distribution* 4- abundance: *relative, (absolute if line transect)*	Short- and long-term		++++	+++	Method in development.
Pictures and video	3- distributional range 4- abundance	3- distributional range: *occurrence index, spatial and temporal distribution* 4- abundance: *relative, (absolute if line transect)*	Long-term	line transect aerial	++	+++	Method and technic in test, not standardised yet.

Table 3- Synthesis for the different cetacean's monitoring methods about the material and human resources involved, an indication about volume storage of data and time needed to process the analysis, and the level of skills needed (+ = low, +++ = high).

Method	Material needed Colour legend: in black "investment" ; in orange "operational "	Platform	Minimum n. Of persons needed	Data storage (volume)	Data processing and analysis (time))	Skills
Visual Line transect "distance sampling" **boat**	- binoculars - GPS, watch - instruments to estimate or measure the distance of the animals from the boat (reticulate binoculars, measuring stick) - observation forms or computer - corner quadrants or angle board	Vessel dedicated (like motor or sailing boat) or not dedicated ("fix line" like ferries or oceanographic vessels)	4	++	++	++
Visual Line transect "distance sampling" **aerial**	- observation forms computer with dedicated software a person to enter data in real time, and/or dictaphone - clinometer - GPS,	Airplane small, high-wing, that can fly slowly while remaining within the limits of safety, equipped with bubble windows (to allow the observer to look under it) and can carry at least three people (two observers and a data recorder).	3 + pilot	++	++	+++
Photo-identification	- observation forms or computer or mobile phone - GPS, - camera with lens	Vessel small or relatively small boat (outboard or an average zodiac boat) with a sufficiently low bridge over the water to take pictures at the correct angle.	1 (3)	+++	+++	+

Method	Material needed Colour legend: in black **"investment"** ; in orange "operational "	Platform	Minimum n. Of persons needed	Data storage (volume)	Data processing and analysis (time))	Skills
Land based method	- binoculars or telescopes - observation forms or dictaphone or computer - watch - theodolite or clinometer camera for photogrammetry - Compass or quadrant angles or angle boards	Land	1 (2)	+	+	++
Acoustic line transect	- binoculars - GPS, - observation forms - hydrophone coupled to stereo amplifier - sound-recording instrument and power source	Vessel Irrespective of the type, which is able to hold a constant speed and a course for use in transect. Preferably silent.	1 (2)	+++	+++	+++
Acoustic fixed point	- binoculars - GPS, watch - observation forms - hydrophone coupled to stereo amplifier - sound-recording instrument and power source	Beacon, buoy Or vessel	(1)	+++	+++	+
Telemetry	- beacon - crossbow or long pole	Vessel	1 (2)	+	++	++
Biopsy	- crossbow or gun and bolts - storage and cleaning material - freezer/frozen storage	Vessel small or relatively small boat (outboard or an average zodiac boat) with a sufficiently low bridge over	1 (2)	+	+++	++ Need specific skills

Method	Material needed Colour legend: in black "investment" ; in orange "operational "	Platform	Minimum n. Of persons needed	Data storage (volume)	Data processing and analysis (time))	Skills
		the water to shoot at the correct angle.				
Stranding	- stranding forms - camera - tape measure - sampling kit (knife, shears, packaging materials) - dedicated dress, safety gloves, safety glasses - freezers - fixing solution such as formalin, ethanol, DMSO	Land	1	+	+	++ Need to make sure this is handled by a trained and authorized scientist or veterinary
By-catch	- GPS, watch - observation forms - camera - tape measure - sampling kit (knife, shears, packaging materials)	Vessel	1	++	++	++
Unmanned Autonomous vehicle (drone and submarine AUV)	- drone or submarine AUV	Vessel	1 (2)	++	++	+++ Need specific skills
Pictures and video	- high resolution camera	Airplane	(1) + pilot	+++	+++	++

Table 4 – Characteristics of cetacean's monitoring methods in regard to indicator 3, 4 and 5 of the IMAP process : at which level they are implemented (population or individuals) and at which spatial scale they correspond the best (small or large area). The darker the colour, the best suited characteristics and the lighter the colour, the more adaptation you have to implement this method for that area or level. Method implemented on individuals can be designed (network, large samples size) in order to give results at the population level (for indicator 5). In cells is given an indication of the time frame and frequency of the campaigns implementing the described methods at the corresponding spatial scale.

Cetacean monitoring method	Population level	Individual level	Large area	Small area
Visual Line transect "distance sampling" **dedicated boat**			1 or 2 / 6 years	Yearly or seasonal
Visual Line transect "distance sampling" **dedicated aerial**			1 or 2 / 6 years	
Visual Fix line transect by ferry or oceanographic vessel	X		Yearly, seasonal or monthly	
Acoustic line transect			1 or 2 / 6 years	Yearly or seasonal
Dedicated observers on opportunistic platform			Yearly or seasonal	Yearly or seasonal
Photo-identification	X		(network) Yearly or several years	Yearly or seasonal
Telemetry				
Biopsy	X			
Land based method				Yearly or seasonal
Acoustic fixed point	X		(network)	Yearly or seasonal
Stranding	X		(network)	Seasonal, monthly
By-catch	X		(network)	Seasonal, monthly

Tableau 5 - Compatibility with other species monitoring for the indicator 3, 4 and 5 5 (X: method compatible with others; 0: method not compatible with other species)

Cetacean monitoring method	Seabirds at sea	Turtles at sea	Sharks	Other big fish (tuna, sunfish, swordfish, ray)	Floating Marine Litter
Line transect "distance sampling" **dedicated boat**	X	X	X	X	X
Line transect "distance sampling" **dedicated aerial**	X	X	X	X	X
Fix line transect "distance sampling" **by ferry or oceanographic vessel**	X	X	X	X	X
Dedicated observers on opportunistic platform *(line transect)*	X	X	X	X	X
Photo-identification surveys	X	X	X	X	X
Land based method	X	0	0	0	0
Acoustic line transect	0	0	0	0	0
Acoustic fixed point	0	0	0	0	0
Telemetry	X	X	X	X	0
Biopsy	X	X	X	X	0
Stranding	0	0	X	X	0

By-catch	X	X	X	X	X

3.2. General considerations

3.2.1. Scientific consideration on sampling and analysis

18. To ensure that the chosen method and the study design will be able to provide data to answer to the question posed with a useful level of precision, a power analysis should be run. It is useful to use existing data if any during this step. And the power analysis helps in indicating the ability of the statistical procedure and the available or planned data to reveal a certain level of change i.e. the ability to detect a trend of a given magnitude. Concretely the power analysis will help to plan studies to calculate the necessary sample size (e.g. the length of time series of abundance estimates), or the coefficient of variation (CV) of those estimates.

19. The use of existing software programs, as "TRENDS" (freely available at (https://swfsc.noaa.gov/textblock.aspx?Division=PRD&ParentMenuId=228&id=4740) helps greatly in the process. But as cetacean's species are highly mobile, spread over vast areas which led to difficulties to cover the whole population or their whole range, another method to increase power to detect trends is to design a trend-site survey design. This site is sought to maximize precision by focusing on a smaller area to survey and increased the effort in the chosen area. The smaller area could correspond to a representative part of the range of the stock or to a stock identified at a smaller spatial scale as demographically independent populations. Finally, one of the most common methods to increase our ability to detect precipitous declines are to increase survey frequency (annual for example). Other useful methods are tested, more during the analysis, as to change the statistical decision criterion.

20. Many of the methods here described work under certain assumptions (equal coverage, homogeneity of capture, detectability, etc) and a great care should be taken in dealing with these assumptions since the beginning of the implementation. Associated data should be collected in order to calculate the correction factors if needed.

3.2.2. Complementarity of monitoring methods

21. There is an interest in implementing several methods, as they can be complementary in spatial or temporal scales and for the different species. This should be defined case by case, according to the objectives, the species, the area and the means (human resources, platform and funds). As the objective of monitoring population of cetaceans is to detect trends over time, it has then to be considered to choose one or several methods and to plan to implement campaigns on a regular basis in order to get several results over time. Often, large-scale dedicated campaigns are more expensive than non-dedicated campaigns or small-scale campaigns. For example:

- **a large-scale** (the whole waters under national jurisdiction of a country at least, entire basin, entire seas) **visual line transect distance sampling dedicated survey** made with a vessel or an airplane will give you surface estimate of abundance and distribution of several visible and numerous species (whales and delphinids). In the meantime, if the campaign is boat-based, you can add a hydrophone to the vessel to collect **passive acoustic** data on abundance, distribution and presence/absence of deep diving species (sperm whale, Ziphiidae) and/or acquire data to correct availability bias for the deep diving species. As those large-scale dedicated campaigns might be one of the most expensive methods, they are often implemented at least once or twice per decade.

- In parallel **non-dedicated vessel - or aerial- based line transect surveys** should be implemented to get data and results on a yearly basis (with one or two samples a year for oceanographic campaigns, even one sample per month for ferry). This will allow you to know inter-annual variability (year with typical, rich or poor abundance) and to correct the results of your dedicated large-scale survey the year it is implemented.

- When an important or representative **smaller area** is defined (MPA, Important Marine Mammal Are, etc), based on the results of this/these previous large surveys, you can implement **visual and acoustic line transect**

distance sampling surveys in this small representative area. Ideally, seasonal monitoring programmes should be conducted at this scale (at least during winter and summer periods).

- And finally, you can focus on some species and launch **individual-based tracking**, implementing photo-identification, biopsy and/or telemetry programmes. Those methods are highly complementary to the previous ones.

3.2.3. Trained and qualified personal

22. These methods are rigorous and high quality designed, implementing standard protocols and awaiting standard data. So, people implementing one of these methods at sea should be trained to acquire the requested skills and knowledge to do it in the correct way. If necessary, funds for training must be included in the program's budgets.

3.3. Standard Monitoring methods of living animals

3.3.1. Visual monitoring method

23. For visual surveys, it is important to consider observer skill and experience. Observers may vary in sighting efficiency; hence, training is important to obtain consistent results in species identification, counting of individuals and measuring information (distance, angle, time of diving…). An observer training must be scheduled upstream to visual monitoring campaigns.

3.3.1.1. *Line transect "distance sampling" method*

24. In line transect "distance sampling", a survey area is defined and surveyed along a sampling design of pre-determined transects ensuring equal coverage of the area. The perpendicular distance of each detected animal to the transect is measured and consequently used to obtain a detection function, from which an estimate of the effective width of the strip that has been searched can be calculated. Abundance is then calculated by extrapolating estimated density in the sampled strips to the entire survey area by means of dedicated software. The calculated number is therefore an estimate of surface abundance in a defined area at the time of the study. Assumptions relating to specimens' detectability and observer perception (availability and perception biases) need to be addressed and various methods (respectively telemetry data and two-platforms surveys) have been developed to accommodate these.

25. This method, either boat- or aerial-based, is mainly used to collect data in order to answer to abundance and distribution questions on cetaceans (indicator 3 and 4). When the platform is dedicated to the mission of collection of data on cetaceans, the whole process of implementation is better robust, namely quantity of effort, equal coverage probability with dedicated sampling design, bias on detectability, etc. When observers go aboard a non-dedicated platform, the data collection are not designed and can not provide all necessary data to ensure a robust results and data to detect trends, Finally, observers on opportunistic platform collect complementary data that could be less robust to answer to the indicators. But this has to be assessed in a case by case cost-benefice study, as in several occasions, something interesting can be launch with existing platforms and analysis exist taken into account the heterogeneity in the effort.

3.3.1.1.1. Dedicated boat-based survey

Principle

26. Systematic surveys carried out from a boat constitute a powerful method primarily aimed to estimate the surface abundance and distribution of cetacean species over large areas. The boat follows a path corresponding to a predefined sampling plan, which covers the area of study as homogeneously as possible and records all cetacean sightings. The minimum amount of effort required to perform the analysis depends on the density of animals in the study area. The amount of effort can be calculated before designing the sampling plan. Often it is required that at least 40 sightings of one species is needed to get reliable results with lower uncertainties depending on the species and the area investigated. To cope with assumptions (perception, availability and responsiveness), often a two-platform surveys is implemented,

corresponding to two different teams of observers working independently of each other on the same platform. Comparing their specific data helps in correcting the perception bias.

Human resources

27. The Line transect method required that 180° in front of the vessel is continuously observed during all daylight hours. This required that at least two trained observers are watching at all time, and to allow resting and mealtime, it is required at least two teams rotating each two hours. So, for long lasting mission, a team of 4 trained observers is a minimum, the best option is at least 3+3 allowing a better coverage and a person also dedicated to record the sightings and all associated information. For double platform then, a supplementary team of 3 observers is requested.

Material needed

28. The equipment needed are as follows:

- A boat with the required characteristics (adequate height, speed…) to carry out the mission for the planned duration, the survey area and the desired collection protocol.

- Binoculars (and for double-platform, a high-power ("big eyes") binoculars on a tripod or other support).

- Compass or angleboard.

- Instruments to estimate or measure the distance of the animals from the boat (reticulate binoculars or a video camera for photogrammetry, or measuring sticks or ruler, etc.).

- Observational forms and a computer with a dedicated software for data (both navigation and sightings) storage.

- A watch.

- A GPS.

Implementation

29. The first phase is the preparation of the campaign, with training of people if needed, design the sampling scheme according to densities of cetaceans (if known) and habitats. Also, everything concerning authorization request and logistic should be considered largely before.

30. Effort should be precisely known, so start and end are recorded (hours and geographical positions). During effort, observers scan the water for cetaceans while the vessel steams along predetermined transect lines at constant speed and heading. Often the speed is at 10 knots for large vessels, but it can be 8 or 6 knots for smaller vessels. When cetaceans are seen, the observers record data such as the species, location (latitude and longitude) of the encounter, general behaviour of the animals, estimates of the number of cetaceans in the group, measure the distance from the observation point and calculate the angle from the bow. The sighting data are later analysed using distance sampling statistical models and imported into a Geographical Information System (GIS) for further spatial analysis.

31. This method is reliable when wind, sea state and visibility are adequate to detect small dolphins, and the limit if often put to Beaufort wind less or equal to 3. Also, Douglas sea state (from 4) can be a limitation to detect small delphinids.

This type of monitoring may require some authorizations procedures, depending on study area (environmentally protected zones, cross border areas).

Advantages

32. the advantages are:
- Allow representative coverage of areas.

- Different types of sample designs are available according to the characteristics of the study area and the census itself. The design of the sampling plan can be done using software DISTANCE (http://www.**distancesampling**.org).

- Protocols for data collection are standard and widely used; they are tested and improved continuously.

- Analytical methods are also standard, tested and constantly improved in order to minimize the influence of potential biases.

- Often, large vessels are required to cover large areas (vessels can remain at sea for many days, which can stay on course and maintain speed regardless of the sea state and can board enough personnel to allow rotation of the observer teams and secretaries). However, this method can also be applied to small areas with smaller boats (sailing vessels, motorboat).

Limitations

33. Limitations are:
- This method is expensive, labour intensive and give little spatial coverage.
- Responsive species movement prior to detection (i.e. attraction to, or avoidance of, the vessel) is difficult to predict but can generate substantial bias in estimates of abundance if it occurs. It must then be tested and calculated during the analysis.
- Theoretically, the line transect should not be interrupted: the boat must be in "passing" mode, that is to say, it does not stop or turn away, which could lead to potential biases. Therefore, species identification and counting of individuals in groups can sometimes be difficult and it is incompatible with the collection of ancillary data, such as photographs for photo-identification, biopsies. It may be possible to make a part of the sampling plan in the "closing" mode where groups of easily identifiable and countable cetaceans are then approached on "off effort" before resuming the transect path in "on effort". In this case, it is important to estimate the bias introduced in the protocol by this manoeuvre and preserve it for conditions with real difficulties.

3.3.1.1.2. Dedicated aerial-based survey

Principle

34. Working by aerial means (airplane) is a powerful method, primarily aimed at assessing the abundance and distribution of marine species over large areas or areas inaccessible by boat (far offshore area, harsh weather conditions, etc.). The platform used in most cases is a small airplane with two observers aboard and a data recording. The airplane follows the path of a predetermined sampling plan to cover a large area according to the equal coverage probability, noting all cetacean sightings.

Human resources

35. At least 3 trained "aerial" observers should constitute the team in one airplane, 2 observers and 1 real time data recorder. In case of the double platform two more researchers are needed

Material needed

36. Material required are as follows:
- A small, high-wing airplane with two motors, that can fly at 90 knots while remaining within the limits of safety. The airplane must be equipped with bubble windows (to allow the observer to look under the plane) and can carry at least three people (two observers and a data recorder) beside the pilot.
- Observation forms and a computer with a person to enter the data reported by observers in real time, or a dictaphone.
- Two clinometers, one for each observer.
- printed angleboards
- A GPS
- A computer with dedicated maps and software.

Implementation

37. The first phase is the preparation of the campaign, with training of people if needed, design the sampling scheme according to densities of cetaceans (if known) and habitats. Also, everything concerning authorization request and logistic (localisation of airports, availability of fuel) should be launch largely before.

38. The pilot of the plane is in charge of following the flight plan defined and surveyed along pre-determined transects. Two observers sit at the rear seats equipped with bubble windows on the left and right side of the plane scan the water for cetaceans. The third scientist, the navigator, sit in the front at the co-pilot seat, is responsible for the flight plan too, entering effort data, environmental conditions and sightings data in real time into a laptop during the flight. When cetaceans are seen, the observers record data such as species, estimated group size and declination angle when animals are abeam (the angle between the observation's point and t the vertical line between the sea surface and the plane). The sighting data are later analysed using distance sampling statistical models and can be imported into a Geographical Information System (GIS) for further spatial analysis.

39. This type of monitoring required a lot of authorization procedures specifics to aviation, in particular in cross border areas and also concerning airport use and fuel availability.

Advantages

40. The main advantages are:
- This technique is usually more profitable than large surveys over large areas, which would be conducted from the boat.

- Large areas can be covered in a short time and remote areas are reached quickly to study them (although the distance depends on the autonomy of the aircraft).

- Some sea conditions, such as waves, interfere much less when working from the airplane than from a boat.

- Provide opportunities to detect wildlife in real time and refine species identifications and group size count using a circle-back approach.

- The movement reaction issue (avoidance or attraction) is generally non-existent (if the aircraft is high enough and passes only once).

Limitations

41. Main limitations are:
- Visibility must be excellent (good sea conditions, clear sky, no glare, etc.).

- There are difficulties in identifying some species and counting large aggregation of cetaceans, merely small delphinids, due to the altitude and / or speed of the aircraft, which allow only few seconds to the observers to collect all the data. Pictures or video can help for those points.
- Sometimes the availability of appropriate aircraft characteristics (slow flight, high wings, sufficient autonomy, etc.) or fuel for such kind of aircraft, is rare.
- Data collection by air is expensive, particularly in remote regions away from airports.
- Aerial surveys are logistically difficult to implement and incur high costs from aircraft hire and staffing and can be limited by flight regulations and safety considerations.
- This technique is not the best one to study deep diving species like sperm whale or beaked whale, staying a long time not visible at surface or subsurface; nevertheless, data collected for deep divers can be corrected with the data on the proportion on time the animals are unavailable to be seen.

3.3.1.1.3. Not dedicated boat-based survey, or Fix line transect "distance sampling" by ferry or regular oceanographic vessel's campaigns

Principle

42. Surveys are conducted along fixed transects using passenger ferry or oceanographic vessels as platform of observation. Teams of trained marine mammal observers (MMO) board either a passenger ferry which conducted almost identical transects from month to month or an oceanographic vessel conducting regularly the same design over the same area (for example yearly national small pelagic fish stock assessments campaigns). Data collection of occurrences of marine mammals are conducted on "passing" mode, that is to say, it does not stop or turn away. The method implemented is the line transect and the purpose of the method is to repeat the same transects in the long-term.

43. On those kind of vessel, reliable data on distribution and abundance can be collected, depending on the type of routes and regularity of crossing. For example, in the Pelagos Sanctuary, the ferries run almost all year round, on numerous routes crossing part of the area, ensuring a good temporal coverage. Also, oceanographic small fish stock campaigns often follow a tied coverage of their area of interest. Those data may be of great interest to answer to indicator 3 and 4 in those conditions.

Human resources

44. The Line transect method required that 180° in front of the vessel is continuously observed during all daylight hours. This required that at least two trained observers are watching at all time, and to allow resting and mealtime, it is required at least two teams rotating each two hours. So, for long lasting mission, the number of 4 trained observers is a minimum, the best option is at least 3+3 allowing a better coverage and a person also dedicated to record the sightings and all associated information.

Material needed

45. The needed materials are:

- Passenger ferry using fixed lines allowing repetitions or oceanographic vessel implementing on a regular basis the same (or equivalent) design in the same area
- Binoculars
- Compass or angleboards
- Instruments to estimate or measure the distance of the animals from the boat (reticulate binoculars, measuring sticks and clinometer).
- Observational forms and a computer.
- A GPS.

Implementation

46. Observer's team conducted the survey from the deck of engine control room of the vessel or outside in a free of obstacle's observer point. They are divided on each side of the ferry/oceanographic vessel and collect data of cetacean's occurrence continuously on both sides. When "on effort", they scan carefully the area (with a focus on the 180° to the front of the boat) by eye and using binoculars, so as to detect visually cetaceans present on surface.

This type of monitoring required some agreements with ferry companies/oceanographic/fishery institutions.

Advantages

47. The advantages are:

- This method, in a representative sector, gives relevant indicators of what occurs surroundings (in terms of distribution and indices of abundance).
- It is a cost-effective means of providing wide coverage over protracted periods. Furthermore, the use of these platforms allows to realize a monitoring all year round or yearly and at a lower cost.
- The regularity with which the crossings are made allows to repeat the operation as much as desired to refine a study.
- in some areas, ferry routes make a kind of sampling design relatively tied, allowing a good coverage of the area (ex.: Pelagos Sanctuary), and also oceanographic small fish stock campaigns often follow a tied coverage of their area of interest.

Limitations

48. The limitations are:

- The major limitations are that there is rarely any control over the routes taken which are already designed, nor the speed of the vessel, and the vessel typically cannot divert from its track to confirm species identity or group size.
- Sometimes the required number of even only 2 observers cannot be allowed aboard, depending on the size of the vessel
- The application of this method is strictly speaking incompatible with the collection of ancillary data focusing on individual animals, such as photographs for photo-identification or biopsies.

3.3.1.1.4. Dedicated observers on opportunistic platform (military, custom, navy, whale-watching boats)

Principle

49. One or more observers board an opportunistic platform and benefit from the platform route to make observations without logistical implementations. Platforms can be boat-based or aerial-based.

50. Ideally, the effort should be significant to obtain a large number of observations and cover as homogeneously as possible the different values used in the environmental variables' analysis. So, the platform should go at sea on a regular basis, and within the same area to be of some interest in monitoring objective of distribution and indices of abundance. So, military or custom's vessel, airplane or helicopter can be targeted, as well as whale-watching boats.

51. This method, not dedicated to cetaceans studies, are less robust to answer to the assumptions needed to get reliable and precise results in terms of indicator 3 and 4. Nevertheless, the fact that the same area is regularly sampled in the same way, allows to gain knowledge on occurrence, presence and to compare these results between seasons and years.

Human resources

52. Depending on method implemented, size and authorization of the platform, at least 1 trained observer is required, and the higher the number of observers, the higher the quality of visual coverage and data recording.

Material needed

53. The needed materials are:

- Binoculars.
- Compass or angle-boards
- Instruments to estimate or measure the distance of the animals from the boat (reticulate binoculars, measuring sticks, clinometer).
- Observational forms and a computer.
- A watch.
- A GPS.

Implementation

54. Observers team conducted the survey and scan carefully the area, with a focus on the 90° to the front of the boat, and with a focus below and perpendicular to trackline for aerial platform. Searching visually cetaceans present on surface has to be done by eyes and binoculars are used to precise parameters such as species, numbers, etc. During every observation period they record the begin and end of effort, the environmental condition and sightings data such as species, estimated group size, behaviour GPS location. Depending on the platform and its mission, ancillary data may be possible to collect. This type of monitoring required some agreements with other structures.

Advantages

55. The avantages are:

- Platforms of opportunity are often used to survey areas at low cost. In some cases, costs may be relatively small because boats and equipment can be minimized without compromising the reliability of the results of a simple, but adequate data collection protocol.
- Data collected from an opportunistic platform can still be used to assess habitat use and to estimate the abundance of animals through spatial modelling. In addition, the use of environmental characteristics to estimate abundance or relative abundance can potentially increase the accuracy of results. Finally, some platforms allow photo-identification or acoustic data to be taken.

Limitations

56. The limitations are:

- The major limitations are that there is rarely any control over the routes taken, the speed of the vessel, the ability of vessel to divert from its track to confirm species identity or group size and even to take ancillary data (photo-identification). But this may vary greatly depending on the type of platform and mission.
- Monitoring implementation can be a low priority in initial objectives of the platform.
- The use of this kind of data should be done carefully, because there might exist a lack in the sampling design with uncovered area, heterogeneity in effort coverage across the range of values for the explanatory variables, etc.
- area covered might be small and unrepresentative for cetaceans

3.3.2. Passive acoustic monitoring

57. All cetaceans produce sounds like "clicks" for echolocation or "whistles" (frequency modulated sounds) for intraspecific communication. Passive acoustic methods allow the near-continuous detection and monitoring of those sounds. The monitoring of these sounds allows for the collection of information on spatial and temporal habitat use, as well as estimation of relative density for some species and even abundance for sperm whale.

3.3.2.1. Passive Acoustic "line transect" (towed hydrophone)

Principle

58. One array with at least two hydrophones are towed by a moving boat. Listening and recording can be continuous or by samples. The array enables to determine angle at perpendicular distance, which is the base of the analysis of the "line transect" method. The trajectory of the boat should be constant in speed and heading, following a predefine design or random transects.

59. The area covered is bounded by the probability of detection by the hydrophone and the frequency and power of the sound made by the animals.

60. This is the most effective method to survey sperm whale, as they are long-deep diving species, and they use "clicks" during the entire duration of their dives. Acoustic data from sperm whales can be used to assess both relative and absolute abundance and also distribution, provided that the appropriate equipment and survey design is followed. For other species, acoustic results might be complementary to visual for indicator 3, but not for indicator 4 as methods to relate sounds to abundance of animals are not efficient yet.

Human resources

61. At least one passive acoustic operator is needed, or more for a 24 hours work.

Material needed

62. The needed materials are:

- A boat, motor or sailing one, which is able to hold a constant speed and heading for a transect and be silent or can stop the engine often (for sampling).
- A whole acoustic acquisition chain:
    - hydrophone array composed of at least two hydrophones (even two arrays of hydrophone) coupled to stereo amplifiers and which is within a pipe that can be towed.
    - A DAQ system (convert the signal from analogue to digital format and convert in quantization)
    - A computer with a software analyzing sounds.
    - and a power source to power the system

- The relevant data forms.
- A GPS.

Implementation

63. The first phase is the preparation of the campaign, with training of people if needed, design the sampling scheme according to densities of cetaceans (if known) and habitats. Also, everything concerning authorization request and logistic should be launch largely before.

64. An acoustic acquisition chain is setup, comprising a tow cable into which is incorporated a linear array of two pairs of hydrophones, a deck cable that connects to the tow cable and carries signals to wherever the PAM station is set up. The electronic equipment at the PAM station provides power to the system, amplifies and digitises signals before feeding signals to one or more PCs that provide the user interface (software) and store the data. If continuous acoustic detection is chosen, the vessel starts the transect with the acoustic acquisition chain in position. The start of the effort is when the acoustic detection of animals is launch.

65. If sampling procedure is used, that means that regularly a listening period is implemented. For example, the standard is to listen for 2 minutes during each 15 minutes. Often, the speed of the boat is decreased at minimum in order to reduce engine noise and noise of the water flowing on the hydrophone. Using hydrophone at sea is often linked to special authorizations to acquired.

Advantages

66. The advantages are:

- This method is cost-effective, autonomous and it provides valuable information without disturbance to wildlife or their habitats.
- The detected radius can be very large for some species: most Mysticeti can be detected at tens or hundreds of kilometres. Depending on the equipment used, the ambient noise and the characteristic of the water for acoustic propagation, dolphins can be detected at distances up to 3 km in good conditions.
- The acoustic approach potentially detects the presence of a cetacean that is not visually observable because it is too far, it remains underwater, it moves at night or the weather conditions deteriorate. This method offers a valuable alternative for monitoring biodiversity when traditional (e.g. visual) surveys are impractical or impossible.
- Acoustic work can easily be done on a great type of vessels, from small boats or even opportunistic platforms to large vessel.
- This technique is not intrusive, and the necessary equipment is not particularly expensive.
- This approach records sound for documentation or future analysis and it is easier to standardize and automate data collection.
- A key benefit of active acoustic methods lies in their fine spatial resolution and their ability to collect data on multiple species simultaneously and nearly continuously from a moving vessel.
- Acoustic data are largely independent of collection error and inter-observer bias.
- A mobile approach grants larger geographic coverage.

Limitations

67. The limitations are:
- This method relies upon animals being vocal.
- Methods to relate sounds to abundance of animals are not well developed. In case of numerous animals, it is impossible to know which individual emits the sound and it is very difficult to know the number of animals in a group.
- Difficult identification for close species, mainly small dolphins (e.g. striped dolphin and common dolphin)
- Acoustic behaviour depends on the activity of a group, not necessarily the number of individuals, which can move without making any sound.
- Ambient noise and the noise generated by the research vessel can make the acoustic detection of an animal difficult. Detection probability is also a function of background noise, with acoustic interferences such as masking potentially species identification and group size estimation.
- Requires specialist data collection equipment.
- The volume of data typically generated by passive acoustic methods is enormous and requires significant investment in storage and after in post-processing.
- Small towed hydrophones are not suitable for the detection of low-frequency and infrasonic sounds simply because the vibrations and movements of hydrophones mask these sounds.
- Almost all hydrophones are sensitive to frequencies from a few hertz. This is why, it is often necessary to use a high-pass filter to remove low-frequency noise.

3.3.2.2. Fix passive acoustic

Principle

68. One (or more) hydrophone(s) is installed in one (or more) fixed strategic sites, either on the ground, or on a boat or a floating platform. Opportunistic or non-dedicated platforms or stations can be used. Sound recording is done continuously or at a regular frequency (sampling). Positioning at least three hydrophones also allows triangulation to precisely locate the animal emitting the sounds. The more hydrophones, the larger the area covered. So, network of several hydrophones is necessary to increase the interest of such tool for monitoring the presence and indices of abundance of several species.

Human resources

69. At least one acoustician should build the acoustic acquisition chain. Then, depending on the situation (coastal or at sea), a ship with pilot should be needed and one diver will setup the system out at sea. The same people might be needed when the equipment has to be changed (batteries if any, hard drive when it is full…).

Material needed

70. The needed materials are:
- A stereo hydrophone amplifier coupled to a transmission cable, a DAQ converter (digital and quantization of the signal), a hard drive to store data, a power source to power everything and finally a protection unit and fixations to install all equipment.
- A thermometer and a probe coupled to the sub-sea installation to enrich the data.

Implementation

71. The site is identified, the type of fixation is defined (depending on ground type, currents, etc) and the hydrophone system is installed. An existing underwater structure can be used, but caution should be made on the noise made by the structure, the more silent the better. Divers may install the acoustic system which will collect data for a predetermined period, mostly depending on capacity storage or power supply of the batteries. Then records (data) are being recovered for analysis. The system can stay for short, medium or long period. The recovering of the data and the changing of the batteries can sometimes be done without removing the whole system. Using hydrophone at sea is often linked to special authorizations to acquired.

Advantages

72. The advantages are:

- Passive hydroacoustic is ideal in long-term monitoring programs and can run on continuous 24-hour cycles, independently of weather conditions. By recording all animals moving close to a given listening station, it is possible to study temporal variations, ranging from the annual scale, to the monthly and daily scale.
- This technique is non-invasive, and the cost of basic equipment is not very high.
- Acoustic data are largely independent of collection error and inter-observer bias.
- The system can be automated and requires no human presence on site. It is easier to standardize and automate data collection.
- Detection over 360° and in almost all weather and light conditions.
- If the installed system is permanent, detection and temporal coverage will work 100%.
- Depending on how the hydrophone is positioned, the material, the water characteristics of sound propagation and the ambient noise, the monitoring area for dolphins is about 3-6 km because there is no noise from the boat. Tracking sperm whales and the Mysticeti can be extended to tens of kilometres.
- The system can sample regularly or continuously areas that are difficult to access.
- Concerning the surface system on a floating platform:
    - It can be self-contained with a power supply from solar panels or wind turbines.
    - Data can be transmitted via VHF waves or Wi-Fi, allowing real-time application.
    - Settings can be changed easily by easily accessible instruments (gain, filters, etc.).
- Concerning the system deployed on the seabed:
    - Discreet and less vulnerable to surface activities.

Limitations

73. The mimitations are:

- Detection probability and receiver performance are also a function of background noise, with acoustic interferences such as masking potentially hampering species identification and group size estimation
- This method relies upon animals being vocal.
- In this fixed method, the coverage is limited to the "immediate" vicinity of the system.
- Corrosion, fouling, and damage from currents, tides, storms, or fishery operations can all affect the longevity and efficiency of acoustic instruments.
- Methods to relate sounds to abundance of animals are not well developed. When animals are in a group, it becomes difficult to identify the individual that issued the sound and how many animals are present. There is a risk of multiple detection of the same group.
- Areas subject to strong tidal currents should be avoided due to noise or risk of damage to facilities (current, debris, etc.).
- Noise near the coast can mask the acoustic detection of an animal.
- Acoustic behaviour depends on the activity of a group, not necessarily the number of individuals, which can move without making any sound.

- As part of a network of permanently installed hydrophones to detect all species, including those that emit very low or very high frequencies, the cost of the equipment required is very high.
- It is hard to differentiate between small dolphins' species
- Concerning the surface system on a floating platform:
  - Susceptible to all weather conditions on the surface;
  - Vulnerable to all activities taking place in the area (possibility of degradation or loss of the equipment) and preferably protected from free access of people.
- Concerning the system deployed on the seabed:
  - The power supply is complicated (cable? battery to change?);
  - Need to dive in the site to change settings, difficult access to instruments;
  - What type of data transmission: by cable or storage?

3.3.3. Monitoring based on focal tracking of individuals

74. The previous methods described work more at a population level. Some specific monitoring focus on individuals. When the samples are numerous, they can give results at the population scales. Most of these methods are complementary to the previous ones, providing information to help to define 'population' for example, apart for photo-identification that can produce population estimates directly, through mark-recapture. Biopsy provide valuable data to the indicator 5.

3.3.3.1. Photo-Identification (or photo-ID)

Principle

75. Scientists use the photo-identification to distinguish cetaceans from each other and recognize them. The technique relies on being able to obtain good quality photos of animals' body parts that constitute unique recognizable markings during their whole life. The animals are photographed and catalogued individually based on natural markings criteria (e.g., pigmentation on the body, shape of the dorsal fin) and personal markings (scores, notches and scars) that identify them. A number of assumptions are made, particularly relating to recognizability, representativeness of sampling and capture probabilities that should be homogeneous. When an already identified individual is re-sighted, or photographically re-captured, this can provide a response to various issues, such as: population size, site fidelity, distribution, movements, social structure, etc. This means that there is a need for sorting, storing pictures and associated data within a catalogue which should be regularly updated.

76. Photo-identification is a good method to estimate population size (indicator 4) through mark-recapture models, and for specific areas that populations or part of populations occupy during one or more seasons of the year. It is also one of the methods to provide population parameters e.g. survival and calving rate.

77. The standard software program for mark-recapture analysis is programme MARK (http://www.cnr.colostate.edu/~gwhite/mark/mark.htm), which includes a wide range of models to estimate population size, survival rates and allow to correct some of the bias against the assumptions.

Human resources

78. At least one trained observer/photograph will take pictures of the cetaceans and indicate to the pilot of the vessel how to move the vessel in order to ensure good photo-identification (speed, heading, position in comparison of the animals…). The post-treatment of pictures requests one skilled person at least, and is time-consuming, in order to get a final catalogue of photo-identified animals and the matrix of recaptures which is the base of any analysis.

Material needed

79. The needed materials are:

- A boat with a sufficiently low bridge over the water to take pictures at the correct angle.
- Observation forms and, ideally, a computer.
- A watch.
- A GPS.
- A camera with a lens (up to at least 200mm, ideally up to 300 or 400 mm). Digital cameras with high resolution (at least 6 megapixels) are highly recommended.
- a computer and a hard drive to store all the pictures and moreover the catalogue of photo-identified animals

Implementation

80. On the boat, researchers take pictures of natural markings on animals at certain angle and from certain parts of the body depending on the species (e.g. flanks for delphinids, tail for sperm whale) of all individuals encountered.

81. The analysis of the images is time-consuming and requires great concentration and attention to detail. Every individual is listed in a catalogue of photo-identification, allowing comparisons. Scientist has to compare the photo of an individual with all the photos which are in his database and update regularly his existing catalogue and the matrix of re-capture. In an attempt to facilitate the process of matching, some software has been developed to make the comparison automatically. The principle is that the software presents a number of candidates (possible matches) with a certain probability/similarity, which safes time to the researcher by not needing to go through the whole catalogue. Nevertheless, the researcher takes the final decision about a positive match.
Photography may require some specifics authorizations procedures as well as regional partnerships may require some agreements.

Advantages

82. The advantages are:
- Relatively easy data collection protocol.
- Non-intrusive method of "marking" animals.
- A systematic sampling plan is not always necessary but is preferable.
- Standard and tested analysis methods exist, that provide reliable results as long as the hypotheses are tested or the bias are well estimated.

Limitations

83. The limitations are:

- Only applicable for species with long-lasting identifiable natural marks.
- Natural marks must be unique, recognizable and not change.
- Heterogeneity of capture probability.
- The collected data is a photograph of a wild animal in motion; it is not easy to take a good quality photograph with targeted criteria without good relative experience.
- Required several captures. If there is not enough recaptures, analyses are difficult and sometimes give unreliable results.
- Require a large quantity of data and a long-term study and is time-consuming for the cataloguing part.

- Difficulty of application in low-density areas.
- This method generates mark-recapture estimates of the total number of individuals in the study area. However, the total size of the population may be greater if all the animals in the population do not frequent the monitored area.

3.3.3.2. Telemetry

Principle

84. There are two types: satellite telemetry (Argos) and radio wave (VHF) telemetry. This technique consists in attaching a transmitter to an animal and following its movements remotely by satellite or via a receiver VHF or acoustics which can be installed aboard a ship or a plane.

85. Thanks to the beacons which transmit every hour/day their signals to the satellites, scientists acquire knowledge on the localization of the animal. These techniques allow to study animals in their world and to obtain information on feeding behaviour, distribution, reproduction area and migratory routes. These beacons also allow to record other data such as temperature, pressure, luminosity, swimming speed and sounds.

86. Information on the movements and distribution of individual animals can help to identify important habitats (feeding areas), migration routes and to define boundaries between populations. So, these data can provide complementary results to the indicator 3 at least and help to define the study area to monitor a population in the frame of the indicator 4.

Human resources

87. At least at sea, one person should have skills to attach/deploy the system on the animals. To detect the animal, and follow with VHF, at least 3 people are needed.

Material needed

88. The needed materials are:
- transmitters (Argos or VHF)
- small or relatively small boat (outboard or an average zodiac boat) with a sufficiently low bridge over the water to approach correctly the animal.
- beacon, crossbow or long pole
- In case of radio telemetry, a receiver VHF or acoustics to set up on a platform (vessel, aircraft) that follows the animal tagged.

Implementation

89. An animal will be detected and approached nearby, in order to attach (suction cup) or deployed the transmitter. Usually suction cups are pressed on the body using a pole, meaning to approach the animal to touch its body, whereas for Argos transmitters it is deployed in pulling on the animal with a crossbow a device with a clip that will be embedded in the subcutaneous fat of the animal.

90. For coastal species the approach can be made from a rubber boat directly, and for more pelagic species a large vessel can act as a base and a rubber boat can be towed and be used to approach the animals. For a device using VHF, the vessel will follow the animal at distance in order not to interfere with its behaviour and also in order to recover the device when it will naturally get off the animal.

Because this method has a direct impact on cetaceans, it requires request of authorization prior to implementation.

Advantages

91. The advantages are:
- These instruments allow to collect a lot of information not allowed by other methods (behaviour, movements) and without human interference.
- This method allows to study movements of animals on a large distance, in isolated area and under the water surface.

92. For satellite telemetry:
- Operate on a very vast area and allows to study movements of animals on a large distance;
- Independent from weather conditions;
- Possibility to obtain additional information;
- No need of an observation platform following the animal at sea;
- Allows to know species presence in an unexplored area;
- Allows to obtain information summaries about the animal's activities during long periods.

93. For radio telemetry:
- Relatively low-cost;
- Small-sized system and relatively non-invasive system;
- Operate on a wide area;
- Relatively independent from weather conditions.

Limitations

94. The limitations are:

- This method is intrusive, either by its approach nearly to touch the animal but also through the system to attach the device (mainly satellite transmitters) to animal body
- Information is obtained on few individuals and depend on performances of equipment used, as well as the accessibility of mammals. A lot of individuals must be tagged to draw any general conclusion, and this is often not possible
- The implementation of this method requires important logistical support because it requires an installation directly on the animal, which is a particularly difficult operation for rare and fast animals.
- This method is intrusive for animals, with infection risks.
- Only animals which can be correctly approached are equipped and required that the animal is at the surface for the data transmission

95. For satellite telemetry:
- Expensive method;
- Limited support of non-intrusive mechanism on animal and limited time-life.

96. For radio telemetry:
- Required to maintain a platform following the animal at close distance;
- limited autonomy;

3.3.3.3. Biopsy

Principle

97. This method consists in collecting on living animals at sea a fragment of skin and blubber. This can be done by throwing with a crossbow darts with tip, dart gun, riffle or even a pole with biopsy tip or skin swabbing when dealing with bow riding animals for example.

98. Such samples allow to gather information on biodemographic parameters (indicator 5):

- To determine the sex of the animal
- To determine the genetic specificity of individuals (fragment of DNA) of the same species. Based on that, analyses of kinship, matrilinear links, and social structure can be run.
- To obtain information on the reproductive status of individuals (e.g., pregnancy for females) based on the level of hormones.

99. Other information can be gain:

- on feeding level (isotope)
- on level of contamination in heavy metals and other pollutants (such as organochlorine contaminants)

100. Several parameters included in the indicator 5 can be obtained through the analysis of the skin and blubber collected with the biopsy method: sex ratio, pregnancy rates. Also, the genetic structure of the animals allows to better determine the limit of a "population", or a sub-population, which helps to know when looking for the distribution or abundance of this population.

Human resources

101. At least one pilot, one shooter and it is highly recommended to have a photographer to be able to identify the animal sampled, which may provide the opportunity, for instance, of monitoring the healing process. A fourth person can take care of the samples when the biopsy has succeeded.

Material needed

102. The needed materials are:

- A small or relatively small boat (outboard or an average zodiac boat) with a sufficiently low bridge over the water to shoot at the correct angle.
- Crossbow or gun and bolts, darts with tip.
- Storage and cleaning material (products)
- Freezer or storage frozen.

Implementation

103. Animal targeted should be approached nearby. Biopsies are realized by means of an arrow (pulled by a crossbow or an airgun) which, pulled with some force, take a piece of skin and fall into the water where it is then recovered with the sample. In the same time, a photo allowing to identify animal is taken to obtain a complete documentation for each animal. It should be noticed that the material (skin and blubber) is right away stored following a strict protocol which can differ depending on the planned analyses (genetic, hormone, isotope): alcohol in one case, freezing in another.

104. As for photo-Identification, for coastal species the approach can be made from a rubber boat directly, and for more pelagic species a large vessel can act as a base and a rubber boat can be towed and be used to approach the animals whereas the large vessel stays away.

Because this method has a direct impact on cetaceans, it requires demands of previous authorization applications.

Advantages

105. The advantages are:

- Give access to information very difficult to obtain in another way (genetic, hormones, isotope)

- Biopsy sampling tends to be relatively affordable and can be easily paired with additional methods to maximize data collection opportunities.

Limitations

106. The limitations are:

- A strong disadvantage of biopsy is that it is invasive because the animal will be approached very near and the biopsy itself (i.e. results in physical lesions), which restricts sampling to the size and age classes (and species) that can be ethically targeted under existing permitting restrictions.
- The lifestyle of cetaceans, which spend only some fractions of their life on-surface limit strongly options to collect tissue from alive animals.

3.3.3.4. Land based tracking

Principle

107. This method consists in collecting data from a fixed point on the coast, following individuals crossing the area watched from the point of observation. Ideally, the point of observation must be high. Such tracking allows studying distribution, behaviour, use of the habitat and movements of focal cetaceans, without impact of boat presence on the natural behaviour of animals. This method is suited for the study of a coastal resident population or migrations close to the coast.

108. This method is most efficient for coastal population or resident groups. It can give results on distribution and habitat use, in link with indicator 3.

Human resources

109. At least 3 persons should be in charge of the observation and measures. One can make the measures of the group/animal followed, the second record notes, and the third one observes other part of the sea to detect other animals.

Material needed

110. The needed materials are:
- Binoculars or a telescope on a tripod.
- Observation form or Dictaphone.
- Watch or clock.
- Compass or angleboard and an instrument to measure the distance between the animal and the observation post (e.g., clinometer camera for photogrammetry, theodolites).

Implementation

111. One or more observers position themselves at a strategic point of view (headland, cliff, strait, entrance of a bay) and collects data on animals and weather. Observations can be made with naked eye or with binoculars or telescopes but is dependent on a calm sea and on a good atmospheric visibility.
This type of monitoring does not require some special authorization procedures as long as the observation point is free of access.

Advantages

112. The advantages are:

- Land-based methods are non-invasive, enabling the monitoring of marine mammals without risks of observer-induced disturbance.
- This is the least expensive techniques (no costs due to platform navigating at sea) used. It can therefore be implemented often and so allow a long-term monitoring.
- The land-based method can be easily standardized and realized all year round, according to observation conditions.

Limitations

113. The limitations are:

- The field of study is limited to the area covered visually (naked eye or binoculars); the prospecting area is thus limited.
- Land-based methods are normally constrained to relatively conspicuous species that regularly come to the surface within sight of land.
- Investigations on fine-scale distribution are constrained by the difficulty in determining the precise geographical position of cetaceans. Theodolites are widely used in such studies, but there are limitations to their use. In particular, measurement readings can often be long, and the collection is made on a centre of gravity of a small group rather than on individuals. In addition, such groups can be spread over tens or hundreds of meters; a single position is rarely representative of all individuals.

3.4. Standard monitoring of strandings and by-catch animals

114. The monitoring of strandings and by-catch deal most of the time with dead animals. A lot of data can be collected which will be used in the three indicators: as a first step, the collection of strandings and by-catch information aids the construction of a species list of cetaceans present in the area (or surroundings for strandings) and a rough measure of status and seasonal variation in abundance. Then, the analysis of carcasses gives a lot of information on demographic parameters.

3.4.1. Stranding

Principle

115. Stranding is a monitoring method that is continuous all year round, with qualified people ready to go on each stranding event of cetaceans when it occurs and is detected. Parameters of the animals are measured, and biological samples are taken when possible and stored.

116. This method was the first one to be used by scientists as monitoring method, because strandings occur all the time and animals arrive on the coast, so they are easier to approach than living animals at sea.

117. Stranding of cetaceans represents an extremely precious scientific material for the knowledge of these species difficult to study in their natural environment. Study of carcasses, realization of autopsies and complementary analyses on biological samplings can supply information on the presence of a species, its distribution, demography of populations, feeding regime, health status of the animal (food, diseases, contamination), death causes, impact of anthropological threats (incidental catches, ship strike). These data will be used mainly for the indicator 5.

118. It is of crucial importance to fund this monitoring on long term and in a structured way. A network of referenced people localised all along the coast and working in the same manner, linked to a coordinator, is the base of an efficient monitoring network of strandings. An animation and steering committee would allow the network to function properly and guarantee the system's sustainability.

Human resources

119. People trained to do the measurements and take biological samples according to specific standard protocols, available to reach the stranded animals as soon as it is detected. Within the network there should be also veterinarians to examine carcasses, detect the causes of mortalities and place to store the biological samples (freezer).

Material needed

120. The materials needed are:
- Stranding forms
- Camera
- Tape measure
- Sampling kit (knife, shears, packaging materials)
- Refrigerated box and freezers network
- Dedicated dress, safety gloves, safety glasses
- Heavy equipment allowing to move carcass if necessary (bulldozers, rendering truck, car)

Implementation

121. When a cetacean stranding is reported, one or more person is on the scene to prevent the approach of people and animals to the carcass and take measures and biological samples. This method requires a specific training for participants. A warning procedure must to be established to be effective. A stranding network must be developed to be efficient and bring useful data.
Approaching and dealing with dead animals as well as protected species need special authorization.

Advantages

122. The advantages are:
- Stranding bring even frequently information, even if these are often limited and non-predictable due to their nature.
- Availability of the whole body and organs for analyses and conservation (tissue bank).
- Some species are known only by stranding and rarely observed at sea.

Limitations

123. The limitations are:
- Not predictable and intervention must be realized on short time for sanitary reasons and for autopsy to be exploitable from a scientific point of view, so require having an available person at the right time.
- Interventions on alive animals represent security and health risks for animals and rescuers. For animals, distress and stress engendered by stranding may cause unpredictable and dangerous behaviour. Also, sanitary risks and disease transmission between rescuers and the animal are real.

3.4.2. By-catch

Principle

124. Marine mammals are frequently captured in fishing gear. "By-catch" means cetaceans accidentally captured by commercial fishing, sometimes but rarely by recreational fishing. Scientific observers can be embarked on board professional fishing ships, to observe captures and fishing conditions, and to take measures and biological samples.

125. Analysis of the measures and samples collected on carcasses provide a lot of information on demography (indicator 5): size of animals, age at maturity, rate of pregnancy, sex ratio…

Human resources

126. People trained to do the measurements and take biological samples of cetaceans according to specific standard protocols. Often, they might take other measures on other species when going on a commercial fishing vessel as observer. One person might go on one vessel for a period. This means that the most vessels to be monitored, the most people trained and authorized to board.

Material needed

127. The needed materials are:

- GPS, watch
- observation forms
- camera
- tape measure
- sampling kit (knife, shears, packaging materials)
- freezer

Implementation

128. One observer embarked on board of a professional fishing vessel. His work consists in collecting scientific data relative to the operation of fishing. He intervenes when a cetacean is captured to take data on the animal. If possible, he takes biological samples, stored them and go back at land with them. To realize sampling on the individuals of marine mammals and bring them on land if useful and feasible, administrative authorization requests are necessary.

Advantages

129. The advantages are:

- By-catch bring crucial biological information on "healthy" animals (compared to strandings who include sick animals), even if these are often limited and non-predictable due to their nature.
- All the animals by-caught might be "fresh" as they were alive few days before and biological samples might be taken from all of them, insuring availability of good quality samples for analyses.
- An observer aboard a fishing vessel will bring data on species and number of animals that are by-caught, enabling to assess the impact of this threat for cetaceans (provide complementary information for indicator 3 and 4).

Limitations

130. The limitations are:

- The event of by-catch is rarely predictable, there might be no by-catch

- Difficulty in going aboard fishing vessel sometimes, because of willingness of fishing captains, size of the vessel or authorization,

- Difficulty in doing the measurements and taking biological samples in some small sized fishing vessel, and also in storing samples in a freezer.

- Intervention on a carcase in a moving vessel represents security risks for people. Also, sanitary risks and disease transmission between people and the animal are real.

3.5. Emerging Monitoring technologies

131. As technologies are improving fast, new studies using them are launch. As these are relatively recent, case by case tested and relying upon technology's capacities (namely pictures resolution, autonomy of AUV, artificial intelligence software to analyses thousands of images, etc.) no standard method is yet approved or define. But as this field is of growing interest and development, and as these technologies may be use within the standard methods already presented in terms of improvements or adding values, these technologies will be shortly presented in this document.

3.5.1. Unmanned underwater and aerial vehicles

3.5.1.1. Sampling from Drone (pictures, blow…)

132. Advances in aerial drone technology offer new opportunities for studying cetaceans remotely and noninvasively. These instruments are light-weight, portable platforms piloted remotely from the ground/deck of a vessel, and allowing surveys of remote, hard-to-reach areas within small time windows.

133. Drones or Unmanned Aerial Vehicles (UAVs) can be used to take pictures or videos by applying the line transects method (visual), to answer abundance and distribution questions. As survey by aircraft, the protocol consists to program to follow a flight plan defined and surveyed along pre-determined transects based on GPS waypoints to form a full coverage survey grid. The drone takes a collection of images with an overlap in coverage of the survey area, and records flight information such as GPS coordinates and altitude in the EXIF header of each image file.

134. UAVs are a promising tool for animal surveys. Indeed, this technology has many advantages:

- potential for carrying out relatively large-scale aerial image-based surveys at often a fraction of the cost of manned aerial surveys, and without many of the safety issues associated with manned aircraft;
- low cost of UAV systems compared to manned aircraft may also allow greater flexibility in survey design, for instance by flying two or more platforms at specific time lags rather than employing the circle-back maneuverer;
- ability to repeatedly collect high-resolution aerial imagery, with extremely low disturbance to animals;
- possibility to be used in areas where manned aerial operations are difficult and dangerous, and allows to survey sites with no airfields;
- may eliminate observer bias in the data collection phase;
- less subject to flight restrictions due to weather conditions;
- results are easily replicated and have minimal impact on the surrounding environment.

135. However, this technology has some limits:

- the longer manual data post-processing times still pose some challenges (in terms of efficiency and costs);
- environmental and survey-related variables, such as light conditions and wind, can affect detectability. Several studies are in progress to quantifying detectability and certainty in animal detections/identification using UAV technology;
- the majority of available UAVs is only useable over limited ranges (i.e. within line-of-sight), at slow speeds, and under small payloads;
- stringent and country-specific civil aviation regulations and complex permitting processes can limit their adoption for scientific applications;
- the covered surface is still lower than the one from a plane;
- impossibility to fly in high winds (wind speed must be less than 25 knots on the ground);

- depending on autonomy of the drone, a vessel can be needed as platform to take off and land, which increase the costs.

136. A drone can be also used as tool to approach an animal realized from a boat. It can allow to study behaviour by achieving better visibility or to take a sample such as in the blow of a whale. This system allows to non-invasively collect mucus microbiota samples safely and reliably, by minimizing external contamination such as air and seawater from outside the blowhole. This type of samples is used for hormonal analysis for example and can help for the indicator 5.

3.5.1.2. Marine AUVs and glider

137. An AUV is a marine craft pre-programmed to conduct underwater missions without constant supervision or monitoring by a human operator. They allow observations of species in their natural environment, with highly accurate vertical and horizontal geo-positioning and the ability to instantly react to the observed environment.

138. Ocean gliders are autonomous winged underwater vehicle that collects ocean data using buoyancy-based propulsion and can remain at sea for weeks to months at a time surveying over spatial scales from ones to hundreds of kilometres. Modern gliders can be fitted with cameras, mobile tracking systems, or acoustic loggers/echosounders. Some robots automatically detect those sounds, identify the species based on characteristics of the sounds, and report which species have been heard to scientists on shore via satellite in near real time.

139. Robots are powerful tools for accessing environments too dangerous or too remote for human exploration. They can complement conventional forms of sampling by providing long-term, fine-resolution coverage of areas that are impractical or too expensive to survey, without constraint from weather conditions or sea states. Some instruments can remain unattended for several weeks to months, offering an unsurpassed level of autonomy.

140. Their biggest drawbacks are their high costs, slow speeds, and limited dive times. Furthermore, their energy storage and power consumption are some limits.

141. AUVs and ocean gliders are valuable for generating long-term datasets in remote locations but can be challenging to deploy and recover.
Launching an AUVs or glider within the sea may be constrained by some authorizations.

3.5.2. Pictures and video

142. Digital cameras delivering stills and video feeds can be used as a support to observers in order to gain some precision if needed. For example, they can be used during a sighting to precise group size count or identification of species. Conducted in a more continuous way, they may help in enhancing encounter rates, although usually within a narrower search swath located immediately beneath the plane. These technologies are helpful in being used in parallel, to combine the advantages of human observations for scanning larger regions with the advantages of later re-analysis and reassessment of images and videos.

143. Several studies are in progress to test if those technologies alone could be used as monitoring methods. Tests are in progress to allow an automatic detection and determination of cetaceans, but methods are not yet operational. Aerial videography benefits from standardized methodologies that can be replicated, but is time-consuming and very costly, because the determination of cetaceans has to be done by an operator.
Taking pictures or video may be constrained by some authorizations.

Conclusion

144. Monitoring cetaceans is a hard task, based on the fact that they are highly mobile and spread in vast areas. Methods have been developed to collect data to follow the evolution, mainly of their distribution, their numbers and their demographic characteristics. Monitoring such parameters imply a lot of knowledge, skills and resources. Each method has its advantages and disadvantages, and approaches may frequently complement one another in providing a more complete picture of the status and distribution of a particular cetacean species.

145. A least strandings monitoring should be organized, with a strong network, everywhere for baseline data on cetaceans (distribution, presence, indices of abundance, genetic analysis). Then a first visual and acoustic survey should be organized over large scale for a knowledge about the global context, which could be repeated regularly several years later (6 to 10). Ferries and oceanographic vessels should be used as non-dedicated platforms if they cover an area on a regularly basis which can be important for cetaceans. Then more focused monitoring programme covering smaller, but representative or important areas should be launch on a yearly basis, including visual and acoustic with some biopsy and photo-ID.

146. Furthermore, the aim of the monitoring programmes is also to get a global vision of the situation at the Mediterranean level. So national programmes should ensure standardization, in method/platform/period with neighbouring countries as much as possible. Even, promoting the implementation of transnational and coordinated monitoring ensure a better effective conservation of cetacean's populations (Authier *et al*., 2017). Initiatives such as the ACCOBAMS Survey Initiative, or the existing "Fixed line transect Mediterranean network" coordinating protocols and database of the different teams working on ferries should be encouraged and supported. This kind of initiatives allows easily to merge all the data for further analysis at a regional or sub-regional level. Standard strandings networks and photo-identification catalogues should also be implemented at the sub-regional level, following the recommendations of Decision IG.23/6 on the 2017 MED QSR (COP 20, Tirana, Albania, 17-20 December 2017) concerning harmonization-standardization-synchronicity of monitoring and assessment methods and improvement of availability /accessibility of the datasets.

147. Before embarking upon a monitoring programme, it is prudent to determine precisely what information can be gained and what limitations exist. A lot of practical and operational adaptation can be found on a case basis. A lot of monitoring programmes already exist, being a source of advises that should be ask for in order to gain at quality, logistical and cost levels.

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B. Guidelines for monitoring Mediterranean Monk seal

Table of Contents

I. Introduction

1. Background

1. In 2008, the Contracting Parties to the Barcelona Convention - namely 21 Mediterranean countries and the European Union (EU) – decided to apply the ecosystem approach (EcAp) to the management of human activities that may affect the Mediterranean marine and coastal environment for the promotion of sustainable development (UNEP/MAP, 2007). It is an ecological strategy for the integrated management of land, water and living resources that promotes conservation and sustainable use in an equitable way, with the aim to ensure that human use of ecosystems is kept within the limits of capacity of ecosystem. The ultimate objective of this approach is to achieve the Good Environmental Status (GES) through informed management decisions, based on integrated quantitative assessment and monitoring of the Marine and Coastal Environment of the Mediterranean.

2. In 2016, the Contracting Parties also agreed to design an Integrated Monitoring and Assessment Programme (IMAP) with a list of regionally agreed good environmental status descriptions, common indicators and targets, with principles and clear timeline for its implementation according to the 6 year-EcAp cycles structure. Building and implementation of a regional monitoring system is the main goal of IMAP to gather reliable and up-to-date data and information on the marine and coastal Mediterranean environment. By adopting IMAP, Mediterranean countries committed to monitor and report on Ecological Objectives (EOs) and their related common indicators (CIs), in synergy with the EU Marine Strategy Framework Directive (MSFD), covering three components: i) biodiversity and non-indigenous species; ii) pollution and marine litter; and iii) coast and hydrography.

3. One of eleven ecological objectives is “Biodiversity is maintained or enhanced” (EO1). The term ‘maintained’ is key to the quantification of GES for EO1. This condition has three determining factors:

a. no further loss of the diversity within species, between species and of habitats/communities and ecosystems at ecologically relevant scales;
b. any deteriorated attributes of biological diversity are restored to and maintained at or above target levels, where intrinsic conditions allow;
c. where the use of the marine environment is sustainable.

4. Among five common indicators related to biodiversity (EO1) fixed by IMAP, three are about marine mammals including the Mediterranean monk seal:

- Common indicator 3: Species distributional range;
- Common indicator 4: Population abundance of selected species;
- Common indicator 5: Population demographic characteristics (e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates)

2. Purpose and Aims

5. As top predators in the Mediterranean Sea, the monk seals are an important element of marine biodiversity. Their abundance and distribution are known to respond to various natural and anthropogenic drivers. Role of long-term monitoring programmes in assessing population states are widely recognized and several programmes covering the North-East Atlantic marine environment including plankton, fish, seabirds and marine mammals already in operation. Monitoring efforts of Mediterranean monk seals are regional due to their scattered distribution range. The largest subpopulation inhabits the eastern Mediterranean Sea in Greece and Turkey. The second largest aggregation located at Cabo Blanco. The third subpopulation inhabit the archipelago of Madeira and the small unknown number of seals might inhabit at the eastern Morocco therefore every working group has a different monitoring strategy regarding their regional differences.

6. The aim of this document is to provide guidance to monitor Mediterranean monk seal in relation to the IMAP common indicators, i.e distribution, abundance and population demographic

characteristics (i.e. Body size or age class structure, sex ratio, fecundity rates, survival/mortality rates) at the Mediterranean and national scale.

7. These monitoring guidelines are for the surveys to be conducted in the areas where the Mediterranean monk seal populations actively occur/inhabit.

3. Common Indicators related to Marine Mammals including the Mediterranean monk seal

8. A common indicator is built in the context of the Barcelona Convention and it "summarizes data into a simple, standardized, and communicable figure and is ideally applicable in the whole Mediterranean basin, or at least on the level of sub-regions, and is monitored by all Contracting Parties. A common indicator is able to give an indication of the degree of threat or change in the marine ecosystem and can deliver valuable information to decision makers (IMAP, 2017)".

9. Among five common indicators related to biodiversity (EO1) fixed by IMAP, three are about marine mammals:

- Common Indicator 3 - Species distributional range:
  This indicator is aimed at providing information about the geographical area in which marine mammal species occur. It is intended to determine the species range of cetaceans and seals that are present in Mediterranean waters, with a special focus on the species selected by the Parties. The main outputs of the monitoring under this indicator will be maps of species presence, distribution and occurrence.
- Common Indicator 4 - Population abundance of selected species:
  This indicator refers to the total number of individuals belonging to a population in a specified area in a given timeframe. Methods for estimating density and abundance are generally species-specific and ecological characteristics of a target species should be considered carefully when planning a research campaign. In this document, target species refers to the Mediterranean monk seal.
- Common Indicator 5 - Population demographic characteristics (e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates):
  This indicator aims to provide information about demographic parameters as the age structure, age at sexual maturity, sex ratio and rates of birth (fecundity) and of death (mortality). These data are particularly difficult to obtain for marine mammals. Monitoring effort should be directed to collect long-term data series covering the various life stages of the selected species. This would involve the participation of several teams using standard methodologies and covering sites of particular importance for the key life stages of the target species.

4. Monitoring methods

4.1. Monitoring strategy

10. Due to the very critical status of the Mediterranean monk seal, any type of monitoring activity of the species should be conducted under the supervision of the national authorized legislative bodies.

11. The Mediterranean monk seals spent most of their time in the water, however, monitoring them in the aquatic environment is a challenging job and provide little information on the population. On the other hand, they marine caves while haul out to rest and breed and this period is the best option to collect data on the species. The most suitable method to monitor the Mediterranean monk seals in their cave is to use non-deterring camera traps in order to minimize disturbance while monitoring.

4.2. Time, Place and period

12. In general, monitoring should be performed all year round. However, if there is any restriction to due to season, location of cave, camera trap availability, the effort should be concentrated in monitoring only the breeding caves during the breeding season, which almost exclusively takes place between August to December in the Eastern Mediterranean Sea. There are, however, not enough scientific evidences to propose that the breeding of the Mediterranean monk seals is strictly seasonal and could therefore show a regional difference elsewhere.

4.3. Equipment

13. The following is the basic equipment needed for cave monitoring

- A boat preferably and inflatable one is essential to reach the seal habitats
- Camera trap with PIR-based motion detector
- Silicone sealant to be applied to the camera traps for extra protection against excess humidity
- Waterproof dry bag and container to carry the camera traps and other electronic equipment
- Flash memory card (16 GB or higher)
- Personal Free diving equipment (ABC equipment)
- Underwater torch
- Hand hold GPS to record the position of the caves
- Photo-trap cave-wall mounter (preferably made of chromium, custom-built)
- Protective equipment as required (such as (life vest, helmet, etc.)

14. For land-based surveys a photo camera with telephoto-lens (200-400 mm) high magnification binocular may also be used

4.4. Maintenance of Equipment

15. The most important equipment of monk seal surveys is camera trap. It is not waterproof but is weather resistant. As camera-traps are deployed for long times in a cave environment that is extremely humid, additional protection should be applied such as sealing the joints of the body with silicon sealant. Placing a small umbrella like protection may be considered to prevent equipment from dripping water. Batteries of GPS and underwater torches are checked before every survey. Setup of camera-traps should also be set considering the status of the environment in which the camera traps are to be deployed. Metal (containing) equipment should be lubricated against corrosion after every use. After the camera trap recovery, memory cards and batteries should be removed from the traps and are cleaned to remove sea salt.

4.5. Monitoring methods

4.5.1. Primary monitoring methods

4.5.1.1. Cave survey and monitoring

16. As mentioned before, the best monitoring method of the Mediterranean monk seals is to observe them in their haul out habitats (i.e. marine caves). Within this scope, cave surveys should be conducted to identify caves that are suitable for monk seal use. Then, the caves that are actively used by monk seals are monitored by camera-traps in order to minimize disturbance while monitoring the population.

4.5.1.2. Surveys to explore resting/breeding habitats

i. In areas not surveyed before

17. Surveys should be conducted in areas not investigated before to explore caves which meet the requirements and descriptions of a Mediterranean monk seal cave (IUCN/UNEP, 1998). Active surveys should be carried out on coasts where the geography is suitable for cave formation. For that respect, karst steep topographies are of great importance. The surveys should be done using a boat manned preferably by four people; two swimming along the coast of interest in search of caves; one recording the data and one steering the boat. The monk seal cave might may have underwater entrance with very narrow passage and a long corridor, so it is not always easily recognizable from surface. The large and narrow openings, crevices and holes between the rocks should therefore be checked carefully. When an entrance is found, a team member should enter the cave with necessary precautions taken in order not to disturb the animals. Caves with underwater entrances should always be investigated by free diving. Noisy equipment, such as scuba diving equipment are not recommended for cave investigations as the disturbance created by the bubbles can deter the seals. If the entrance of a cave is too long to be enter on apnea, SCUBA equipment may be used only for exploration.

ii. In areas surveyed before

18. If the area has already been surveyed before and an available information about the marine caves are available to identify the caves to monitor, the procedures explained in the section above can be neglected. However, in any case, surveys are recommended to cover the whole area at least once as Mediterranean monk seals can also use protected and deep crevices for resting.

4.5.1.2.1. Cave Inventory

19. Information of newly explored caves should be recorded in both a field survey (Annex 1) and a cave inventory protocol sheets (Annex 2). The cave inventory protocol includes the coordinates of the cave and various characteristics of the cave related to the Mediterranean monk seal monitoring including number of entrances, resting platforms, air chambers, its photograph, total length, its sketch where possible etc. Each cave should also be classified according to the categories described by Gucu et al. (2004).

4.5.1.2.2. Selection of caves for monitoring

20. The height of the ceiling and width of the inner space of actively used caves are taken into consideration to evaluate the risk that the camera could be exposed to strong waves while selecting a cave for monitoring. In order to prevent loss of camera-traps, the caves that has ceiling lower than the maximum wave height are not used for monitoring. Combination of various factors such as the season, accessibility, cave type (potential, active or breeding) and cave characteristics, number of available camera traps is effective of selection of caves for monitoring. However, if year-round monitoring is not possible, then emphasis should be given to the breeding caves during the breeding season, as fecundity is utmost important population parameter to be monitored.

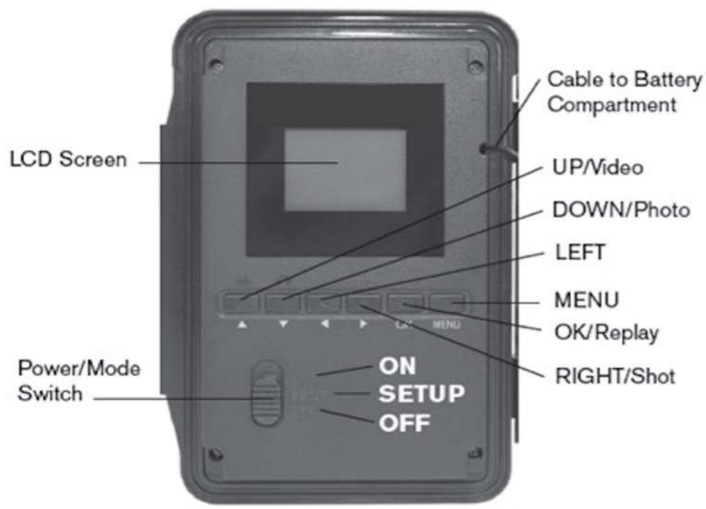
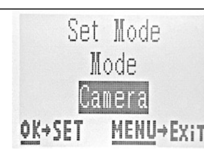
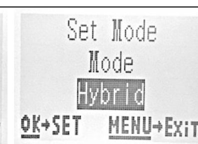
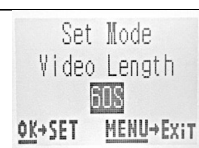
4.5.1.2.3. Camera trap set up, deployment, and recovery

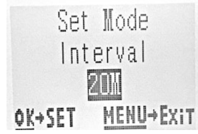
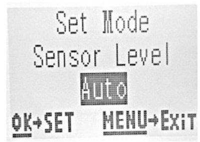
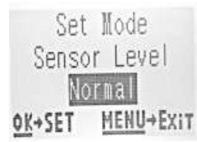
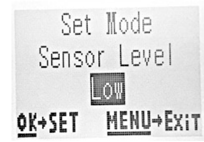
21. Commercially available camera traps have photograph, video and hybrid modes. The hybrid mode allows both still photos and videos to be captured at each trigger so may be good for data collection on behaviour. Camera image size should be in the highest resolution as high-quality photographs are needed for the photo-identification analyses. The length of the video captures should be set considering the duration of deployment, battery life and the size of the memory card.

22. Data and time stamp of the camera-trap is crucially important for the data stored in the memory cards. The built-in clock should be set with care and stamp mode should be set on “ON”. Some camera-traps has built-in temperature and moon stamps, which may be useful to have more information about the in-cave seal behaviour.

23. Most commercial camera-traps will take a photo (or record a video clip) automatically at your choice of time intervals to prevent the card from filling up with too many redundant images and to prolong battery life. The interval between two consecutive activations may be set at 20 minutes and longer in order to minimize disturbance (Gucu 2009). Sensor setting is set to auto or to normal/medium if the auto option is not available as in the case of some models. If the other fauna (bats, rats, etc.) is observed in the cave, a low sensitivity of sensor settings may be used to avoid unnecessary activation of the camera trap by this fauna (Table 1).

Table 1. The basic camera set-up for monk seal cave survey/monitoring

Basic camera trap set-up for monk seal cave survey/monitoring	
Front view	**Back view**
	
Settings	**LCD screen view**
Camara Mode	 
Camera image size	
Video length if hybrid mode is set	

Event interval	
Sensor level	   or

24. Location of the camera-traps is determined in order to get appropriate photos that cover the right location where the animal hauls out most of its time in the cave. The number of traps used in a cave changes based on size and morphology of the cave. The caves with wide inner space where the haul out platforms are larger than the camera view angle is monitored with sufficient number of camera traps.

25. Photo-trap cave wall mounter is placed to the suitable location by nailing its legs. When the suitability of location is assured, it is permanently fixed by covering the legs with white cement. After drying of cement, camera trap is fixed to the mobile arm of the wall mounter by using screws. At last, tilt angle of the trap is checked, the paper cover over the PIR sensor is removed and the trap get activated. The camera trap is strengthened with plastic cable ties.

26. Depending on combination of various factors such as the season, accessibility, cave type (potential, active or breeding) and cave characteristics, camera traps are left in caves for one to the maximum of three months. During recovery, camera trap used is usually replaced with a new one, as the camera trap used is usually worn out due to the conditions in the cave. However, the flash card is replaced only if there is no spare camera-trap available and previous one is going to be kept in the cave for the next survey.

4.5.2. Secondary monitoring methods

27. The methods below are used in the Mediterranean monk seal monitoring, but the output is usually very limited. So, these methods are considered as complementary to the primary monitoring methods.

4.5.2.1. Land based survey

28. Land based survey is conducted by a team of two observers during daytime at a high point on land where presence of the monk seal is confirmed or previously reported. During the observations, information is collected on date and start and end times of observation, name and coordinates of observation point, weather conditions (taken at hourly intervals or when it changes), time of seal sighting, seal morphology and behaviour. Photos/videos are taken when possible. Survey lasts over 1 hour and is stopped if a seal does not appear after 2 hours of observation or, when the sighted seal disappeared from sight. As well as during cave surveys and monitoring, weather conditions (sea state, wind force and direction, and visibility) are also factors limiting the land-based surveys.

4.5.2.2. Opportunistic monitoring

i. Dedicated observers on opportunistic platform (i.e oceanographic vessel)

29. Surveys are performed by dedicated observers during daytime while the vessel is in transit. An observer is placed on the bridge of the research vessel, searches for the presence of the

monk seal using both naked eye and binoculars. During the observations, information is collected on date and start and end times and coordinates of observation, weather conditions (taken at hourly intervals or when it changes), time of seal sighting, number of seals, morphology and behaviour. Photos/videos are taken when possible. These observations are carried out when the research vessel is cruising at speeds not greater than 12 knots and weather conditions are relatively fair.

ii. Stranding

30. Information on stranded animal is recorded including the ID number, observation date, stranding location, latitude and longitude coordinates, length and weight of the animal (where possible to measure), age class, sex, stranding condition (live or dead), and other observational comments, including evidence of injury or human interaction. Photos/videos are taken where possible. Morphological features are mapped to a seal identification sheet. Data on stranding contributes the mortality rate estimations while evaluation demographic structure of the population.

4.5.3. Synthesis tables

Table 2. A synthesis table listing the different monitoring methods that can be used to monitor each common indicator.

Related to common indicators				
Monitoring methods	**CI 3** Species distributional range	**CI 4** Population abundance	**CI 5** Population demographic characteristics	**What to survey/monitor**
Surveys to explore resting/breeding habitats	x	x	x	• Seal presence/absence • Seal habitats • Seal habitat use • Basic demographic
Cave monitoring	x	x	x	• Basic demographic structure, parameters and trends • Seal habitat use • Seal behaviour Individual identification • Monitoring the habitats • Low cost • Can be used for public awareness
Land based surveys	0	0	x	• Seal presence/absence • Seal habitats • Seal habitat use • Behaviour
Dedicated observers on opportunistic platform (i.e. a research vessel)	x	x	x	• Seal presence/absence • Seal habitats
Stranding	x	x	x	• Input to basic demographic structure (specifically mortality rates)

Table 3. A synthesis table listing the different data analyses methods that can be used for each common indicator. X: the method is relevant ; 0: the method is not relevant

Data analyses methods/ Related to indicators	**CI 3 Species distributional range**	**CI 4 Population abundance**	**CI 5 Population demographic characteristics**
Photo-identification	x	x	x
Demographic analyses	0	x	x
Population Viability analyses	0	x	x
Mark-recapture analyses	0	x	0

Table 4. Synthesis table listing the equipment for the different research methods. X represents the equipment is used, 0 respresents the equipment that is not used

	Primary monitoring methods		**Secondary monitoring methods**		
Equipment	**Surveys to explore resting/breeding habitats**	**Cave monitoring**	**Land based survey**	**Opportunistic monitoring (from a vessel)**	**Opportunistic monitoring (stranding)**
Research vessel/ Inflatable boat	X	X	0	0	0
GPS	X	X	X	X	X
Photo/video camera	X	X	X	X	0
Underwater torch	X	X	0	0	0
Personal free diving equipment (mask, snorkel and fins) (ABC equipment)	X	X	0	0	0
Camera trap with PIR-based motion detector	X	X	0	0	0
Flash memory card	X	X	0	0	0
Photo-trap cave-wall mounter (chromium, custom-built)	X	X	0	0	0
Silicone sealant	X	X	0	0	0
Waterproof dry bag and container	X	X	0	0	0
Life vest	X	X	0	0	0

Various tools (such as plastic cable tie, nails, pliers)	X	X	0	0	0
Binoculars	0	0	X	X	0

Table 5. Synthesis table listing the equipment for the different monitoring methods.

Monitoring methodology	Advantage	Disadvantage
Surveys to explore resting/breeding habitats	▪ Updating/Identification of habitats ▪ Updating/recording of habitat use	▪ High cost and logistic challenges
Cave monitoring (with camera traps)	▪ Recording of basic demographic; structure, parameters and trends ▪ Recording of natural behaviour individual identification ▪ No/minimal disturbance ▪ Monitoring the habitats ▪ Low cost ▪ Can be used for public awareness	▪ Equipment is prone to water and damage ▪ Medium quality population estimates
Land based surveys	▪ Updating/Identification of habitats ▪ Updating/recording of habitat use ▪ Input to basic demographic structure ▪ Low cost and challenges	▪ Poor individual identification ▪ Low quality of population estimates
Dedicated observers on opportunistic platform (i.e. a research vessel)	▪ Updating/Identification of habitats ▪ Updating/recording of habitat use ▪ Input to basic demographic structure	▪ Poor individual identification ▪ Low quality of population estimates
Stranding	▪ Input to basic demographic structure (specifically mortality rates)	▪ Poor individual identification

5. Data analyses

5.1. Photo-Identification

31. Estimation of the population size of the Mediterranean Monk seals has a critical importance to assess status of the species. However, it is very challenging job considering their small numbers and isolated nature, therefore, methods used in cetacean studies such as tagging or observation from boats are not applicable for this species. Photo-ID on the other hand is another commonly used method on numerous species which is a practical alternative for monk seal studies.

32. The Mediterranean monk seal has distinguishable unique pelage patterns, scars, natural marks, that can be identified through high-resolution photographs and video footages taken by camera-traps. Pelage colour is not used to identify seals as it is dark and shiny when the seal just hauls out and gradually turns light grey as the animal get dried during resting. Obtained photographs are sorted by date and time to be able to identify seals photographed at the same time. Captured images are controlled and photographed seals are grouped regarding their sex and the morphological categories based on Samarach and Gonzalez (2000), Dendrinos et al. (1999), Ok (2006). The details of the morphological categories are given below in section 3.2.1. Morphological features mapped to a seal identification sheet (Annex 1). These sheets include dorsal, ventral, lateral drawings of the seals which can be full-filled manually. Finally, the sheets compiled in an identification catalogue that involves basic characteristics of the identified individuals such as sex, name, morphological stage, date of the first sight and habitat information.

5.2. Demographic structure

33. The demographic structure of the population is explored by using the approaches explained below.

5.2.1. Minimum estimated age

34. The minimum ages of the individuals are estimated according to the method given by Gucu et al (2004). Estimated minimum age in years; Aest= (P-D)/365+X where

D: Date of the first sight.

P: Days transpired since the first sighting

X: the age of the individuals at the first sighting.

35. In order to estimate minimum age of an individual in years, the age of the individuals at the first sighting (X) is estimated by choosing one of the morphological categories described in Table 6.

Table 6. Modified morphological categories of the Mediterranean monk seal (taken from Ok, 2006).

Stage	Characteristics of the category	Period (years)	Photo/illustration Photos taken from Dendrinos et al. 1999 Illustrations taken from Samaranch and Gonzales, 2000
1	skinny (pup-premolted; pms)	0.00-0.03	
2	fat (pup-premolted; pmf)	0.03-0.08	

3	pwm moulting (pup-preweaned; pwm)	0.08-0.14	
4	pup-preweaned (pw)	0.14-0.33	
5	youngster- weaned (y)	0.33-2.50	
6	subadult (sa)	2.50-6.00	
7	adult female young (afy)	6.00-7.00	
8	adult male young (amy)	7.00-8.00	
9	adult female elder (afe)	8.00-20.00	
10	adult male elder (ame)	9.00-20.00	
11	senesce female (sf)	20.00- -	Not available

5.2.2. Fecundity

36. Fecundity of the population is calculated using the formula formed by Akçakaya et al. (1999)

Ft = Pt+1/At

Ft: Fecundity at time t.

Pt+1: Number of pups born at time t+1.

At: Number of parents at time t.

5.2.3. Annual birth rate

37. Annual birth rate of the population is calculated according to Gazo et al. (1999)

ABRt = Pt/ AFt

ABRt = Annual birth rate at time t

Pt = Number of pups born at time t

AFt = Number of sexually mature females (categories starting from 7 in Table 2) at time t

5.2.4. Survival and Mortality rates

38. Number of individuals and deaths (mainly stranded animals) are recorded for each year and used to calculate the annual mortality rate and subtract from one to obtain overall survival rate to the next year. Following formula of Akçakaya et al. (1999) summarizes the calculation:

$S_t = 1 - (D_{t+1} / N_t)$

S_t: Survival of the individuals at time t.

N_t: Number of individuals at time t.

D_{t+1}: Number of deaths at time t+1.

5.3. Additional Advanced methods

5.3.1. Population Viability Analysis

39. Population viability analysis is used to explore current and future status of the Mediterranean monk seals including the threats faced by species, risk of their extinction or decline, and their chances for recovery, based on species-specific data as described by Akçakaya et al. (1999). Various types of population models can employ depending on the structure of the population. A stage-structured stochastic population model is used as its groups individuals in a population according to their age or morphological characteristics, allowing vital rates (survival and fecundity) by age or stage-class to be integrated in the model (Akçakaya 2000). Model results are summarized in terms of population trajectories and risks of decline within different time durations and different parameters.

5.3.2. Mark-recapture Analyses

40. Data derived from photo-Identification is exploited in mark-recapture analyses. In this approach, re-sighting events of seals with distinctive markings are used to study the movement patterns, site fidelity, and population size (Karlsson, Hiby, Lundberg, 2005). More specifically, the marking recapturing index (Lancia et al., 1994) is used considering 2-sample closed population model of Lincoln-Petersen (Lincoln 1930). The first step is to capture and mark a sample of individuals. Marking methods depend on the species. In monk seals, identified individuals are assumed as marked individuals. The assumption behind mark-recapture methods is that the proportion of individuals identified in first control recaptured in the following period represents the proportion of identified individuals in the population as a whole.

6. Quality control

41. All the survey protocols filled are cross-checked between at least two members of the survey team. Photographs taken by camera-traps are scored by different researchers taking into account various factors such as image resolution, level of distinctiveness, visibility of natural marks. In order to test the accuracy of the photo-identification, the same set of photographs are assessed by different researchers. Each national monitoring group has its own quality control protocols. Although especially photo-identification methods used are similar, the selection, scoring, and matching of

images are varied greatly amongst research groups. Therefore, it is recommended that a common protocol in quality control should be developed between the contracting parties.

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Annex 1: Field survey protocol

Date:	Time start:	Time Stop:						
Team:	Survey type:							
Weather Conditions								
Wind speed:	Calm	Medium	Strong					
Cloudiness:	Bright	Partl	Cloud	Rainy				
Sea Condition								
Wave direction:								
Wave strength:	Cal	Mo	Ro	Sto	Sw			
Turbidity:	Clear	Blui	Green	Brow				
Tide	Up tide	Normal	Low tide					
The Coastal	From:	To:	**GPS file:**					
Caves Discovered								
Cave	L	L	Remark					
Other survey remarks								
Event	Time	**Lat**	**Lon**					

Annex 2: Cave inventory sheet

Cave code	**Cave name**	**Discovered by**
Cave Info		
Latitude	Longitude	Photo frame
Total length in meters (opening to far end)		
Number of seal (s) :	Sighting Code :	Odor :
Number of chambers	With air:	Without air:

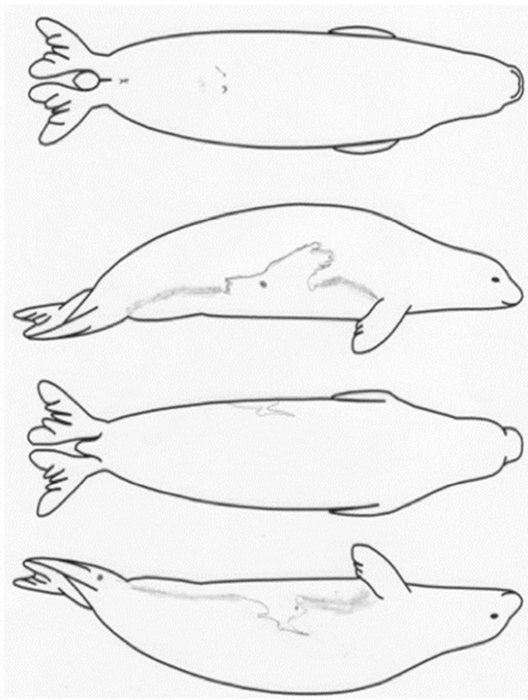
Cave entrance information							
Entrance #	Surface	Underw	Land	Depth	Height	Width	Direction

Platform information								
Platform	Positio	Length	Width	Texture	Suitabil	Feces	Fur	Track

Seal Evidence					
Platform	Depression	Track	Fur	Feces	Other

Sketch of the cave

Annex 3: identification sheet



Code	: Y1
Sex	: Female (Youngster)
Sighted in	: Zafer Burnu
Cave(s) used	: Z1
Number of photos	: 20
Identification	: Ventral discoloration

C. Guidelines for monitoring sea birds in the Mediterranean

Table of Contents

Executive Summary

1. Conservation and wise use of marine ecosystems requires managing human activities. Sound scientific knowledge is needed to allow for adequate measures to be put in place. Monitoring and assessment of biological populations, and of the ecological conditions on which they depend, becomes essential to achieve the conservation objectives.

2. In the Mediterranean region, the UN Environment/MAP Barcelona Convention ***Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria*** **(IMAP)** defines the roadmap to deliver the implementation of the Ecosystem Approach Process (EcAp process), between 2016 and 2021, to assess the status of the Mediterranean Sea and coast, as a basis for further and/or strengthened measures.

3. In relation to seabirds, IMAP proposes to monitor and assess the following common indicators (CIs): **CI 3: Species distributional range** (EO1); **CI 4: Population abundance of selected species** (EO1); **CI 5: Population demographic characteristics** (EO1, e.g. body size, age class structure, sex ratio, fecundity rates, survival/mortality rates). IMAP recommends monitoring and assessing those common indicators for a selection of representative species, 11 in total, organised into 5 functional groups.

4. **Functional groups** aim to combine information on different species to illustrate the effect of common factors. Each functional group represents a predominant ecological role (e.g., offshore surface-feeding birds, demersal fish) within the species group. For the purpose of these guidelines, the most relevant functional groups are coastal top predators, inshore benthic feeders, offshore surface-feeders, inshore surface feeders and offshore (surface or pelagic) feeders.

5. It is recommended that competent authorities develop a **monitoring strategy**, detailing the species, data, methodology, sites and timeframe. It should also specify the uses of the collected data. Ideally, the monitoring strategy will be implemented through successive multi-annual work plans. It is advisable to keep things simple and aim for the long term; a few species monitored in a reasonable number of representative sites over many (20+) years is likely to provide more informative results than in the case of more ambitious approaches with a variable effort over shorter periods of time.

6. The choice of monitoring method will depend on the species and data being sought. Counting birds at colonies (**colony census**) is the single most effective way of obtaining numerical information on species abundance and population trends over time. The number of colonies, and their spatial distribution also provides information on species distribution range. Censuses should be carried out regularly every 5 – 10 years and must be done professionally to keep disturbance to a minimum.

7. Outside of the breeding colonies, **counting bird numbers** at particular sites where birds aggregate (for roosting, bathing, etc.) can provide a good indication of their abundance, especially if censuses are carried out simultaneously at several sites in a particular area. Birds' presence may be influenced by external factors, so good knowledge of local conditions and a large sample size can help improve accuracy of the estimates. Similarly, **shearwater rafts** at sea near the breeding sites can be used as a proxy for breeding numbers at those sites, but there is large variability in the size of those rafts, so they do not necessarily represent differences in population size at the site. This method can complement other techniques, but it is not recommended on its own to estimate bird abundance.

8. **Migration point counts** allow for the assessment of the total abundance of birds passing through narrow points at sea. This method can only be expected to provide reliable estimates at a few strategic points like the Strait of Gibraltar but may be less accurate elsewhere. Detectability can be an issue, but it could be improved using distance sampling methods. Counting birds at migration points does not allow to establish a link with national populations, so its use is limited.

9. **Ship-based surveys** in set transects at constant speed are a very effective method to monitor seabird distribution and abundance, particularly when the probability of detection is estimated at the same time using the method of distance sampling. Ideally, the surveying team should have free use of a vessel and control over its course of travel and speed. Seabird distribution can be heavily disrupted by the appearance and activity of the survey vessel; fishing boats are the least suitable for surveying, as they tend to attract a

large number of species. When surveying, it is recommended to record the activity of the own as well as other vessels, especially if they are fishing.

10. **Aerial surveys** are another effective method to study distribution and non-breeding abundance on a large scale but may not be a preferred method in the Mediterranean context. Plane time can be very expensive, and the distance and speed of the survey may limit the ability to detect or identify difficult species. It is important to record all events (e.g., presence of fishing boats) during the surveys. Distance sampling methods should be used to estimate density.

11. **Citizen science** (opportunistic observations) and **fishermen questionnaires** are supplementary methods to obtain additional information on seabird distribution. Effectiveness of these methods is limited; their value increases when boat-based observations are provided by regular collaborators and when the exact location (coordinates) is recorded.

12. **Capture–mark–recapture methods** are highly effective in providing robust estimates of demo-graphic variables, but they require adequate planning and long-term commitment (at least 5 years, ideally 10 or more), as well as highly specialised teams. This restricts the use of CMR methods to a relatively small number of sites and species. The team should also collect data *in situ* on the breeding biology of the species under study to allow for the development of population models.

13. **Tracking** methods are increasingly popular and may be extremely useful to unveil the movements and behaviour of a small number of individuals. However, those individuals may not be necessarily representative of the whole population, so sufficiently large sample sizes may be required. Tracking provides presence-only data at a medium to very high cost; their effectiveness to monitor bird abundance is limited, but they can help find/identify hotspots of seabird activity.

14. **Automated trail cameras** can be used to provide data on breeding success and on the causes of failure (e.g., predation). This method is very effective in obtaining information, and multiple cameras can be deployed at several colonies. There are associated costs in the cameras and in the number of human hours required to analyse the images or videos. The use of **drones** allows for the estimation of the total area occupied by the breeding colony, as well as total number and several estimations of density. Some preparation is needed before the start of the breeding season. Surveys should be stopped at the first evidence of disturbance/stress.

15. **Comprehensive censuses** should cover all (most) breeding sites and should be carried out regularly, every 5 to 10 years. More intensive work can only be carried out at a few sites at a time: selected sites should be representative of the range of ecological conditions available in the country or region. Also, care is needed when extrapolating to the whole area of results from a few sites.

16. Survey effort should be timed to coincide with the **peak of detectability** of each species. The biggest effort must be directed at continuing the **time series** of previous monitoring activities. Most statistical analysis methods can cope with one gap in the series, but few can manage two consecutive gaps (seasons) without data.

17. Use of the monitoring **data** should be defined in the monitoring strategy. Data collection should be straightforward and clear, and it should remain constant for as long as possible, for consistency in the time series. The types of statistical analyses should be clear from the beginning, and they should be shared with the team doing the field work to increase the quality of the data.

18. **Reporting** must follow the UN Environment/MAP Barcelona Convention integrated data and information system and should be based on the structure of the Common Indicator Fact Sheets. For EU Member States, the specific reporting scheme of article 12 of the Birds Directive requires them to provide data on the actual state and trends of bird populations, with the next report due in 2019.

1. Introduction

19. UN Sustainable Development Goal 14 "Life below water" urges to conserve and sustainably use the oceans, seas and marine resources for sustainable development. To achieve this goal, it is necessary to manage human activities and to promote the conservation and wise use of marine ecosystems. Monitoring and assessment, based on scientific knowledge, become indispensable tools in order to assess the status of any marine system and to put in place adequate measures.

20. The Ecosystem Approach (CBD 2000) integrates the management of human activities and their institutions with the knowledge of the functioning of ecosystems. It requires to identify and take action on influences that are critical to the health of marine ecosystems, thereby achieving sustainable use of ecosystem goods and services and maintenance of ecosystem integrity (Farmer et al. 2012). To inform management planning adequately, it is especially important that assessment methods and management tools can incorporate new knowledge, new monitoring methods (to tackle the problem of covering large areas) and indicators into assessments, but still maintain comparability with previous assessments so that any change in the status can be measured and quantified (Borja et al. 2016).

2. Policy framework

21. In the context of the Mediterranean, the United Nations Environment Programme / Mediterranean Action Plan adopted in 2017 its Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria, IMAP (Decision IG.22/7). IMAP describes the strategy, themes, and products that the Contracting Parties of the Barcelona Convention are aiming to deliver over the second cycle of the implementation of the Ecosystem Approach Process (EcAp process), between 2016 and 2021, in order to assess the status of the Mediterranean Sea and coast, as a basis for further and/or strengthened measures.

22. In relation to seabirds, IMAP proposes to monitor and assess the following common indicators:

<u>Common Indicator 3: Species distributional range</u> (EO1);

<u>Common Indicator 4: Population abundance of selected species</u> (EO1);

<u>Common indicator 5: Population demographic characteristics</u> (EO1, e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates)

23. IMAP recommends monitoring and assessing those common indicators for a selection of representative sites and species, which can showcase the relationship between environmental pressures and their main impacts on the marine environment. For seabirds, these are summarised in Table 1 below:

FUNCTIONAL GROUP	SPECIES	
coastal top predators	*Falco eleonorae* *Pandion haliaetus*	Eleonora's Falcon Osprey
intertidal benthic-feeders	*n.a.*	
inshore benthic feeders	*Phalacrocorax aristotelis desmarestii*	(Mediterranean) Shag
offshore surface-feeders	*Larus audouinii*	
inshore surface feeders	*Larus genei* *Thalasseus (= Sterna) bengalensis*	Slender-billed Gull

	Thalasseus (= Sterna) sandvicensis	Lesser Crested Tern Sandwich Tern
offshore (surface or pelagic) feeders	*Hydrobates pelagicus* *Calonectris diomedea* *Puffinus yelkouan* *Puffinus mauretanicus*	European Storm-petrel Scopoli's Shearwater Yelkouan Shearwater Balearic Shearwater

24. It is also recommended that the Contracting Parties include at least the monitoring of those species with at least two monitoring areas, one in a low-pressure area (e.g. marine protected area/ Specially Protected Area of Mediterranean Importance (SPAMI)) and one in a high-pressure area from human activity.
25. In the context of the European Union, Commission Decision (EU) 2017/848 [1] sets the criteria, methodological standards, specifications and standardised methods for monitoring and assessment of biological diversity. It establishes the need to define the criteria, including the criteria elements and, where appropriate, the threshold values, to be used for each of the qualitative descriptors of Good Environmental Status (GES). Threshold values are intended to contribute to the determination of a set of characteristics for GES and inform their assessment of the extent to which it is being achieved. It further establishes that monitoring and assessment should be based on the best available science. However, additional scientific and technical progress may still be required to support their further development and should be used as the knowledge and understanding become available.

3. Species aggregation – functional groups

26. The use of functional groups for monitoring and assessment purposes results from the work of the Joint ICES/OSPAR Working Group on Seabirds (JWGBIRD) (ICES 2015). Functional groups aim to combine information on different species in order to illustrate the effect of common factors. The rationale for this classification is that it is expected that natural and anthropogenic factors are likely to act similarly on species that share the same food types and display similar feeding behaviours and are those, subject to the same constraints on food availability. Several regional conventions for the protection of the marine environment have adopted the use of functional groups of species (e.g., OSPAR, HELCOM), and they also feature in the revised Commission Decision on the Marine Strategy Framework Directive (2017/848/EU).
27. IMAP defines functional groups as ecologically relevant sets of species, in particular (highly) mobile species groups, such as birds, reptiles, marine mammals, fish and cephalopods. Each functional group represents a predominant ecological role (e.g. offshore surface-feeding birds, demersal fish) within the species group. For the Mediterranean region, and for seabirds in particular, the most relevant functional groups are:

coastal top predators – birds of prey and other large predators at the top of the food chain in the coastal environment, so not necessarily true seabirds *stricto senso*. In an unperturbed environment, a typical representative would be the White-tailed Eagle (*Haliaetus albicilla*), a predator of seabirds, as well as mammals and fish that historically suffered from prosecution and has now become rare in the region. Two other birds of prey, Osprey (*Pandion haliaetus*) and Eleonora's Falcon (*Falco eleonorae*) typically

[1] Commission Decision (EU) 2017/848 of 17 May 2017 laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardised methods for monitoring and assessment, and repealing Decision 2010/477/EU

nest on sea cliffs. Although ecologically their niche may be broader, they are considered to belong to this group for monitoring and assessment purposes.

intertidal benthic-feeders – typically shorebirds (including Spoonbill *Platalea leucorodia*), ducks, geese, swans and gulls that mostly walk or wade while feeding. In the Mediterranean region, such birds generally associate with wetlands or saltpans, rather than being characteristically coastal or marine. IMAP does not identify any particular species as belonging to this functional group, so none will not be considered for these Guidelines.

inshore benthic feeders – birds that dive to the seabed to feed, generally on demersal fish. In the Mediterranean region, this group is best represented by the Mediterranean Shag (*Gulosus (=Phalacrocorax) aristotelis desmarestii*), an endemic form estimated to number only 10,000 individuals and showing a comparatively local distribution. Mediterranean Shags have historically suffered a succession of declines and recoveries and may be heavily affected by human pressure, both as a result of habitat occupation and of bycatch in fisheries.

offshore surface-feeders – birds (e.g., gulls) that feed in the top layer of the water column on the outer part of the continental shelf or in the open sea. The Mediterranean endemic Audouin's Gull (*Larus audouinii*) is the most characteristic species of this functional group in this region. The species was once rare but has seen a substantial recovery (especially in the western Mediterranean), as a consequence of the increased availability of fishing discards and of the protection of its nesting habitat.

inshore surface feeders – restricted as feeders to the surface layer of the water column and occurring mostly near the shore. In the Mediterranean region, this niche is occupied by the Slender-billed Gull (*Larus genei*), Lesser Crested Tern (*Thalasseus (= Sterna) bengalensis*) and Sandwich Tern (*Thalasseus (= Sterna) sandvicensis*). The former two, whilst not being endemic as species, have geographically and numerically significant populations in the Mediterranean. Their specialised association to low-lying coasts and shallow waters has traditionally made them vulnerable to habitat transformation.

offshore (surface or pelagic) feeders – open seas are typically the realm of seabirds that feed across a broad depth range in the water column (albatrosses, petrels, penguins). In the Mediterranean, they form a small group of endemic species that are extremely important for conservation: the Balearic Shearwater (*Puffinus mauretanicus*) and the Yelkouan Shearwater (*Puffinus yelkouan*) are both globally threatened. Together with Scopoli's Shearwater (*Calonectris diomedea*), which is also endemic, they fall frequent victims to bycatch in longline fisheries and are also threatened on land by introduced predators in their breeding colonies. The European Storm-Petrel (*Hydrobates pelagicus*) is the sole representative in our region of the cosmopolitan group of storm-petrels; these are small but long-lived and truly oceanic seabirds that feed on plankton and act as effective indicators of the general state of the marine environment.

4. Monitoring strategy

28. For effective use of limited resources, it is crucial that competent authorities develop a monitoring strategy, which can provide detail on important aspects such as species, sites, methods and timing and regularity. It is also important to decide on the uses of the collected data. Ideally, the strategy will be implemented through successive multiannual work plans that will integrate pre- and post-field work, as well as the development of the monitoring activities that need to be undertaken.
29. Based on the species composition, area and available resources, a monitoring strategy should cover the following aspects:

a) **Species** – as a minimum, the representative species of each functional group (Table 1) should be monitored on a regular basis, if present in the country. It is possible to add more species to the mix, but such a decision must take into account that effective monitoring requires a long-term commitment, which may be difficult to meet for prolonged periods of time. Also, the decision to monitor additional species should not put at risk the monitoring of the standard species set, as these benefit from the fact that they are being monitored on a wider scale (e.g., whole Mediterranean region), which adds value to the data obtained at national or local scale.

b) **Data** – the nature of the data to be collected varies with the common indicator and is specified in the Common Indicator factsheets. A monitoring strategy should consider possible data in the form of numerical values of distribution (total area occupied, number of squares, maps), abundance (number of birds present, number of apparently occupied nests, etc.; relative density), breeding productivity (young fledged per egg laid, young fledged per breeding attempt) and general demography (annual survival rate, juvenile recruitment rate, age class ratio). Wherever possible, it is recommended to collect supplementary data on environmental pressures that may be biologically relevant, as already in practice in some countries. Such data may include colony surveys for evidence of predation or evidence of anthropogenic waste (e.g., plastics) in seabird nests, as well as blood and/or feather sampling for evidence of contaminants in adult birds or their young.

c) **Methodology** – an assessment of population size can be obtained either by counting the total number of individuals at a given time or by counting numbers at selected periods of sampling, and then calculating the total number through extrapolation. The latter method (i.e., sampling + calculating) is by far the commonest, but it requires an appropriate design of the sampling periods / sites, plus the use of robust statistical methods for the calculation. A monitoring strategy should be specific about the sampling methods, the monitoring techniques and the calculation procedures. It should also describe how different methods should interact, e.g. by calculating an annual population trend value (through stratified and representative sampling) and combining with a comprehensive, large-scale census every 5 or 10 years.

d) **Sites** – the monitoring strategy shall define the spatial dimension of its sampling effort. Whole-area censuses can only be carried every number of years (usually, between 5 and 10), whereas the annual effort of obtaining data on population trends or on breeding performance will have to be limited to a smaller sample of representative sites. Even within single (large) colonies, it is often necessary to obtain detailed data from a randomised selection of squares. The number and location of colonies monitored will influence the results [2], so it is important that the strategy considers the representativeness of each site in relation to the general context. It is generally recommended to treat the data with robust statistical methods that bear in mind the relative weight of each site in the wider context of the entire population.

e) **Timeframe** – the timing and repeatability of monitoring activities will vary according to species and area. In general, the monitoring strategy should aim at obtaining data *ad infinitum*, or at least for as long as

[2] Tobler's first law of Geography (spatial autocorrelation) applies: "Everything is related to everything else, but near things are more related than distant things" (Tobler 1970).

threatened species or sites remain in that status. For that reason, the strategy should aim at obtaining the most valuable data (e.g., overall productivity with preference over first egg laying date), and the multiannual work plan should guarantee that the necessary monitoring takes place at least once every year. For effective monitoring, the strategy should also take into account the issue of seasonality and propose the ideal timing for each sampling to take place. Ideally, the work plan should seek to optimise and combine samplings for different species, wherever possible, to maximise the outcome.

30. In general, it is advisable to keep things simple and aim for the long term; a few species monitored in a reasonable number of representative sites over 20+ years is likely to provide results that are far more informative than in the case of more ambitious approaches with a variable effort over shorter periods of time.

5. Monitoring methods

31. The choice of monitoring method will depend on the species and data being sought. For seabirds in the Mediterranean region, the following methods may be considered:

Colony census

- All seabirds invariably need to visit land in order to nest, and most breed colonially. Counting birds are colonies is the single most effective way of obtaining numerical information on their abundance (Common Indicator 4), and thus of their population trends over time. The number of colonies, and their spatial distribution also provides information on species distribution range (Common Indicator 3).

- In medium (250-1000 breeding pairs) to large colonies (> 1000 b.p.), it will be difficult to accurately assess the exact number of birds present. In these cases, it is recommended to record and plot the entire area of the colony (e.g., by using drones, see below), and to monitor the spatial evolution of the colony over time.

- For very large colonies (e.g., > 5000 b.p.), it is recommended to define smaller squares (e.g., 20 x 20 m, 50 x 50 m, 100 x 100 m or larger, depending on the species and the geography of the site) and to count every single nest inside the square, to obtain a measure of density. By repeating the same procedure on a number of squares, it is possible to obtain a measure of the average density, as well as its standard deviation. Such values can be used to calculate the total population of the colony, by multiplying the total number of squares by the average density ± standard deviation.

- For burrow-nesting species (storm-petrels, shearwaters), it is good practice to estimate the average number of nests per burrow, as a single burrow or cave may contain several breeding pairs or nests.

Land-based roost (aggregation) counts

- Several species, particularly of gulls, terns and cormorants (shags), aggregate at predictable sites after feeding or for roosting, bathing, etc. Assessing bird numbers at those sites can provide a good indication of their abundance (Common Indicator 4), especially if censuses are carried out simultaneously at all sites where birds aggregate in a particular area. This method is not without its drawbacks, as bird presence may be influenced by external factors such as weather, season, day of the week, etc., so good knowledge of local conditions and a large sample size can help improve accuracy of the estimates.

- Similarly, the well-known tendency of some seabirds, particularly shearwaters, to form rafts at sea near the breeding sites can be used as a proxy for breeding numbers at those sites. It is also known, however, that there is large variability in the size of those rafts, due to weather, time of year and local characteristics of each colony, so they do not necessarily represent differences in population size at the site. Given the number of potential biases (disturbance, time of day, weather conditions), this method should only be considered as supplementary to other monitoring methods, because it may not be indicative of abundance. The rafting behaviour at well-known breeding areas, though, may be useful to inform the management of marine extensions to breeding colonies, in terms of phenology, spatial extension, etc.

Migration point counts

- As birds travel between different areas (e.g., during migration), geography may force them to funnel through certain narrow points, where they become easier to detect and to count. One such place in the Mediterranean region is the Strait of Gibraltar, the only connection between the Mediterranean Sea and the Atlantic Ocean and a necessary gateway for all species whose populations move between the two. A small number of similar places exist in the region (e.g., Bosphorus, Dardanelles, northern Tunisia, strait of Otranto) but their accuracy in tracking bird numbers is probably less reliable. Bird abundance passing on migration near such places can be used as a proxy for their total abundance (Common Indicator 4). However, issues of detectability (only a proportion of all birds passing near the watchpoints can be seen from land) and representativeness (the breeding sites of passing birds cannot be known) make this method not entirely suitable for monitoring seabirds in the Mediterranean. Combined analyses of all watchpoints on a regular (annual) basis, and a long time series, may be able to reflect real population changes.

Ship-based surveys

- Systematic surveying of marine areas in search of seabirds has historically produced good results in the detection of hotspots of activity, generally associated to foraging behaviour. Observations of seabirds in set transects at constant speed are particularly useful if the probability of detection is estimated at the same time using the method of distance sampling (Buckland et al. 2001). This method allows for the estimation of the density of each species per transect (or per fraction of transect). Multiple estimations of density can be combined and averaged for each unit of space (e.g., 10 x 10 km or 1° x 1° cells), so they can be mapped and analysed spatially. This provides useful values of bird distribution (Common Indicator 3) and abundance (Common Indicator 4).

- This well-known method requires free use of a vessel that can offer good visibility, ideally with vantage points as used for cetacean surveys; line ferries are used in several places with positive results, but their inability to change course limits their effectiveness for seabird monitoring. Seabird distribution can be heavily altered by the appearance and activity of the survey vessel; fishing boats are the least suitable for this purpose, as they tend to attract a large number of species. When surveying, it is recommended to record the activity of the own as well as other vessels, especially if they are fishing.

- To make the data comparable inter-annually, it is important that surveys are carried out at the same time each year, and with efforts that are comparable. In addition, this monitoring must be coupled with measurements of environmental variables, particularly of the water mass (temperature, chlorophyll, etc.), to make it possible to link the inter-annual variability of observations to environmental conditions.

Aerial surveys

- Similar to ship-based surveys but on another scale, aerial surveys are used to collect distribution and abundance data on seabirds, particularly of species with high detectability (e.g., gannets Morus sp.) or low mobility (e.g., auks Alcidae). Using distance sampling methods, aerial surveys can provide abundance data over large sections of the ocean and are thus quite effective, albeit expensive. However, in the Mediterranean region and for our set of species, aerial surveying is arguably not the most suitable method. Detectability can be potentially quite low (e.g., of storm-petrels, shearwaters) and identification at species level may be very difficult, almost impossible in some cases (e.g. Balearic vs. Yelkouan Shearwater, or Sandwich vs. Lesser Crested Tern). For difficult species, the use of HD cameras for photo ID will undoubtedly improve identification (as successfully tested in e.g., France).

- As with other surveys, it is important during aerial transects to collect data on environmental variables to enable habitat modelling and testing of hypotheses.

Citizen science (bird portals, logbooks, opportunistic observations)

- Opportunistic observations of seabirds collected non-systematically by amateur ornithologists, seafarers or the general public can provide additional information on bird distribution (Common Indicator 3). Such data can rarely be used to estimate densities, and therefore abundance, because they generally lack essential information on the space covered (transect) or the observation effort (time). Their value lies in their ability to provide information on spatial distribution and is particularly useful in detecting change in the distribution of rapidly expanding species.

Questionnaires (fishermen, seafarers)

- Through the use of questionnaires, it is possible to obtain useful information from fishermen or professional seafarers. The value of this information is generally qualitative and not quantitative, so it is most useful when it involves data on seabird distribution (Common Indicator 3), particularly on the location of nesting sites / colonies. Occasionally, the collaboration of fishermen can provide additional info on breeding phenology or success, although the burden of the collection of demographic data must remain with objective methods such as colony counts by experienced staff possibly with the assistance of cameras near nests.

Capture – Mark – Recapture

- Capture – mark – recapture (CMR) methods provide robust estimates of demographic variables such as individual survival, recruitment and emigration (Amstrup, McDonald & Manly 2005). They require adequate planning and long-term commitment, because seabirds are generally long-lived. For this activity, highly specialised teams are required that can capture and ring a sufficiently large number of birds over a long sequence of years (at least 5 years, ideally 10 or more), and who can analyse the data using specific software (Program MARK: White & Burnham 1999). This restricts the use of CMR methods to a relatively small number of sites and species.

- In most cases, the same team of professional biologists collect data in situ on the breeding biology of the species under study (e.g., no. of eggs laid, hatching success, chick survival, breeding success) that add to the information on demography and are essential for the development of population models. Also, by taking additional data during the same fieldwork, e.g., samples of feathers/blood to monitor contamination by pollutants, it is possible to test hypotheses and develop population models that will contribute to our understanding of variations of the "Common Indicator 5 (demography)".

Use of tracking methods (VHF, GPS, PTT) to locate important sites

- With the development of tracking technologies, the movements and behaviour of many individuals of several seabird species have been unveiled. In the Mediterranean region, the most intensively studied species with this method are Scopoli's and Yelkouan Shearwaters, Audouin's Gull, Eleonora's Falcon and Osprey. Tracking only provides information about the unique movements of tagged individuals, so a large sample size may be needed to extrapolate those movements to the rest of the population. Despite the limitations, tracking data can be particularly useful in assessing the distribution of birds in a population or in finding their breeding sites (e.g., the discovery of new colonies) (Common Indicator 3). On the negative side, this method is expensive and can only provide presence-only data from a fraction of the population.

- Tracking data can be analysed against environmental variables, either collected in the field or from remote sensing, for functional habitat modelling or testing of hypotheses.

Trail cameras

- Automated trail cameras can be situated strategically at nesting sites to obtain timed data about breeding biology and behaviour with limited disturbance. Importantly, trail cameras can also provide data on breeding success and on the causes of failure (e.g., predation), so they can provide very useful additional data to inform and test data from Common Indicator 5 (demography), as described previously. This method is very effective in obtaining information, and multiple cameras can be deployed at several colonies. However, there are associated costs in the cameras themselves and in the number of human hours required to go through the recorded images or videos.

Drones

- The use of drones to assess breeding numbers at a given site is increasingly popular and constantly being developed. This method allows for the estimation of the total area occupied by the breeding colony (Common Indicator 4), as well as total number and several estimations of density if the necessary arrangements have been put in place before the birds settle to start breeding (see Sardà-Palomera et al. 2017). For asynchronous species (e.g., Eleonora's Falcon) it may useful to survey the colony several times in order to obtain data from all phases of the breeding cycle and count in all nesting attempts.

6. Territorial coverage

32. A monitoring strategy should recommend the spatial scale of the monitoring effort – should all areas be monitored all the time? Or, given limited resources, is it better to concentrate on a few sites and extrapolate to the whole? The answers to these questions depend on the geographical characteristics, and on the species being monitored. In general, it is advisable to carry out regular censuses that cover all (most) breeding sites and attempt to count all the birds; such censuses should be carried out regularly, every 5 to 10 years.
33. For more intensive work, such as a capture–mark–recapture scheme, or monitoring with trail cameras or drones, work can only be carried out at only a few sites at a time. In the selection of those sites, it is important to follow two criteria: (i) the sites should be representative of the range of ecological conditions available in the country or region, so that good sites as well as not-so-good sites are included; and (ii) extrapolation to the whole area of results from a few sites must be done with care because that the country is likely to be ecologically diverse.

7. Sampling design and representativeness

34. To obtain precise estimates, it is necessary to plan the sampling effort adequately. This is particularly important when the whole area cannot be surveyed and only a selection of squares (cells) can be visited to obtain data. Survey effort should cover a sufficient number of cells that (a) represents the entire spectrum of ecological conditions, and (b) is statistically robust to allow for analysis of the data. The same strategy applies to the local scale, in choosing the number of squares to count nests in a large breeding colony, or on a large scale, in surveying marine areas using transects.
35. Sampling should take place over enough cells, and preferably in the same cells or transects, every time. Through this spatial consistency, a data log of bird counts at each spatial unit will develop over time that will allow for further analysis in the future, if conditions change.

8. Timing and regularity – the importance of long-time series

36. Survey effort should be timed to coincide with the peak of detectability of each species, for optimal results. Peaks of breeding activity vary seasonally and often during the course of the day for all species, and a monitoring strategy should account for that variability whilst trying to integrate different monitoring activities into a single work plan. In any case, it is important to record all relevant details (day of week, time of day, activity of fishing vessels, disturbance events, etc.) when carrying out the surveys, so that they can be taken into account during the analysis of the data.

37. The value of monitoring becomes increasingly important as the time series becomes longer, because the ability to detect change also increases. Therefore, the biggest effort must be directed at continuing the time series of previous monitoring activities, which must remain unaltered with the same methods and in the same places unless there is good reason to change.

38. Most statistical analysis methods can cope with one gap in the series (generally equivalent to one season without monitoring), but few can manage two consecutive gaps (seasons) without data. Time series interrupted in this way are generally irreparable and end at that point.

9. Data management, analysis and control

39. Use of the monitoring data should be defined in the monitoring strategy. This aspect should be integrated in the design of all monitoring activities, and it should be taken into account when they are carried out. Data collection should be straightforward and clear, and it should remain constant for as long as possible, for consistency in the time series. Ideally, a data analyst should form an integral part of the monitoring team, and they should be able to inform survey design. This strategy will improve the overall efficiency of the team.
40. The types of statistical analyses should be clear from the beginning, and they should be shared with the team doing the field work. With an increased understanding of the whole process, individual observers will put more attention into collecting additional or supplementary data about the conditions at the time of conducting their activity; this will increase the quality of the data.

10. Reporting

41. As part of IMAP's integrated assessment, Contracting Parties to the Barcelona Convention are required to report on the quality and status of the marine environment under their jurisdiction. Reporting must follow the UN Environment/MAP Barcelona Convention integrated data and information system and should be based on the structure of the Common Indicator Fact Sheets. IMAP encourages Contracting Parties to use up-to-date tools for data exchange.
42. In the context of the European Union, article 12 of the Birds Directive 2009/147/EC (EU 2009) requires that EU Member States report on the implementation of the national provisions taken under this Directive. This includes providing data on the actual state and trends of bird populations, and must be done every six years, starting in 2013, so the next report is due in 2019. The Birds Directive applies to all species of naturally occurring birds in the wild state in the European territory of the Member States, and a detailed report has to be completed for all regularly occurring species in the relevant seasons, including breeding, wintering and passage.

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Annex I Comparative table.: Characteristics of monitoring techniques

Monitoring technique	Suitable species	Common Indicator(s)	Personnel requirements	Equipment	Recommendation
Colony census	all	4 – abundance (3 – distribution range)	trained staff/volunteers; at least one team (2-3 people) per colony; ideally several teams working simultaneously in several colonies; coordination	boat to access islands or difficult places; binoculars; camera / drone	▪ single most effective technique; ▪ should be carried out regularly every 5 – 10 yrs; ▪ must be done professionally to keep disturbance to minimum
Land-based roost (aggregation) counts	*Puffinus* (rafts) *Calonectris* (rafts) *Phalacrocorax* *Larus* *Sterna*	4 – abundance	single trained observer or, preferably one team (2-3 people) per site; ideally, several teams working simultaneously in several sites; coordination	binoculars / telescope; access to viewing points	▪ no substitute for colony census (especially true for shearwater rafts) ▪ suitable for non-breeding species ▪ weather, season and local conditions may affect numbers ▪ should be repeated regularly
Migration point counts	*Puffinus* *Calonectris* *Larus* *Sterna*	4 – abundance	trained observers; at least one team (2-3 people) per watchpoint; ideally several teams placed strategically to maximise cover	binoculars / telescope; access to viewing points	▪ reliable estimates only expected at few places like Strait of Gibraltar, Bosphorus, etc. ▪ no link to breeding (national) populations

					▪ partial detectability; could be improved by using distance sampling
Ship-based surveys	all	3 – distribution range 4 – abundance if additional data taken	1-3 trained observers to cover 180° view; binoculars	vessel with good visibility (e.g. for watching cetaceans); control over vessel course/speed of travel; binoculars	▪ very effective method to study distribution and non-breeding abundance ▪ vessel time very expensive, so less optimal solutions often used ▪ ability to fix course/speed of travel needed for density estimation ▪ fishing boats change bird distribution and behaviour and should be avoided ▪ important to record all events (e.g., presence of fishing boats) during survey ▪ important to collect data on environmental variables, especially of the water mass (temperature, salinity, chlorophyll, etc.).
Aerial surveys	most species	3 – distribution range 4 – abundance	1-2 trained observers to cover 180° view; binoculars	low-speed aeroplane with good visibility; control over plane course/speed of travel; binoculars	▪ effective method to study distribution and non-breeding abundance on large scale ▪ plane time very expensive

					▪ ability to fix course/speed of travel needed for density estimation ▪ distance/speed limits ability to identify difficult species ▪ important to record all events (e.g., presence of fishing boats) as well as environmental data during survey
Citizen science (bird portals, logbooks, opportunistic observations)	all	3 – distribution range	volunteers with varying degrees of training		▪ low effectiveness; only supplementary info expected ▪ most valuable data from boat-based observations ▪ important to record exact location (coordinates)
Questionnaires (fishermen, seafarers)	all	3 – distribution range (5 – demography)	volunteering professionals; interviewing staff		▪ limited effectiveness ▪ value increased when collaboration becomes well established over time
Capture – Mark – Recapture	all	5 – demography (4 – abundance)	professional team (2-3 people) with ringing licence; data analyst	ringing equipment; access to colonies	▪ very effective method to obtain demographic data ▪ monitoring must be maintained for >5 yrs ▪ work at breeding colonies should can be combined with collection of data on breeding biology for

					comprehensive demographic analyses ▪ during fieldwork, important to collect additional data (e.g., blood/feather samples) for analysis of environmental factors
Tracking methods (VHF, GPS, PTT) to locate important sites	all	3 – distribution	professional team (2-3 people) with ringing licence; data analyst	tagging devices; ringing equipment; access to colonies	▪ extremely useful method to unveil individual movements / behaviour ▪ not necessarily representative of whole population, so large sample size required ▪ presence-only data ▪ medium to very high cost
Trail cameras	all	5 – demography	small professional team (1-2 people); image/video analyst	trail cameras (several); access to site	▪ can be used to provide data on breeding success and causes of failure (e.g., predation) ▪ effective and relatively low cost, but require long man hours of lab work analysing images/footage ▪ useful as supplementary method ▪ low disturbance
Drones	all	3 – distribution 4 – abundance if additional data taken	small team (1-3 people) with licence to fly drone;	flying drone; HD camera	▪ very useful to assess total area of breeding colony (for estimation of density)

			image/video analyst		▪ some preparation before breeding season essential ▪ survey should be stopped at first evidence of disturbance/stress

D. Guidelines for monitoring marine turtles in the Mediterranean

Contents

ACRONYMS AND ABBREVIATIONS

ASM	Age at Sexual Maturity
CCL	Curved Carapace Length
CF	Clutch Frequency
CI	Confidence Intervals
CMR	Capture-Mark-Recapture
CS	Clutch Size
DE	Number of dead embryos
EES	Number of empty egg shells
ES	Emergence Success
GI tract	Gastro Intestinal Tract
GPS	Global Positioning System
IP	Incubation Period
IUCN	International Union of Conservation of Nature
PE	Number of predated eggs
PIT	Passive Integrated Transponders
RMI	Remigration intervals
RMU	Regional Management Units
RNI	Re-nesting (inter-nesting) intervals
SCL	Straight Carapace Length
SSF	Small-Scale Fleets
TED	Turtle Excluder Device
UAV	Unmanned Aerial Vehicle
UE	Number of unfertilized eggs

1. INTRODUCTION

1. Two species of sea turtle – the loggerhead turtle and the green turtle – regularly occur and breed in the Mediterranean Sea. The breeding activities of both species are regularly monitored in the main nesting areas of ten countries; namely, Cyprus, Egypt, Greece, Israel, Italy, Lebanon, the Libyan Arab Jamahiriya, the Syrian Arab Republic, Turkey and Tunisia. The species' distributional range, population abundance and demographic characteristics are generally estimated according to nest counts in those above countries. A recent approach has been to divide all species of sea turtle into Regional Management Units (RMU; Wallace et al. 2010), identifying Mediterranean RMUs for loggerhead turtles (RMU:11) and green turtles (RMU:17).

2. Sea turtles are a long-lived species; they can take more than two decades to reach maturity. They also use different habitats at different age classes. Post-hatchlings mainly use pelagic habitats as developmental areas and remain offshore until they reach large juvenile size (<40cm Curved Carapace Length (CCL). However, once their CCL exceeds 30 cm, they start to shift their developmental areas to neritic habitats. The monitoring of sea turtles must therefore be conducted not only on beaches but also in the water, as they migrate between feeding grounds and spend the winter months.

3. The monitoring of sea turtles is mostly performed using these techniques: **(i) counting the number of nests during nesting period, (ii) collecting stranded turtles, (iii) in-water capture-mark-recapture studies, and (iv) boat and aerial surveys.**

4. Nesting female sea turtles and their clutches in particular, have been used as indicators of population size and trends (Bjorndal et al., 1999; Broderick et al., 2002; Margaritoulis, 2005; Türkozan & Yilmaz, 2008). Nesting activity has the potential to address two indications that specifically relate to the Barcelona Convention Decision on Common Indicators (IG.22/3), namely:

- Common indicator 4 (CI4): Population abundance of selected species
- Common indicator 5 (CI5): Population demographic characteristics

5. Sea turtles inhabit the shallow waters along coasts and around islands, but most are highly migratory, particularly as juveniles, and are found in the open sea. After the nesting season, species in temperate areas migrate to warmer waters, to avoid cold temperatures. In addition, only female turtles are observed on the nesting beaches; males and juveniles never come ashore (Heppell et al., 2003). Consequently, determining empirical estimates for the number of juveniles is extremely challenging.

6. For instance, boat surveys and aerial surveys can be used to estimate the number of turtles on the surface as Visual Counting Surveys and then the total number can be extrapolated. These techniques give an indication in accordance with the Barcelona Convention Decision (IG.22/3), in particular:

- Common indicator 3 (CI3): Species distributional range

7. These monitoring activities can be classified as: 1- Monitoring carried out on beaches; 2- Monitoring carried out at sea and 3- Monitoring that takes place in rehabilitation centres and/or labs.

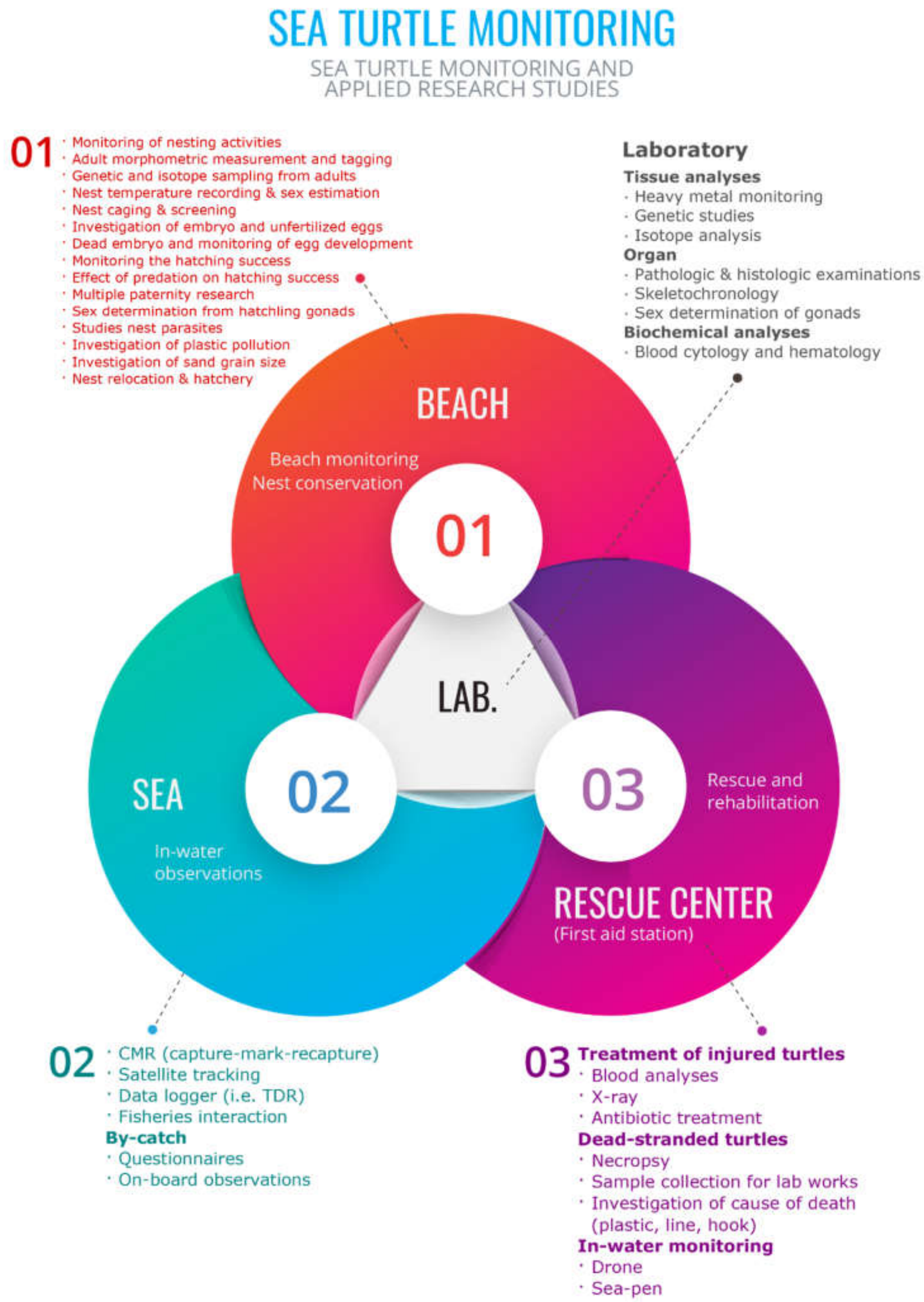

Figure 1. Spatial sea turtle monitoring and research activities

8. Sea turtles exhibit high nest-site fidelity. Research on migratory behaviour and the distribution of sea turtles shows that adult turtle fidelity to breeding sites is also a component of homing behaviour. It has also been directly observed, mainly in females, through flipper and satellite tagging (Margaritoulis, 1998; Broderick et al., 2003; Casale et al., 2013; Schofield et al., 2013). Site fidelity is even stronger in adults, as they appear to return to the same foraging ground after reproductive migration (Godley et al., 2003; Lazar et al., 2004; Broderick et al., 2007; Zbinden et al., 2008; Schofield et al., 2010a; Schofield et al., 2010b; Casale et al., 2013). Site fidelity can be monitored using standard flipper tagging and satellite tagging.

1.1. Distribution Ranges of Sea Turtles

1.1.1. Nesting Site Distribution of Loggerhead Turtles

9. Loggerhead turtle (*Caretta caretta*) nesting occurs over a wide area, with more than 96% of clutches laid in Cyprus, Greece, Libya and Turkey, which host the major nesting rookeries for this species in the Mediterranean. Lower levels of nesting take place on the Mediterranean shores of Egypt, Israel, Italy, Lebanon, Syria and Tunisia, with minor and infrequent nesting occurring along the western basin coastline of France, Italy, Spain and their offshore islands. Sporadic nesting is also recorded on the Aegean coast of Turkey and on the coast of Albania. If all the surveyed years are included, there is an average total of 6751 loggerhead turtle clutches per year, with 8179 in more recent times (Casale et al., 2018).

1.1.2. Nesting Site Distribution of Green Turtles

10. Green turtle (*Chelonia mydas*) nesting is restricted to the eastern Mediterranean and has only been recorded in Crete, Cyprus, Egypt, Israel, Lebanon, Syria and Turkey. There are 13 major nesting locations with an average of 1650 green turtle clutches per year, if all surveyed years are included and 2204 in more recent times (Casale et al., 2018). The principal green turtle rookeries are located in Cyprus, Syria and Turkey with minor nesting aggregations occurring in Egypt, Israel and Lebanon. The nesting sites in Turkey and Cyprus account for more than 90% of all green turtle nesting in the Mediterranean.

1.2. Population Abundance and Trends

11. The first parameter that needs to be analysed is population abundance and its trend in nesting populations. The nest counts and number of females nesting on the beaches, as mentioned above, need to be recorded using the same methodology. The population abundance in the sea has to be determined via in-water observations.

12. **Loggerhead turtle:** A more accurate comparison between past and current nest counts at 16 index nesting sites, which was included in a recent IUCN Red List assessment of the Mediterranean loggerhead turtle subpopulation as an RMU, reported a positive trend and was classified as of Least Concern (Casale, 2015). The abundance of adult females on the beach can be calculated from nest counts, clutch frequency (the number of clutches laid by a female in a nesting season), remigration intervals (the number of years between two consecutive nesting seasons) and adult sex ratio. The most recent available data provides an average of 8179 nests per year at the monitored nesting sites (Casale et al., 2018) and estimated 15843 adults (CI95%: 6915-31,958) (Casale and Heppell, 2016). Abundance estimates at sea, where juveniles represent the majority of the population, have been conducted through several spatially limited aerial surveys. Casale and Heppell (2016) attempted to provide at least the order of magnitude of a possible range of values for the total population abundance (including adults): from 1,197,087 (CI95%: 805,658-1,732,675) to 2,364,843 (CI95%: 1,611,085-3,376,104).

13. **Green Turtle:** For green turtles, a rough comparison of average nest counts at seven nesting sites between the same two arbitrary periods described above, indicates an overall positive trend. In Cyprus, an increasing proportion of neophytes (nesting females captured for the first time and assumed to be in their first year of breeding) was observed (Stokes et al., 2014), suggesting an increasing population. Monitoring programmes for green turtles at sea have yet to be established.

14. The most recent available data provides an average of 2204 nests per year at monitored nesting sites (Casale et al., 2018). Casale and Heppell (2016) estimated 3390 adults (CI95%: 1894-6552) with a population abundance from 261,727 (CI95%: 176,284-391,386) to 1,252,283 (CI95%: 679,433-2,209,833).

1.3. Population Demographics

15. Population demographic parameters need to be collected from nests and nest environments, as well as from in-water observations.

1.3.1. Monitoring of Development and Incubation Period

16. The monitoring of nests and embryos are also important and vary among the beaches. The incubation duration of clutches negatively correlates with nest temperature for both species of sea turtle (Godley et al., 2001a; Mrosovsky et al., 2002; Kaska et al., 2006) and is highly variable among the Mediterranean beaches. For example, viable hatchlings from loggerhead nest

temperatures as low as 26.5 ºC (with an incubation duration up to 79 days) have been recorded in Sicily, Italy (Casale et al., 2012a), whilst the longest incubation duration for loggerhead turtles in the Mediterranean (89 days) has been recorded twice on Marathonissi beach (Laganas Bay, Zakynthos) (Margaritoulis, 2005; Margaritoulis et al., 2011). At the opposite end of the temperature range, nest temperatures as high as 33.2ºC in Cyprus (Godley et al., 2001a) and with an incubation duration as short as 36 days in Calabria, Italy (Mingozzi et al., 2007) have been observed. Nest temperature measurements have also been carried out for green turtles and the nests were usually deeper than those of loggerhead turtles (i.e., Kaska et al., 1998; Candan & Kolankaya, 2016).

17. The parameters that need to be monitored here are as follows:

- Inter-nesting (or re-nesting) intervals (RNI) which is between 12.7-19.9 days,
- Remigration intervals (RMI),
- Clutch frequency (CF), the number of clutches deposited by a female in a single season,
- Incubation periods (IP),
- Hatchling sex ratios and,
- Hatching success and hatchling emergence success (ES%).

1.3.2. Recording the Clutch Size and Hatching Success

18. For loggerhead turtles in the Mediterranean, substantial differences exist in terms of clutch size, with the smallest females and clutch sizes observed in Cyprus and the largest females and clutch sizes observed in Greece. The number of clutches laid per season range between 1–5 clutches per season for loggerheads at Alagadi, Cyprus (Broderick et al., 2003) and this parameter could be associated with re-nesting interval. The mean clutch size for loggerhead turtles ranges from 64.3 to 126.8 eggs, among the different Mediterranean sites.

19. The mean clutch size among the different Mediterranean sites ranges from 108 to 120 eggs for green turtles (see references in Casale et al., 2018).

20. The monitoring and recording of nest depth, diameter, humidity, hatching success, clutch size, fertilization rates and mortality rates is essential.

1.3.3. Spatial and Temporal Monitoring of Sex Ratio

21. The sex ratio of hatchlings on the beaches and the sex ratios in adult and sub adult stages are important when monitoring the population of both sea turtle species. When estimating the sex ratio of the hatchlings, the most commonly used methods are nest temperature measurements and gonad histology. Laparoscopy can also be used for hatchlings and at later ages. The monitoring of the temporal and spatial changes of the sex ratio on the beaches is also very important when taking the possible effects of global warming into account.

1.3.3.1. Loggerhead turtle sex ratio estimations

22. The pivotal temperature (the egg incubation temperature at which both sexes are produced in equal numbers) for Mediterranean loggerheads assessed in laboratory and field conditions, is about 29-29.3°C and is similar to other populations elsewhere, with a pivotal incubation duration (at which both sexes are produced at equal numbers) of 53 days from laying to hatching (Kaska et al., 1998; Mrosovsky et al., 2002). Other studies carried out under natural conditions, (Fuller et al., 2013) found a slightly lower (28.9ºC) pivotal temperature and a longer incubation duration than expected (56.3 days), due to the effect of metabolic heating generated by the whole nest.

23. By applying different indirect sex determination methods, loggerhead hatchling production at most Mediterranean nesting sites are likely to be highly female-biased, with the major rookeries in Greece, Turkey, Libya and Cyprus producing 60-99% females (see references in Casale et al., 2018). Interestingly, gonadal histology as a direct sexing method, although possibly biased by the field sampling protocols and applied only in a limited number of cases, showed less skewed loggerhead hatchling sex ratios (55.6-79% females). Conversely, male-biased hatchling production occurs in some sites, such as Marathonissi beach in Zakynthos, Greece (Margaritoulis, 2005; Zbinden

et al., 2007; Margaritoulis et al., 2011) and Kuriat Island in Tunisia (Jribi & Bradai, 2014) and in some years may also be possible at other sites.

24. Spatio-temporal variations in sex ratios have also been reported (Kaska et al., 2006; Katselidis et al., 2012; Fuller et al., 2013), with more male hatchlings being produced from the nests laid at the beginning and the end of nesting season (May and August, respectively), than from those laid in the middle of nesting season (June-July). Eggs at the top of a nest are also likely to be exposed to more heat from the sun and produce relatively more females than those at the bottom of a nest (Kaska et al., 1998). Beach sand colour (albedo), sand grain size and shading by vegetation are all important factors when determining hatchling sex ratios (e.g. Kaska et al., 1998; Hays et al., 2001; Zbinden et al., 2007; Fuller et al., 2013).

1.3.3.2. Green Turtle sex ratio estimations

25. Clutch temperatures in green turtle nests range from 28.3 ºC with an incubation period of 59 days in Turkey (Candan & Kolankaya, 2016) and as high as 32.5 ºC and an incubation period of 43 days in Cyprus (Kaska et al., 1998; Broderick et al., 2000). Mean incubation durations range from 49 to 60 days (Casale et al., 2018). Primary sex ratios tend to be female-biased (70-96% females; (see references in Casale et al., 2018). An operational sex ratio of 1.4M:1F was estimated from a paternity study at Alagadi (Alagati) Beach, Cyprus (Wright et al., 2012).

1.3.3.3. In-Water Sex Ratio Estimations

26. Surprisingly, and contrary to predominant female-biased hatchling production, the sex ratios of juvenile loggerhead turtles in most Mediterranean marine habitats showed no significant deviation from a 1:1 ratio, with the proportion of females ranging between 52 and 56%. The explanation initially given for the discrepancy between strong-female biased hatchling production and almost even sex ratios in juvenile loggerheads was the strong male-biased immigration of Atlantic juveniles into the Mediterranean Sea (Casale et al., 2002; Casale et al., 2006). Overall, a female bias in the juvenile sex ratio (1.56:1) was recorded in the long-term study in the Tyrrhenian Sea, although in some years this ratio has shown no deviation from a 1:1 ratio (Maffucci et al., 2013).

1.3.3.4. Monitoring the Effects of Global Warming

27. Temperature profiles of monitored nesting beaches in the eastern Mediterranean strongly imply a female biased sex ratio for hatchlings (Casale et al., 2000; Godley et al., 2001a; Godley et al., 2001b; Kaska et al., 2006; Zbinden et al., 2007; Fuller et al., 2013). In the context of global warming, even more female-biased hatchling sex ratios may result. However, extremely skewed sex ratios resulting from a moderate increase of incubation temperature may not necessarily be negative for the population dynamics and a greater threat is represented by reduced hatching success at higher temperatures (Pike, 2014; Hays et al., 2017).

28. Measuring nest and sand temperature offers simple and reliable data for sex ratio estimation, a technique for which electronic data loggers are commonly used. Measuring the sand temperature provides information about the general profile of a beach but *metabolic heating* (the heat that embryos produce during incubation) should also be taken into account, as this usually means the nest temperature is higher than that of the surrounding sand.

29. In order not to interfere with the nest after nesting, the best time for placing data loggers is during egg laying. The data logger may be placed at the bottom or the top of the nest, but the most common practice is to place it in the middle of the nest.

30. If a nest is found after the eggs have been laid, the data logger can only be placed in the nest within the first 24 hours of egg laying. Follow the same procedure during nest relocation, when removing the eggs from the nest and returning them. Data loggers can be collected during the nest excavation. Data loggers, their launching, placement into the nest, information retrieval and the downloading of temperature data can be found in the references (Kaska et al., 1998, 2006).

1.3.3.4.1. Monitoring of Beach erosion and Coastal development

31. Coastal development is largely the result of recreational/tourist activity. It is associated with the presence of hotel resorts and other tourism related constructions such as restaurants, bars,

houses and related businesses, typically built along the beach, impacting an originally flexible and adjustable coastal system. There are many examples of these developments on the nesting beaches of sea turtles in the Mediterranean and all such activities and changes in the nesting habitat should be monitored.

32. Beach erosion and beach armouring may also be recorded, as this very much relates to changes in the ecological conditions of the nests and the development of embryos and hatchlings.

33. Coastal development is also associated with the activities that have an impact on sea turtle nesting activity. Driving on the beach and the use of heavy machinery for beach cleaning purposes are common practices and are responsible for alterations in sand characteristics and the destruction of turtle egg clutches.

34. Water sports, a leisure activity closely linked with high tourist activity, can lead to collisions between turtles and speed boats, especially close to nesting areas where turtle density is high. Such recreational activities and their potential impact on sea turtles should be recorded and necessary precautions and mitigation measures need to be taken into account.

35. Coastal development can be easily monitored during beach monitoring studies. The nesting beach can be photographed at the beginning, middle and end of the nesting season and GPS coordinates recorded. This procedure can be repeated each year. Optionally, satellite images from previous years can be used for comparison. Free images are available from different sources (e.g. https://earthengine.google.com/timelapse/).

1.3.4. Growth, Age at Sexual Maturity and Survival

36. Different aging methods result in the similar estimation of Age at Sexual Maturity (ASM), ranging between 14.9-18.6 years for small nesters of 66 cm CCL and 26.3-34.9 years for larger reproductive females of 84.7 cm CCL (see references in Casale et al., 2018). The mean size of female loggerhead turtles nesting in the Mediterranean is 79.1 cm CCL and males appear to reach maturity at a similar size (Casale et al., 2005; Casale et al., 2014). The average ASM for the Mediterranean loggerhead population was estimated at 25 years (range: 21-34 yrs) from the mean values of the eight age-at-length relationships obtained by the above studies, applied to a size at maturity of 80 cm CCL (Casale & Heppell, 2016).

37. Mediterranean loggerheads appear to reach 28 cm CCL at about 3.5 years old, with the growth rates ranging from 11.8 cm $year^{-1}$ in the first months of life to 3.6 cm $year^{-1}$ at the age of 2.5-3.5 years, similar to that of Atlantic loggerhead turtles (Casale et al., 2009). Broderick et al. (2003) reported growth rates of 0.36 cm $year^{-1}$ for loggerhead females nesting in Cyprus.

38. Based on capture-mark-recapture data, the annual survival probability of loggerheads of 25-88 cm CCL was estimated at 0.73 and this was considered to be underestimated by at least 0.1 because of tag loss (Casale et al., 2007b). The annual survival probabilities of large juveniles at four different foraging areas were estimated through a catch curve analysis, resulting in values ranging 0.71-0.86 depending on the area (Casale et al., 2015). These values were considered to be lower than expected from a healthy population and are possibly due to anthropogenic mortality such as bycatch, especially in some areas like the south Adriatic (Casale et al., 2015).

39. For green turtles, the current information on growth rates is limited to adult females showing a slow growth of 0.11 cm yr^{-1} CCL (Broderick et al., 2003).

40. Oceanic nursery areas for post-hatchling and small juvenile turtles (< 40 cm CCL) are largely unknown in the Mediterranean. Loggerhead turtles, especially juveniles, can be found in virtually all oceanic areas within the Mediterranean. Their distribution is fundamentally driven by the circulation system of the Mediterranean as indicated by genetics (Carreras et al., 2006), telemetry (Revelles et al., 2007) and flipper tagging (Casale et al., 2007a; Revelles et al., 2008). Identifying the most frequented areas is not a simple task and at present the best insights are provided by interaction with fisheries. Turtles in the oceanic zones belong to at least three different Regional Management Units (RMUs) (Wallace et al., 2010): the Mediterranean, the Northwest Atlantic and, to a lesser extent, the Northeast Atlantic (Clusa et al., 2014). Juveniles from Atlantic RMUs enter the Mediterranean through the Straits of Gibraltar and mainly distribute across the south of the western basin following the less saline waters from the Atlantic (Millot, 2005). They can also be found in other regions of the Mediterranean, but at much lower proportions (Clusa et al., 2014). Juveniles from the Mediterranean RMU can be found throughout the basin, although their relative proportion is greater in the eastern, central and north-western Mediterranean (Clusa et al., 2014).

41. Adult sea turtles in the Mediterranean are primarily found in neritic areas, and also on the nesting beaches. Loggerhead turtles can be encountered at pelagic areas, but priority should be given to the aggregation areas in neritic habitats, taking time, budget, and human resources into account. Population demographic parameters need to be collected by conducting in-water studies for both species, especially for juveniles and sub-adults.

1.3.5. Data can be collected from Fishermen-Fisheries Interaction

42. There is a large body of data on turtle bycatch in the Mediterranean, which has recently been reviewed, showing that the level of information available is not equal across countries or sub-regions (Casale, 2011). This review estimated more than 132,000 captures and 44,000 deaths in the Mediterranean annually, from all gear combined. The resulting ranking order of different fishing gears for the number of captures per year was: pelagic longline, bottom trawl, set net and demersal longline. For fatalities, the ranked order was: pelagic longline, set net, bottom trawl and demersal longline.

43. Small-scale fleets (SSF), polyvalent vessels of up to 12 m in length, are the dominant fishery segment and account for 80 percent of the total vessels in the Mediterranean and Black Sea (FAO 2016). Sea turtles are at high risk from SSF, possibly due to the long soak durations of gear (Carreras et al., 2004; Echwikhi et al., 2010, 2012; Coelho et al., 2013) and this fishery may be responsible for most of the fishing-induced mortality in the Mediterranean (Casale, 2011).

44. Bottom trawlers cause death by drowning and mitigation measures are represented, among others, by the modification of the gear (turtle excluder device or TED) to enable any captured turtle to exit the net (FAO, 2009; Lucchetti et al., 2016) and by keeping comatose (i.e. semi-drowned) turtles on-board until they recover (Gerosa & Aureggi, 2001; FAO, 2009). However, decompression sickness may represent an additional and overlooked problem (García-Párraga et al., 2014). Pelagic longlines generally cause death after release, as result of internal damage caused by the line and secondarily by the hook (Casale et al., 2008; Parga, 2012; Alvarez de Quevedo et al., 2013). Mitigation measures are represented, among others, by using larger hooks (e.g., circle hooks) (Piovano et al., 2012; Gilman & Huang, 2017), which decrease the catch rate and by removing the gear (especially the line) from the turtle before releasing it (Gerosa & Aureggi, 2001; FAO, 2009). Set nets cause death by drowning, with very high mortality rates due to the long time the net is left

in the water (Echwikhi et al., 2012) and the only mitigation measure available at present it the illumination of the net, so that turtles can see and avoid it (Ortiz et al., 2016).

45. The highest catch rates in the Mediterranean have been observed off the coast of Tunisia, in the Adriatic Sea and in the easternmost part of the Levantine basin, off Turkey, Syria and Egypt (Casale, 2011; Casale et al., 2012b). A regional bycatch project (supported by the MAVA foundation) should be established to update bycatch figures.

2. MONITORING METHODS

46. The monitoring of sea turtles can be performed by:

a) counting the number of nests during the nesting period and monitoring nest parameters
b) collecting stranded turtles and obtaining information from collected tissues
c) in-water capture-mark-recapture studies for population distribution
d) boat and aerial surveys can also be used for the beach monitoring and in-water monitoring of sea turtles

47. To monitor the distributional range, the population abundance and the demographic characteristics of sea turtles, two monitoring methods can be applied:

- beach monitoring: ground based or aerial monitoring
- in-water monitoring: boat based or aerial monitoring

48. Before starting a sea turtle monitoring study, it should be noted that the necessary permits from the National authorizations should be taken from the relevant authorities.

Table 1. Data to be collected, data collection tools, and relevant common indicator.

Common Indicator	**Nesting Beach Monitoring**		**Marine Habitat Monitoring**	
CI3 Distribution range	*Implementation/ Tools*	*Data collected*	*Implementation/ Tools*	*Data collected*
	Beach foot patrol	Yearly number of nests and tracks; nesting success; spatial and temporal distribution of nests	Boat surveys	Number of individuals; size classes; species distribution; habitat use
	UAV or plane surveys	Number of tracks, and identify nests if possible	UAV or plane surveys	Number of individuals; size classes; species distribution
	Satellite-GPS tracking turtles	Migratory corridors, cutch frequency, inter-nesting habitats, feeding grounds	Satellite-GPS tracking turtles	Migratory corridors; wintering areas; nesting grounds; habitat use
	Sand, nest, and sea water temperature monitoring	Sex ratio trends; suitable nesting beaches; nesting periodicity	Fisheries bycatch data	Sex ratio, maturity, distribution of species, size classes;

				number of individuals
	Stranded turtle network	Spatial and temporal distribution and age classes of turtles	Stranded turtle network	Spatial and temporal distribution and age classes of turtles
	Stable Isotope Analysis	Habitat use; estimating origin of feeding ground;	Stable Isotope Analysis	Habitat use
	Monitoring potential nesting grounds	Yearly number of sporadic nest counts		
	Photo ID, flipper tag, PIT tag, genetic tag	Number of individuals; multiple paternity; haplotype diversity		
CI4 Population Abundance	Beach foot patrol	Yearly number of nests counts and the number of nesting females	Boat surveys	Number of individuals; size classes; species distribution
	Photo ID, flipper tag, PIT tag, genetic tag	Number of individuals; multiple paternity; haplotype diversity	Genetic sampling	Mix stock analyses; genetic diversity (mitochondrial and nuclear DNA)
	Monitoring potential nesting grounds	Yearly number of sporadic nest counts	Fisheries bycatch data	Sex ratio, maturity, distribution of species, size classes; number of individuals
			UAV or plane surveys	Number of individuals; size classes; species distribution; habitat use
			Stranded turtle network	Spatial and temporal distribution and age classes of turtles
CI5 Population Demographics	Beach patrol	Hatching and emergence success; predation rate; hatchling sex ratio	Boat surveys	Number of individuals; size classes; species distribution; habitat use
	Photo ID, flipper tag, PIT tag, genetic tag	Number of individuals; multiple paternity; haplotype diversity	CMR studies	Age and size classes, sexing, maturity, health status
	Stranded turtle network	Aging dead turtles through skeletochronology	Genetic sampling	Mix stock analyses; genetic diversity (mitochondrial and nuclear DNA)

			Stranded turtle network	Spatial and temporal distribution and age classes of turtles
			Fisheries bycatch data	Sex ratio, maturity, distribution of species, size classes

49. Both methodologies can be applied for the Loggerhead turtle as well as the Green turtle. Selecting the most appropriate monitoring method depends on the budget, equipment and personnel available. Beach monitoring should be established on all known nesting beaches, on daily basis, during the nesting period. Potential nesting sites may also be monitored once or twice a week. The monitoring of beaches allows for counting the emergence of adult female turtles, their clutches, and the number of hatchlings. Therefore, estimates for breeding populations can be calculated. For ground-based monitoring, the number of people working in the field depends on the size of the beach, while the equipment can easily be acquired on a low budget. For instance, for daily foot patrols, at least three (2-8) people should be considered for a five km nesting beach.

50. The monitoring of in-water populations requires more expensive equipment, such as boat, entanglement net, or Unmanned Aerial Vehicles (UAVs).

2.1. Time and Area

51. Sea turtles are a highly migratory species. They can be found in different habitats at different times of the year. Therefore, the demography and sex ratio of the population changes temporally throughout the year. Breeding, foraging and overwintering areas are the main ones to be monitored.

2.1.1. Breeding Area

2.1.1.1. Nesting Female Population

52. Nest counts, the direct observation of nesting females, and reproductive outputs are observed during the nesting season. The monitoring of nesting beaches starts at the beginning of May and continues until the end of September, every year.

2.1.1.2. Operational Sex Ratio

53. Operational sex ratio is the proportion of ready to mate individuals from both sexes. This requires the direct sampling of individuals from the sea. In the Mediterranean, mating mainly occurs during April and May. Therefore, monitoring activity should start in April and continue until the end of May and it should be conducted every year.

54. The monitoring of the operational sex ratio before April and after May should be avoided, as individuals captured during these periods may represent different populations and the results can be misleading.

2.1.2. Foraging and Overwintering Areas

55. Monitoring sea turtles at foraging and overwintering sites can be conducted annually and throughout the year. Loggerhead turtles can be found throughout the Mediterranean, especially in bays and estuaries. Green turtles can be found in the eastern Mediterranean and are rare in western locations. The best period for monitoring foraging and overwintering areas is during the months of September and October, as the turtles will have completed their post-nesting migration.

2.2. Samples and Data to be Collected from Sea Turtles

Implementation and/or sampling	Data to be collected	Monitoring methodology		
		Beach Monitoring	In-water Surveys	Rescue/ Stranding
Morphometric measurements	• Size class • Age at Sexual Maturity	X	X	X
Tagging *Metal tags* *Plastic tags* *PIT tags* *Photo ID*	• Population size estimates • Inter-nesting period • Migration route	X	X	
Sampling skin	• Genetic analysis • Stable isotope analysis • Trace element analysis • Heavy metal analysis	X	X	X
Sampling scute	• Stable isotope analysis • Trace element analysis • Heavy metal analysis	X	X	X
Sampling blood	• Genetic analysis • Blood biochemistry and health parameters • Sexing juveniles • Blood cell physiology • Stable isotope Analysis • Trace element analysis • Heavy metal analysis	X	X	
Tissue sampling from internal organs and muscles	• Histologic investigation • Genetic analysis • Heavy metal analysis • Marine litter ingestion			X
Parasite – Epibiont	• Health status • Stable isotope	X	X	X

2.2.1. Size measurement of individuals and Tagging

56. Regardless of monitoring methodology, measuring carapace length is an essential tool for identifying the age class of sea turtles.

57. Adult body size varies greatly among different nesting sites for both species. One of the most distinctive characteristics of Mediterranean loggerhead turtles is a smaller adult female size in comparison with other populations worldwide (Tiwari & Bjorndal, 2000; Kamezaki, 2003). Some loggerhead males start to develop an elongated tail at size >60 cm CCL (Bolten, 1999) and a clear dichotomy in this trait is evident in the population in the >75 cm size class CCL (Casale et al., 2005; Casale et al., 2014). For Straight-line Carapace Length (SCL), 70 cm is usually accepted as a mature female. This type of information can only be obtained by the measurement of individuals.

58. Sea turtle measurement techniques, as explained by Bolten (1999), are frequently used. The measurement of carapace length is an important parameter for identifying size classes. The most common measurements are given below:

- Straight carapace length (SCL): A calliper is used to measure straight length. Three types of measurements are available for SCL:

(i) SCL_{min}: measured from the anterior point at midline (nuchal scute) to the posterior notch at midline between the supracaudals

(ii) SCL_{n-t}: measured from the anterior point at midline (nuchal scute) to the posterior tip of the supracaudals.

(iii) SCL_{max}: measured from the anterior edge of the carapace to the posterior tip of the supracaudals.

- Curved carapace length: A tape measure is used to measure straight length. Three type of measurements are available for CCL:

(i) CCL_{min}: measured from the anterior point at midline (nuchal scute) to the posterior notch at midline between the supracaudals

(ii) CCL_{n-t}: measured from the anterior point at midline (nuchal scute) to the posterior tip of the supracaudals.

(iii) CCL_{max}: measured from the anterior edge of the carapace to the posterior tip of the supracaudals.

- Straight carapace width (SCW): A calliper is used to measure the straight width of the carapace. SCW is measured at the widest point and there is no anatomical reference point for the measurement.
- Curved carapace with (CCW): A tape measure is used to measure straight width of the carapace. As in SCW, CCW is measured at the widest point and there is no anatomical reference point for the measurement.

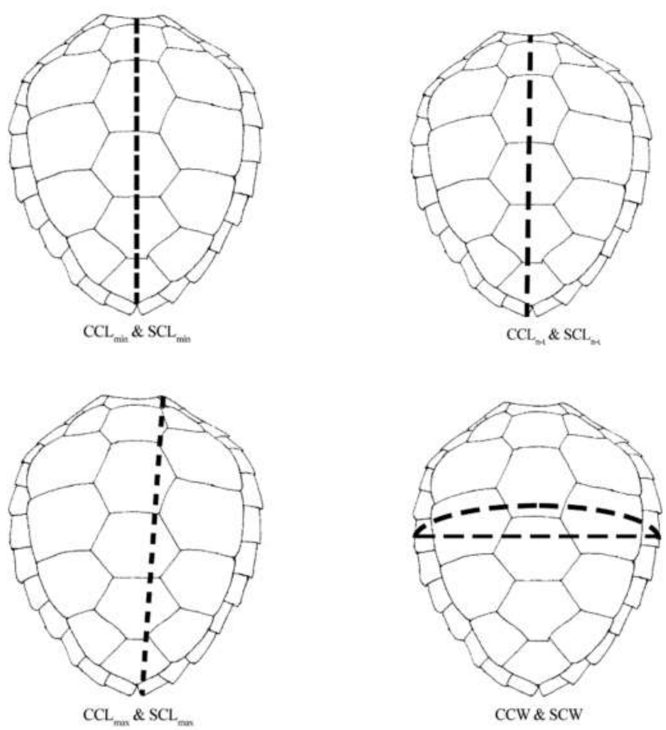

Figure 2. Morphometric measurements of carapace. (For abbreviations see the above text)

59. Tagging is an important tool for monitoring sea turtle populations, as it allows the identification of each turtle as an individual. Each size class of sea turtle, apart from hatchlings, can be tagged. Different types of external tags are available; the most common are Monel, Inconel and Plastic flipper tags. These tags can be found in various sizes and should be selected accordingly to the size of the turtle. A range of tag models can be found at https://nationalband.com/.

60. The advantages of these tags are:

- Visual identification is possible without additional equipment or device by different researchers, fishermen or any person who encounters the turtle. Tag returns are important for monitoring projects
- Cheaper in comparison with other methods.

61. The disadvantages of these tags are:

- High loss rates, especially when the turtle is not properly tagged
- External tags may cause entanglement in fishing nets or marine garbage

62. Passive Integrated Transponders (PIT tag) are also used in monitoring projects. This is an invasive technique that can be applied with a gun or a needle applicator. Sea turtles are tagged with a very small microprocessor. Although the PIT tag remains in the turtle's tissue and may have a low tag loss rate, these tags are not visually identifiable, and an electronic reader is required. Furthermore, PIT tags are more expensive than flipper tags.

63. Photo-identification: Photo identification is an alternative tagging method that is becoming increasingly popular. The methodology is minimally invasive, as it is a technique that basically depends on photographing an individual's scales, creating photo database, and evaluating database photos. Computer programmes for photo-identification are available. This method is currently well developed for green turtles and hawksbill turtles (*Eretmochelys imbricate*) (Carpentier et al., 2016; Calmanovici et al., 2018), and can be used as a viable tool for loggerhead turtles (Schofield et al., 2008). The lateral scale patterns of turtles are commonly used. To obtain the best results, photographs should be taken from the same distance and angle for each individual.

Required Equipment

Measuring the Size

- Notebook
- Pencil
- 150 cm long calliper
- 150 cm long tape measure

Tagging

- Monel, Inconel, or plastic flipper tags
- Tagging Pliers (different pliers for each type of the tags)
- PIT tags
- PIT tag needle applicator or applicator gun
- Electronic PIT tag reader
- Camera

2.2.2. Skin and Scute Sampling

64. Carefully clean the sampling area prior to the procedure. First, gently clean the sampling area to remove all possible epibionts and algae and rinse the area with water. Next, clean with ethanol or another disinfection agent. Using a 6 mm biopsy punch is an easy way to take skin samples. If a scalpel is being used, the turtle should first be restrained and immobilized. After stabilizing, use forceps to facilitate sampling. The biopsy should be no deeper than 0.5 mm. This will prevent bleeding. After sampling, clean the area with betadine to prevent any bacterial infection. Tissue samples should be placed in ethanol (70% or 96%). Use disposable single-use sampling materials and gloves. Using the same sampling materials – such as a biopsy punch or scalpel for different turtles – may transfer DNA from one sample to another. Place the samples in cryovials or Teflon bags and store, frozen to at least -20℃, until analysis.

65. There are two preferred methods for collecting scute samples. The first is by cutting a small piece of keratin with a biopsy punch or scalpel, and second is by shaving. If the turtle is large, use a biopsy punch or scalpel to sample the scute, as this enables different layers of keratin tissue to be collected.

66. After cleaning the area of algae, sand and any other materials, the top layer can be gently shaved then rinsed with distilled water, if possible. A 1X1 cm scute sample is usually sufficient for analysis. Place the samples in cryovials or Teflon bags and store, frozen to at least -20℃, until analysis.

67. If sampling is taken from a juvenile turtle, it can be collected via shaving the scute. The keratin layer is very thin, especially with green turtles. Clean and rinse the sampling area, then start shaving an entire scute by using a knife (5th ventral scute is suitable for this procedure). Approximately 2.00 mm of the keratin should be shaved. Using a wind shield (e.g. umbrella) whilst shaving is beneficial.

2.2.3. Blood Sampling

68. Blood is widely used for scientific purposes, such as:

- Diagnosing a turtle's health status
- Physiologic studies (blood cells, hormones, antibodies, etc.)
- Blood biochemistry studies (electrolytes, blood enzymes, proteins)
- Sex identification (hormones and enzymes)
- Stable isotope analyses
- Genetic analyses
- Toxicological analyses

69. Whole blood tissue comprises two main parts: blood cells and plasma. A study can therefore be made using whole blood, blood cells (haematocrit), or plasma. In each case, a sufficient amount of blood should be collected and stored. If the blood sample is not properly collected and/or is incorrectly stored, the results will not be reliable.

70. Blood sampling should be completed as soon as possible after the capture of the animals; ideally, within 5 minutes of capture and a maximum of 15 minutes. A sea turtle's dorsal cervical sinus is an easily accessible location for taking blood samples. The turtle should be restrained in stable position. The best position is to lift the turtle's back, as this will help to fill the cervical sinus with blood. Gently pull the head forward and downward to stretch the neck.

71. Once the neck is stretched, locate its midpoint. Move 1 cm. towards the nuchal scute, a suitable area for blood collection. Do not insert the needle into the median line of the neck, as this

could strike the vertebral column. When the neck is stretched, two tendons become visible. The needle can be inserted by these tendons, at the lateral sides. Insert the needle vertically. Suction should start after passing the integument. Carefully continue to insert the needle downward, using a small amount of suction until the blood starts to flow. On seeing the blood, maintain the needle in a stable position until sufficient blood is collected.

Required equipment

- 21g Needle and Syringe/Vacutainer
- Heparinized blood tubes
- Centrifuge (for separating blood cells from the plasma)
- Vials and cryo tubes
- Ice box (for transportation)
- Gloves
- An antiseptic (e.g. ethanol)

2.3. Beach Monitoring

72. Beach monitoring should be conducted at night or during morning patrols. Night patrols permit encounters with nesting females, while finding nests at night helps them to be protected from predation, inundation risk, or poaching. Night patrols begin after sunset and may continue until morning. Morning beach surveys start at dawn.

Required Equipment

- Notebook
- Pencil
- Measure tape (30 m or longer)
- GPS
- Headlamp with red-light
- Camera (optional)

73. To determine turtle activities, potential nesting sites should be monitored every two weeks during the summer period. Beaches identified as nesting areas should be monitored every 1-3 days for nest/track counts. During these visits stranded turtles can also be recorded and the necessary samplings conducted.

2.3.1. Beach Monitoring during nesting season

74. Existing and potential nesting beaches should be monitored during the nesting season. Ground-based surveys with a hand-held GPS should be used to map the sea turtle nesting beaches.

- All equipment must be ready prior to beach monitoring.
- At night, only red-lights should be used on beaches; ideally, patrol teams should be silent, and any sounds should be minimal.
- On patrols, avoid large numbers of people.
- To avoid covering sea turtle tracks, patrol teams should walk on wet sand in the ebb tide. Once a track is found, only one person should follow the track, notifying the rest of the team if a female sea turtle is found.
- If a turtle is found, the group should sit quietly, waiting until it finishes laying its eggs and starts to cover the nest.
- It will save time if the location can be marked at this stage.

- The sea turtle should be tagged and measured as soon as it finishes laying. Once the turtle is tagged, it should also be recorded.
- Tissue samples should be collected after tagging. If sensitive samples are to be taken, such as blood, these should be collected first.
- Minimal light should be used to record data, to avoid distracting the female and affecting the nesting activity.
- The location of the nest should be recorded using physical measurements. To obtain three-point positioning, measure the distance from the shore line and also from at least 2 permanent points at the back of the beach. Record the GPS coordinates.
- The nest should be covered with a grid to protect it from predation (eggs dug up by animals searching the beach for food).
- All turtle tracks should be erased, so subsequent teams can clearly see new tracks and are not distracted by tracks and nests that have already been logged.
- The presence of predators (dogs, cats, ferrets, seabirds, foxes etc.) on the beach can be recorded by direct observation and the documentation of tracks. If a predation occurs, it should be recorded immediately. In such cases the actions to be taken are given below:
- The predator should be identified. Egg shells scattered around the nest should be collected and counted to establish how many eggs have been damaged as a result of the predation.
- In cases of infestation in the scattered eggs, specimens (adults, pupae, larvae) should be collected for further examination in the laboratory
- The damaged eggs should be removed from the beach.
- The centre of the predated and distorted nest should be located and opened
- Carefully search for intact (undamaged) eggs.
- In cases of completely ruined nests where intact eggs are observed, excavate a new nest close to the existing one and carefully relocate the undamaged eggs.
- The eggs should be kept in the same position (for transporting over long distances, mark the top of the eggs with marker pen) to avoid them being affected by vibration, rotation or temperature changes. The number of the intact eggs and the GPS coordinates of the new nest should also be logged.
- For every measurement location or sampling collection point, the GPS position should be recorded, and all information should be added to the GIS database

75. Aerial surveys are also an effective way of monitoring of nesting beaches; when the nesting beach is in a remote area, the beach is long, or human resources and equipment are insufficient. Ariel surveys by UAV or plane may be used for counting sea turtle tracks and nests. Surveys can be conducted daily, on alternate days, or on a weekly basis.

2.3.2. Beach Monitoring during the hatching season

76. Data collected during the nesting season is used to estimate the hatching period. This will be confirmed by physical evidence and the observation of tiny tracks leaving the nest towards the sea. The hatching period usually occurs between 45 and 70 days after the first nesting date. Nests that have reached the 40 days incubation period should be monitored.Nest excavations should be conducted 4 days after spotting the first tracks and the following data should be recorded:

a) Live hatchlings
b) Dead hatchlings
c) Yolk sacks still attached
d) Half developed eggs
e) Unfertilized eggs

f) Empty shells

2.3.3. Hatched Nest Excavation

77. Nest excavations are essential for saving hatchlings that are unable to exit the nest because they are not strong enough or due to the nest being closed by an external factor. During a nest excavation, information is recorded about healthy hatchlings, unfertilized eggs, dead embryos, empty shells and live hatchlings that could not exit the nest. Egg shells found in the nest are recorded as empty shells, and eggs with dead embryos inside are recorded as dead embryos. However, the detection of dead embryos early in life can be difficult.

78. Data collected during the nest excavation are given below:

- Early Stage Embryo: An embryo that is smaller than 1 cm. The embryo may have died a few days after egg laying. For this reason, it is difficult to distinguish an early stage embryo from an unfertilized egg.
- When the egg is opened in such cases, a blood clot should be observed, and the egg yolk should be still be attached to the shell. Also, the part of the outer shell should be examined for the clarification of the whitening calcium layer, due to the breathing of the embryo. Furthermore, all or part of the egg colour will be white. If the egg has these characteristics, it is called early stage embryo.

i. Middle Stage Embryo: These are embryos of between 1 and 2 cm.
ii. Late Stage Embryo: These are embryos larger than 2 cm.
iii. Dead Hatchling in the Nest: These hatchings are found in the nest during the excavation process.
iv. Live Hatchling Outside the Nest: These hatchlings are found during field work, or their presence is determined by the tracks they leave.
v. Dead Hatchling Outside the Nest: These hatchlings are detected during field work on the beach, by their traces, which do not reach the sea.
vi. Unfertilized Eggs: Eggs in which the embryo failed to develop. These eggs are yellowish-brown or greyish in colour and show none of the above characteristics.
vii. Empty Shells: Eggs shells left behind by the hatchling after emerging.
viii. Alive Hatchlings in the Nest: Living hatchlings found in the nest during the excavation process.

79. The timing of nest excavations for control is variable. The first nests of the season (April, May and early June) usually have a longer incubation period and it takes longer for hatching to commence in these nests with incubation lasting up to 70 days. The hatchlings that belong to these earliest nests may take 8-10 days to hatch.

80. Nests from the middle of the season have shorter incubation period, when 45 days is sufficient for the incubation process. The complete hatching process may take only a few days, although in some cases it can last as long as 6-7 days. Excavation for these nests should be made 5-6 days after the first hatching. During excavation, live hatchlings that have reached the sea; unfertilized eggs; dead embryos; dead hatchlings; empty shells and living hatchlings still in the nest, should be recorded.

2.3.3.1. Calculation of Hatching and Incubation Period

81. Usually, the surface of the nest collapses 2-3 days before the hatching begins and the egg crumples as the hatchlings begin to emerge, allowing sand to enter. This movement opens a route through which the hatchling can emerge from the nest. At night, the temperature of the sand decreases and the hatchlings start scrambling to the surface. Most of the hatchlings exit the nest on the first night and the rest during the next few days. The hatching process is usually completed within a week.

The incubation period is from the nesting date to the date of the first emergence of hatchlings and is measured on a day-by-day basis.

2.3.3.2. Calculation of Hatching Success

82. The calculation of hatching success:

- Hatching Success = (Empty Egg Shells) / (Total Number Eggs) X 100
- Total Number of Eggs = EES + UE + DE + PE
- EES: Number of empty egg shells; UE: Number of unfertilized eggs; DE: Number of dead embryos; PE: Number of predated eggs

2.3.3.3. Sand, Nest, Sea Surface Temperature

83. It is recommended that sand, nest and sea surface temperatures are monitored to track the effect of climate change. The temperature of these environments is a useful gauge for assessing different parameters.

Sand Temperature	Sea Surface Temperature	Nest Temperature
Affects nest temperatures	Affects nest temperatures (see Girondot and Kaska, 2015)	Sex ratio estimates
Temporal and spatial temperature changes in different beach sections	Breeding periodicity of adults	Assessing hatching success

84. The use of data loggers that record temperature is a common and simple way for monitoring sand and nest temperatures. Sea surface temperature may be recorded, or the data can be requested from national meteorological organisations.

Monitoring sand temperature

85. Data loggers are placed at specific intervals on the nesting beach. For most sea turtle nesting sites, 1 km intervals between each data logger is preferred, buried at a depth of 50 cm, although this depends on the condition of the beach. When placing/planning devices, attention should also be paid to the following:

- Devices should not be placed in the inundation zone.
- If possible, devices should be placed in different zones within the location (e.g. nesting zone, vegetation zone).
- Devices should be placed by the second week of April and collected at the end of September.
- The beach structure is likely to be affected by natural phenomenon; for instance, winds, waves, and inundation. It is therefore advisable to take precautions, such as fixing the devices or covering them with grids.
- Take GPS coordinates of the device locations.

Required equipment

- Data loggers
- GPS
- An interface programme (to programme devices and download data)

2.4. Monitoring of Abundance of In-Water Population

2.4.1. Boat Survey

86. In-water population monitoring is used to estimate the population size, abundance, and sex ratio of a population in a particular area. It is also very useful for collecting biological samples. A research area can be a breeding, feeding, overwintering ground or a mixture of these three areas. This means that different populations can be found in an area. Sea turtles are a migratory species, so the timing of the study is important and should be selected carefully and a standardized methodology should be followed.

Boat based survey: capture-mark-recapture (CMR) method

87. Two common methods are used for in-water surveys. First, a capture net is set in the sampling area. Second, the turtles are captured using the rodeo technique.

(i) In the case of a large study area with low visibility and deeper water, a capture net is preferable.
(ii) The mesh size of the net should be large to avoid the by-catch of other marine animals but small enough to capture turtles.
(iii) The mesh size of the net can be from 10 to 15 cm. Once the net is set, it should be monitored regularly from a boat.
(iv) If the team is sufficiently large and the visibility is high, it is best to swim to the net for this study.
(v) When a turtle becomes entangled in the net, it should be removed and transferred to the boat for measurements and sample collection.
(vi) The turtle should remain on the boat until the net is collected and then released into the sea.
(vii) This study can be used to estimate the size and sex ratio of the population.
(viii) The rodeo technique requires smaller team and can be used in small areas and in shallow waters with high visibility.
(ix) When a sea turtle is spotted from the boat, a swimmer dives and captures the turtle.
(x) The sea turtle is then measured, and biological samples are collected.

Required Equipment

- A Boat
- Entanglement net
- Measurement equipment
- Tagging equipment
- Balance
- Snorkel
- Mask
- Fins
- Ultrasonic-type depth meter
- GPS
- A minimum of five crew members, which can be increased according to type of study, area, and budget

2.4.2. Satellite Tracking

88. A satellite telemetry of adult sea turtles is required for identifying the foraging grounds used by the adults of each population. This technique can also be used to assess the surface time of

turtles at foraging grounds. A parameter is necessary to derive absolute population estimates for aerial surveys.

2.4.2.1. Application of satellite tags and data loggers

89. Satellite tracking is one of the most commonly used techniques for tracking sea turtles, as it can determine migratory corridors, feeding and overwintering areas. It also gives precise information on the localisation of the animal. However, as the cost of the tracking devices is high, this may limit the number of turtles that can be tracked.

90. The Argos tracking system is the most commonly used, but the Iridium satellite device has become a new option in recent years. The systems work in similar way, and a common methodology is used for attaching transmitters to turtles.

91. Before attaching the transmitter to the sea turtle, it should be checked using a small receiver device. If the transmitter is emitting signals, turn the receiver device off and prepare the turtle for the attachment.

92. The turtle can be stabilised in a large tank (1m X 1.5 m). The transmitter is normally attached on the second vertebral scutes. The attachment area on the carapace should be cleaned of epibionts, then rubbed with sandpaper until smooth. Carefully remove any dust and swab the area with acetone, before leaving it to dry for a few minutes.

93. Use a strong glue, such as marine epoxy, to attach the device. Depending on the type of glue being used, it can be mixed prior to application, or on the carapace itself. The glue is also applied to the device but avoid getting it on important parts, such as the magnet connection point or sea water switches. After completing the attachment, leave the sea turtle in the open air until the glue is completely dry. Then it can be released into the sea.

Double check! Make sure the device is switched on before releasing the turtle. Forgetting to check that the transmitter is operational before the release is a common mistake.

Required Equipment

- Satellite transmitter tags (order at least two months before they are needed)
- Container for handling turtle (100 X 150 cm)
- Sandpaper
- Acetone
- Glue (marine epoxy resin)
- Magnets (to switch on and off the tags)

2.4.3. Aerial Surveys and use of UAV

94. Aerial surveys are the best method for determining the abundance of turtles at sea and detecting changes in population, before they translate into changes in nest counts.

95. Aerial surveys necessitate information about time spent on the surface, in order to produce absolute estimates of turtle abundance. Drones, for monitoring nesting activities and making individual counts of sea turtles swimming on the surface, are becoming popular in recent years.

96. Aerial surveys should be conducted every five years at each major foraging ground (Alboran Sea, Balearic Sea, Algerian Basin, Tyrrhenian Sea, Libyan Sea, Adriatic Sea, Aegean Sea, the southern coast of Turkey and the Levantine Sea).

97. Unmanned aerial vehicles (UAVs) or drones are increasingly being adapted for gathering data, at previously unprecedented spatial and temporal resolutions, in diverse geographic locations. This easily available, low-cost tool is improving existing research methods and enabling novel approaches in sea turtle ecology and conservation. For studies on turtle nesting, sea distribution

and behaviour surveys, UAVs can reduce costs and field time, while improving safety, as well as data quality and quantity, over existing methods. They are also expanding into new avenues, such as the surveillance of illegal take (See Rees et al., 2018 for further information).

98. However, there are some limitations on the use of UAVs:

(i) They require a trained pilot
(ii) The battery life of most UAV's is less than 30 min. Therefore, flight time and the monitoring area should be carefully determined before starting the study.
(iii) Meteorological conditions (strong winds, light, etc.)
(iv) Legal limitations (no-flight zones, necessary licences and permissions)
(v) Ethical implications (privacy, effects on animals etc.)

99. Plane surveys are also a useful methodology for estimating sea turtle abundance. However, considering the flight altitude especially in the areas with deeper water and low visibility, plane surveys have challenges identifying species, sex and size classes for sea turtles (Jean et al., 2010; Herren et al., 2018), and other marine animals (Laran et al., 2017).

Required Equipment

- UAV (DJI drones are the most common for sea turtle research)
- Trained UAV pilot
- Tablet, computer
- Remote control device
- Replacement batteries

2.4.3.1. Monitoring Remote Nesting Beaches

100. A UAV can be used for the regular monitoring of remote beaches with low nesting density, especially when the beach is inaccessible. This saves time and gives precise information about sea turtle nesting activities.

2.4.3.2. In-Water Observations

101. UAVs are very useful tools for monitoring in-water populations. They can be used to determine the density and distribution of sea turtles in foraging areas, as well as investigating their behaviour, monitoring and mapping habitats.

2.5. Genetic Structuring

102. Molecular genetic techniques are widely used and there are several non-invasive sampling methods. Although these look simple enough, they require close attention during sampling, due to the possible contamination of DNA from different individuals. Genetic samples can be collected from adult females, hatchlings and dead embryos.

103. Blood and skin are the two most common tissues used for collecting genetic samples. Blood collection is described above. A tissue biopsy from skin is straightforward: tissues are collected from the front or (preferably) the rear flipper using a biopsy punch. If no biopsy punch is available, use a scalpel. A skin sample of 1.5 to 2.0 cm is adequate for genetic analyses. To prevent bleeding the biopsy should be no deeper than 0.5 mm.

104. After sampling, clean the area with betadine to prevent any bacterial infection. Place the tissue sample in 70% ethanol. Always use single-use disposable sampling materials and gloves.

If the same sampling materials are used, such as biopsy punch or scalpel, for different turtles, DNA may be transferred from one sample to another.

105. For genetic analyses, take a small amount of muscle from a dead turtle during necropsy. It is best to collect the same tissue for each research study, if possible.

106. Cheek swabs and carapace scrubbing are other sampling methods. A cheek swab is not ideal, as the mouth of the turtle must be kept open during sampling.

107. When collecting samples for a stable isotope from the carapace, carapace scrubbing can be used. When scrapping a carapace, the white epidermal tissue can be seen on the inner part of the carapace sample. Rinse the carapace sample and let it air-dry for a short period. It is easy to remove the epidermal tissue and store the sample in ethanol.

108. Available information is based on the use of mitochondrial haplotypes and nuclear microsatellites. This allows the individual assignment of loggerhead and green turtles to major nesting areas in the Atlantic (Carreras et al., 2011, 2014).

109. Genetic structuring on nesting beaches and in foraging grounds is better determined by using genetic analyses together with other nesting information, such as remigration interval and clutch frequency through female fingerprinting. This helps to understand the genetic contribution made by nesting beaches to foraging grounds.

2.6. Monitoring Stranding

110. Most research on sea turtles has traditionally been conducted on nesting beaches, even though they spend most of the time in the ocean. The available information suggests that turtles do not distribute homogenously within the sub-basins (Clusa et al., 2014) and that some key parameters, such adult body size and fecundity, vary between females foraging in different sub-basins, although they nest on the same beach (Zbdinen et al., 2011; Cardona et al., 2014). Therefore, detailed information about adult habitat use is critical, albeit some for major nesting beaches is still missing.

111. Stranded turtles are a good data source for collecting various data about sea turtle biology and possible threats. The following information can be collected from stranded turtles:

- The spatio-temporal distribution of turtles
- Tissue sampling for genetic and stable isotope analyses
- Bone sampling for skeletochronology
- Size classes
- Sex
- Threats (cause of deaths)
- Marine pollution (marine litter ingestion; monitoring organic and chemical pollutants in the marine environment).

112. Common protocols are available for data collection from stranded turtles. For example, a detailed protocol for collecting data from stranded turtles, in order to monitor marine litter ingestion, was prepared by the INDICIT consortium. This can be found at their project website https://indicit-europa.eu/indicit-documents/ .

2.7. The Monitoring of Pollution and Pollutants

113. Sea turtles can ingest or become entangled by anthropogenic debris. In contrast to ingestion, entanglement has been reported as an important cause of stranding in the Mediterranean (Tomás et al., 2008; Casale et al., 2010). Studies on marine debris ingestion by sea turtles in the Mediterranean have been reviewed by Casale et al. (2016). It shows that the

occurrence of marine debris varies among studies, with the highest occurrence (80%) reported from turtles caught by pelagic longlines in the central Mediterranean (Casale et al., 2016). Investigations into plastic ingestion can be made using the necropsies of dead turtles but contamination from the environment during the necropsy should be avoided.

114. Before removing the GI tract, tie the anterior part of the oesophagus. Then, tie it above cardiac sphincter and at the beginning of intestine (after the pyloric sphincter). Finally, tie the end of the intestine. In this way the contents of the different GI tract sections will not become mixed.

115. The working space should be cleared before an investigation of the GI tract for possible contamination. Cut each section apart, then measure the weight (and the volume, if required) of the sections (oesophagus, stomach, intestine).

116. Start by cutting each section separately and placing them in a sieve with a mesh size of 1 mm under running water. Collect each foreign object from the contents of each section and place in a container with 50% ethanol. Collect organic materials for diet studies and keep the organic materials in 70% ethanol.

117. Follow the same procedure for each section. Always clean the sieve before starting on another section of GI tract. Measure the empty weight and volume of each section.

118. Clean and dry the collected foreign materials, then measure the weight and volume (if possible). Plastic sheets are needed, and a four-digit precision scale is necessary for measuring micro plastics (from 1 mm to 5 mm in diameter). After measuring, label and keep all samples in a plastic bag.

Chemical Pollutants

119. Chemical pollutants represent a potential threat for sea turtles too. This is especially significant when the several large rivers that flow into different parts of the Mediterranean and its semi enclosed nature are taken into consideration. The presence of heavy metals in sea turtles has been studied in different parts of the Mediterranean Sea. Most of the concentration values were below toxicity levels, apart from the north Adriatic (Franzellitti et al., 2004) and the sea off southern Turkey (Kaska et al., 2004).

120. Recently, Cortes-Gomez et al. (2017) reviewed the metal concentrations revealed in 58 studies among sea turtle species. They summarised the results and reported that the accumulation of pollutants varies between species, the geographic locations and their life-stages. Ross et al. (2017) also reviewed the toxic metal contamination in sea turtle tissues from 95 studies and remarked on the implications for human health. A recent study reported ecotoxicological assessment of stranded loggerhead turtles from blood, skin and scute tissues (Casini et al., 2018). They tested biomarker responses of the selected tissues and contaminant levels in these tissues. Their results also suggest that older animals showed highest levels of erythrocyte nuclear abnormalities, which may indicate a long term ecotoxicological stress in marine environment.

121. Stranded sea turtles are extremely useful for molecular studies, stable isotope analysis and skeletochronology and should be monitored regularly. Carapace length is a parameter commonly recorded from most stranded and rehabilitated turtles. Although stranded individuals are certainly a biased sample, they offer the most cost-effective method for collecting information about size distribution in foraging grounds.

Required Equipment:

- Please see section 2.2. for sampling methodology of blood, skin and scute tissues and required equipment

- For sampling from internal organs, necropsy should be performed. Please see standard protocols of INDICIT Consortium, and Protocols for monitoring interactions between marine litter and marine turtles (UNEP/MAP/SPA/RAC, in press) for sea turtle necropsy. These protocols are planned to be harmonized in 2019. Video tutorials are also accessible at INDICIT Consortium webpage.

2.8. Habitat Use: Stable Isotope Analysis

122. Stable isotope analysis (Carbon (^{13}C), Nitrogen (^{15}N) and Sulphur (^{34}S)) offers an inexpensive method for mass monitoring. The Mediterranean Sea is subdivided into a number of isotopically distinct sub-basins (Cardona et al., 2014), which offers a good opportunity to use stable isotopes as habitat markers both for loggerhead and green turtles (Zbinden et al., 2011; Cardona et al., 2014). Regular collections of tissue samples from nesting females will enable the identification of the foraging grounds used by the females nesting at each major site.

123. The first approach is the collection of tissue samples from adult satellite tagged turtles, tagged at their nesting beaches, and the use of the stable isotope ratios in these samples to characterize the foraging grounds of the turtles (Zbinden et al., 2011).

124. The second approach is the collection of tissue samples of adults and juveniles captured at their foraging grounds and use the stable isotope ratios to characterize them. This approach assures a large sample size from most areas, but there is no way to discriminate between transient and resident individuals, which will reduce the spatial accuracy of the data. The stable isotope ratios of satellite tracked turtles are also useful for identifying potentially transient individuals.

125. The third approach is the use of stable isotope ratios in potential prey from different foraging grounds to characterize them. This is necessary in order to understand the sources of variability among foraging grounds and to make sure that differences in the stable isotope ratios of turtles are because of differences in the isotopic baseline and not because of variances in diet. However, to derive stable isotope ratios in turtle tissues from those of their potential prey is not straightforward, even if prey-to-predator discrimination factors are known.

126. Tissue selection is critical for stable isotope analysis, as diet-to-predator discrimination factors are tissue dependent (Seminoff et al., 2006; Reich et al., 2008; Vander Zanden et al., 2012). Skin is probably the best option, as can be sampled easily from both dead and alive individuals and integrates diet over several months. However, collecting skin samples from most females is unlikely at most nesting beaches due to logistical constraints.

127. Sampling dead hatchlings is easier and less intrusive, but the probability of finding a dead hatchling increases with clutch size and hence this approach may bias the sample in favour of the females using the most productive foraging grounds, as they lay more eggs (Cardona et al., 2014). Egg sampling offers an alternative to avoid such a bias, but this means that each nest has to be excavated once discovered. Furthermore, the methods need to be improved to infer stable isotope ratios in female skin from those in an egg.

Sample Collection for Stable Isotope Analyses

128. The most common stable isotope sampling tissues are blood, carapace and skin from live turtles. Bone samples from dead turtles also contains important information. Each tissue may contain different information about their life cycle.

129. The volume of a sample needed for stable isotope analyses is minimal. Samples of 0.5g to 2.0g samples are sufficient.

130. To collect blood, follow the same procedure as given previously. If samples are to be collected from other tissues, bear in mind that all samples must be collected from the same part of each animal. Tissues collected from different parts of the animals (e.g. a skin sample from the proximal part of the front flipper from one turtle and a skin sample from another's rear flipper) may provide different information and as a result the study samples will not be homogenous.

131. Sampling from the skin: Begin by cleaning the sampling area. Gently remove any epibionts and algae and rinse with water. Using a 6 mm biopsy punch is an easy way to obtain a skin sample. If using a scalpel, restrain and immobilize the turtle and use forceps to facilitate sampling. Place the samples in cryovials or Teflon bags and store, frozen to at least -20°C, until analysis.

132. Sampling from the carapace: There are two methods for collecting scute samples: cutting a small keratein with biopsy punch or a scalpel, and shaving. If the turtle is large, use a biopsy punch or scalpel to sample the scute. In this way, it is possible to collect different layers of keratin tissue. Be careful when using a scalpel, as the blade can break during sampling.

133. Start by cleaning the sampling area of algae, sand any other materials. Gently shave the top layer then rinse with distilled water, if possible. An 1X1 cm scute sample is usually enough for analysis. Try to reach the white epidermal tissue under the keratin layer. After sampling, remove the white epidermal tissue from the scute. Rinse the sample with ethanol and air dry it to facilitate removing the tissue. Place the samples in cryovials or Teflon bags and store, frozen to at least -20°C, until analysis.

134. If the samples are from a juvenile turtle, collect samples with shaving the scute, as the keratin layer is very thin, especially in green turtles. Clean and rinse the sampling area, then start shaving an entire scute using a knife (the 5th ventral scute is suitable for this procedure). Approximately 2.00 mm of the keratin can be shaved. Use a wind shield (e.g. umbrella) while shaving. Place the samples in cryovials or Teflon bags and store, frozen to at least -20°C, until analysis.

Required Equipment

- Biopsy punch
- Scalpel
- Blade (for scratching)
- Vials (for sample storage)
- Teflon bags (for sample storage)
- Ethanol 70%
- 21g needle and syringe/vacutainer (for sampling blood)
- Heparinized blood tubes
- Centrifuge (for separating blood cells from the plasma)
- Vials and cryo tubes

2.9. Contributions from Fisheries

135. Fishing activities are one of the main threats to sea turtles, as they can be caught as bycatch in the various fishing gears. Then again, collaborating with fishermen can be an important monitoring tool. Such partnerships allow researchers to collect data from inaccessible areas, especially from pelagic areas. When limitations such as time, human resources, and budget and so on are taken into account, collecting data from oceanic areas is invariably difficult but the following information can be gathered from fishing operations:

- Distribution ranges in marine habitats
- Demography
- Sex ratio in marine habitats
- Tag return
- Seasonality of marine habitats
- Sampling tissues (e.g. blood, skin, scute)
- Health assessment

136. Researchers are able to collect data on-board during fishing operations. In addition, fishermen may provide important information by self-sampling without the assistance of a researcher. There are also technologies available for *citizen scientists*, such as smart phone applications for collecting data on an entangled or a stranded animal. Smart phones can also be provided to the fishermen to encourage their involvement in monitoring projects. Nevertheless, with or without new technologies, fishermen can collect the following data:

- Entangled sea turtle species
- GPS location
- CCL measurement
- Tag return information
- Tagging
- Photograph of entangled/stranded turtles

137. In addition, collaboration with fisheries researchers and use of their database would be useful for monitoring sea turtles in marine habitats. It should be noted that specific codes (e.g. TURAA00 for turtles) designed by FAO for each species or groups are used in these databases (Sparre 2000).

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E. Guidelines for monitoring non-indigenous species (NIS) in the Mediterranean

Table of Contents

1. Background

1. The Ecosystem Approach (EcAp) process was elucidated in 2008 at the 15th Meeting of the Contracting Parties to the Barcelona Convention, in Decision IG. 17/6, with the vision of "A healthy Mediterranean with marine and coastal ecosystems that are productive and biologically diverse for the benefit of present and future generations", along with an Ecosystem Approach Roadmap, aiming to achieve this vision. Subsequently, the Parties agreed on strategic goals to achieve the Ecosystem Approach vision, on 11 Ecological Objectives (EOs), and on matching Good Environmental Status (GES) descriptions, targets and indicators, including EO 2 (Non-indigenous species).

2. At their 19th Ordinary Meeting (COP 19, Athens, Greece, 9-12 February 2016), the Contracting Parties (CPs) to the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention) adopted the Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP) which describes the strategy, themes, and products that the Contracting Parties are aiming to deliver, through collaborative efforts in the framework of the Mediterranean Action Plan(MAP) , during the second cycle of the implementation of the Ecosystem Approach Process in 2016-2021.

3. The overarching principles guiding the development of the IMAP include (i) adequacy; (ii) coordination and coherence; (iii) data architecture and interoperability based on common parameters; (iv) concept of adaptive monitoring; (v) risk-based approach to monitoring and assessment, and (v) the precautionary principle, in addition to the overall aim of integration.

4. Data and information are gathered through integrated monitoring activities on the national level and shared in a manner that creates a compatible, shared regional pool of data, usable by each Contracting Party. The IMAP information system will ensure the establishment of the regional pool of data and will allow the production of common indicator assessment reports in an integrated manner, following the monitoring specifics and data provided, which ensures comparability across the Mediterranean region. Integration is achieved through IMAP both at monitoring level, through an integrated monitoring system, following common principles and undertaken in a coordinated manner, and at assessment level, with the overall aim to assess the overall status of the marine and coastal environment.

5. The common indicators are the backbone of IMAP which covers 11 ecological objectives including the non-indigenous species (EO2), Citing UNEP/MAP (2017):

'In the context of the IMAP, a common indicator is an indicator that summarizes data into a simple, standardized, and communicable figure and is ideally applicable in the whole Mediterranean basin, or at least on the level of sub-regions, and is monitored by all Contracting Parties. A common indicator is able to give an indication of the degree of threat or change in the marine ecosystem and can deliver valuable information to decision makers.'

1.1 Definitions

6. The following definitions have been extracted from the Decision IG.22/7 (Barcelona Convention, COP19, 2016) entitled ''Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria'' and from the Joint Research Centre (JRC) guidance document on the MSFD Descriptor 2 (Non-indigenous species), citable as Olenin et al. (2010).

7. **Non-indigenous species (NIS; synonyms: alien, exotic, non-native, allochthonous)** are species, subspecies or lower taxa introduced outside of their natural range (past or present) and outside of their natural dispersal potential. This includes any part, gamete or propagule of such species that might survive and subsequently reproduce. Their presence in the given region is due to intentional or unintentional introduction resulting from human activities. Natural shifts in distribution ranges (e.g. due to climate change or dispersal by ocean currents) do not qualify a species as a NIS. However, secondary introductions of NIS from the area(s) of their first arrival could occur without human involvement due to spread by natural means.

8. **Invasive alien species (IAS)** are a subset of established NIS which have spread, are spreading or have demonstrated their potential to spread elsewhere, and have an adverse effect on biological diversity, ecosystem functioning, socio-economic values and/or human health in invaded regions. Species of unknown origin which cannot be ascribed as being native or alien are termed cryptogenic species. They also may demonstrate invasive characteristics and should be included in IAS assessments.

9. The key term "…levels that do not adversely alter the ecosystems" is described as the absence or minimal level of "biological pollution". The latter is defined as the impact of IAS at a level that disturbs environmental quality by effects on: an individual (internal biological pollution by parasites or pathogens), a population (by genetic change, i.e. hybridization), a community (by structural shift), a habitat (by modification of physical-chemical conditions) or an ecosystem (by alteration of energy flow and organic material cycling). The biological and ecological effects of bio-pollution may also cause adverse economic consequences.

1.2 Legislative framework outside EcAp

10. The CBD's (Convention on Biological Diversity) Strategic Plan for Biodiversity 2011-2020 includes twenty measurable Aichi Biodiversity Targets, which need to be met by 2020, including Target 9 which refers to NIS: 'By 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment.'

11. COP Decision VI/23 includes guiding principles for the prevention, introduction and mitigation of impacts of alien species that threaten ecosystems, habitats or species9. Guiding principle 5 on Research and monitoring recognizes that these are required not only to develop an adequate knowledge base to address the problem but are also key to early detection of new invasive alien species.

12. Monitoring should include both targeted and general surveys, and benefit from the involvement of other sectors, including local communities. Research on an invasive alien species should include a thorough identification of the invasive species and should document: (a) the history and ecology of invasion (origin, pathways and time-period); (b) the biological characteristics of the invasive alien species; and (c) the associated impacts at the ecosystem, species and genetic level and also social and economic impacts, and how they change over time.

13. The European Union's Marine Strategy Framework Directive (MSFD) is a wide-ranging framework directive (2008/56/EC) with the overall objective of achieving or maintaining Good Environmental Status (GES) in Europe's seas by 2020 (MSFD, 2008). Eleven high level qualitative Descriptors of GES have been defined in Annex I of the MSFD, including Descriptor 2, for which GES has been defined as 'Non-Indigenous Species introduced by human activities are at levels that do not adversely alter the ecosystem.' Currently, the first six-year cycle of the MSFD is nearing completion, with EU Member States having submitted to the EU Commission their respective Programme of Measures (PoM) prior to their eventual implementation, following the collection of monitoring data for different Descriptors.

14. EU Regulation 1143/2014 lists the Invasive Alien Species (IAS) of Union Concern which should be the target or management measures and in which no commercial trade is allowed. Currently, this Regulation lists only terrestrial and freshwater species, and not marine ones.

15. Parties to the Bern Convention are required to Parties "to strictly control the introduction of non-native species" (Article 11.2.b). The European Strategy on Invasive Alien Species adopted under the framework of the Convention similarly addresses research and monitoring10. Monitoring that is systematic helps build an understanding of the ecological, distribution, patterns of spread and responses of IAS to management.

1.3 Scope and introduction to EcAp Common Indicator 6

16. The scope of this document is to elucidate the monitoring guidelines to address the EcAp Common Indicator 6: "Trends in abundance, temporal occurrence and spatial distribution of non-indigenous species, particularly invasive non-indigenous species, notably in risk areas in relation to the main vectors and pathways of spreading of such species".

17. This Common Indicator was selected by the February 2014 Integrated Correspondence Group on GES and Targets (Integrated CorGest) of the EcAp process of the Barcelona Convention from the integrated list of indicators adopted in the 18th Conference of the Parties (COP18), as a basis of a common monitoring programme for the Mediterranean in relation to non-indigenous species, being preferred over other Common Indicators for Ecological Objective (EO) 2 (Non-indigenous species), such as the 'Ratio between non-indigenous invasive species and native species in some well-studied taxonomic groups.'

18. Common Indicator 6 is a trend indicator, whose main objective is to establish reliable, long-term datasets as a first step of monitoring. In order for this trend indicator to become operational, at least two years of relevant data are necessary, in order to allow a minimal comparison of two annual datasets. In the absence of relevant pre-application (of the trend indicator) data, it is advised to deploy a two-year dataset collected after the optimisation of the indicator.

19. Although the GES for EO2 has not yet been fully elucidated by Contracting Parties, with respect to Non-Indigenous species, UNEP/MAP (2014) establishes the following aspirations:

(i) that no new non-indigenous species are introduced, and
(ii) that the number and composition of non-indigenous species have decreased to such a level where only non-indigenous species which had previously settled at a location are present, i.e. a reference level indicating that the number of non-indigenous species has remained the same in the period of three successive years, assuming that the eradication of established marine NIS is virtually impossible.

1.4. Aims and objectives

20. The main aim of this document is to provide guidance to environmental management practitioners (e.g. environmental authority representatives, researchers, students, Marine Protected Area [MPA] representatives) on field methodologies for monitoring Non-Indigenous Species (NIS) in MPAs and in identified hotspots. This provision of guidance is pursuant to enabling the same practitioners to achieve the goals of EcAp Common Indicator 6, by reviewing recognised good practices in the field of NIS monitoring protocols.

2. Monitoring protocol

2.1 Rationale and strategy

21. Two potential metrics/attributes of the Common Indicator 6 identified within UNEP/MAP (2014) are the following:
(i) Abundance of non-indigenous species
(ii) Temporal occurrence and spatial distribution of non-indigenous species

(i) It is widely recognised that the collection of abundance monitoring data is an expensive process. It is thus recommended to focus monitoring efforts on the recording of all NIS in a particular area – i.e. on the compilation of site-specific NIS inventories. The collection of abundance monitoring data might only be justified in cases of a species exhibiting abrupt spreading beyond a pre-defined threshold. Given the broad geographical range of monitored areas within different Contracting Parties, it is recommended that these thresholds are calculated as a fraction or percentage of the total monitored coastline, rather than as an absolute length of coastline. A relevant threshold example could be the spread of a NIS within a coastal stretch exceeding 5% of the total national coastal extent, or the doubling of the number of coastal monitoring stations at which a NIS has been reported.

(ii) To monitor the trend indicator of non-indigenous species two parameters [A] and [B] should be calculated on a yearly basis. Parameter [A] provides an indication of the introductions of new species (in comparison with the prior year), and parameter [B] gives an indication of the increase or decrease of the total number of non-indigenous species, computed as follows:

[A]: The number of non-indigenous species at T_n (year of reporting) that was not present at T_{n-1} (previous year). To calculate this parameter, the non-indigenous species lists of both years are compared to check which species were recorded in T_n but were not recorded in T_{n-1} regardless of whether or not this species was present in years antecedent to T_{n-1}. To calculate this parameter, the total number of non-indigenous species is used in the comparison (although species names should also be listed).

[B]: The number of non-indigenous species at T_n minus the number of non-indigenous species at T_{n-1}.

22. Trends in both [A] and [B] should be monitored to develop the best management plan for non-indigenous species in an area.

2.2 Spatial and temporal considerations (the 'Where' and the 'When')

23. It is recommended that NIS surveys are conducted within both 'hotspots' areas (e.g. ports and their surrounding areas, docks, marinas, aquaculture installations, heated power plant effluents sites, offshore structures) and within marine areas subject to some form of environmental management, most notably Marine Protected Areas (MPAs).

'Hotspots' are defined as the most feasible entry/introduction points for NIS by virtue of:

(i) a preliminary desk study which identifies particular site-specific features (e.g. a harbour frequented by a considerable number of vessels) or
(ii) an elevated number of NIS already established within the confines of the same hotspot.

24. Typically, hotspots would include site typologies such as harbours, ports, yacht marinas, mariculture cages, offshore structures and thermal effluent discharge locations. Sites not necessarily in close proximity to these 'conventional' hotspots could also be considered within this same category, including locations subject to intense anchoring pressure during the tourist season.

25. In terms of NIS 'hotspots', UNEP/MAP (2014) recommends that NIS monitoring is conducted for at least two hotspot locations per potential introduction pathway, most notably commercial shipping, recreational boating and aquaculture. The same report provides guidance in the form of criteria, which should be applied when selecting candidate hotspot locations, as follows:

- Past research has shown them to be hotspots for non-indigenous species that can be transported with the transport vector concerned;
- The species communities at the two locations do not directly influence each other;
- Vulnerable areas with prospects for 'inoculation' or invasion by new introductions.

In terms of MPAs, a minimum of two sampling stations per MPA are recommended, with the two stations being located within different management zones within the same MPA. In terms of the specific positioning of the two NIS monitoring stations within each MPA, it is recommended to ensure a high degree of geographical and ecological representativity. This can be ensured in a variety of ways, including:

(a) opting for a minimum threshold of physical distance between the two sampling stations, expressed as a percentage of the total lateral extent of the MPA in question (e.g. the distance between the two sampling stations should not be inferior to 25% of the total lateral extent of the MPA);
(b) opting for sampling stations dominated by different marine biocoenoses (e.g. algal-dominated rocky reef versus seagrass meadow);
(c) opting for sampling stations incorporated within anthropogenic or ecological features of interest, with potential candidates including wrecks (which are considered as promoting the

establishment of NIS – e.g. Bariche [2012]), a benthic area heavily impacted by anchoring or a sea urchin barren.

26. The exact geographical location of each selected sampling station in both hotspots and MPAs should be recorded through GPS coordinates, so as to enable consistent sampling on successive occasions.

27. In terms of sampling frequency, it is recommended that hotspots are monitored on a bi-annual/six-monthly frequency, so as to cover both spring and autumn seasons, with the same monitoring survey being conducted after three years.

28. MPAs should be monitored on an annual basis (preferably in spring), given that the rate of introduction of new NIS within MPAs is expected to be lower than that observed within hotspots, such that the latter sites should be sampled with a higher intensity. The rationale behind the preference for the spring season for monitoring purposes is that recruitment in most marine species takes places during this season, and thus conducting monitoring surveys in spring allows for the collection of different NIS life stages which only occur during this time of the year.

29. The following table summarises the recommended spatial and temporal recommended dimensions of the NIS monitoring:

Sampling location typology	**Recommended number of sampling stations**	**Recommended sampling frequency**
'Hotspots'	Two per NIS introduction pathway	Bi-annual/six-monthly
Marine Protected Areas (MPAs)	At least two per MPA	Annual

2.3 Procedures (the 'Which' and 'How')

30. Which NIS to focus upon within the trend analyses is one of the most important considerations to make. The trend indicator (2.1ii), in fact, hinges on the compilation of a preliminary inventory of NIS present within a monitored marine area, which will then also feed into attribute/metric 2.1i. The compilation of this baseline NIS list will also, in turn, allow the identification of reference conditions and thus facilitate a better definition of GES for EO2. This first NIS inventory can be compiled through the exclusive or mixed deployment of any of the following tools:

(a) **Rapid Assessment Survey**. According to Lehtiniemi et al. (2015), rapid assessment is 'a synoptic assessment, which is often undertaken as a matter of urgency, in the shortest time frame possible to produce reliable and applicable results for its defined purpose. Protocols for rapid assessment of marine and coastal biological diversity are available (e.g. UNEP/CBD/SBSTTA/8/INF/13 – Pedersen et al., 2005). Rapid assessment monitoring for targeted species enables direct reporting to management when a notable species is encountered, and the 'field' work can be undertaken by a small group of experts. The method is cost-effective and relevant when prompt management response is sought, but unsuitable for detection of newly arrived introductions;

(b) **Literature review**, specifically of recently published (preferably not earlier than 2010) national censuses or inventories of recorded NIS. For EU Member States, the MSFD IA (Initial Assessment) reports for Descriptor 2 could hold useful relevant information, as well as a number of international and regional (European or Mediterranean basin-scale) databases and lists. These include the European Alien Species Information Network (EASIN) developed by the Joint Research Centre of the European Commission, which facilitates the exploration of non-indigenous species information in Europe (and the entire Mediterranean), from distributed resources through a network of interoperable web services, following internationally recognized standards and protocols. Additional global relevant databases include the CABI Invasive

Species Compendium, the GISD (IUCN Invasive Species Specialist Group and IUCN Global Invasive Species Database) and FISHBASE, whilst additional databases of regional interest include DAISIE (Delivering Alien Invasive Species Inventories for Europe), the CIESM Exotic Species Atlas linked with NIS base, the MAMIAS Database from the Specially Protected Areas Regional Activity Centre (SPA/RAC) of the UNEP/MAP Barcelona Convention and the ESENIAS East and South European Network for Invasive Alien Species. Regional data portal on invasive alien species (IAS) in East and South Europe.

(c) **Citizen science**. With rigorous quality control in place, national and regional citizen science campaigns are ideal for NIS monitoring purposes. Members of local communities, due to their broad geographic distribution and familiarity with their natural environment, can in fact, be of great help to track invasive species in both terrestrial and aquatic systems (Delaney et al., 2008). A renewed drive to identify components of the natural world, through 'bioblitz' events organised round the globe, is bolstering the interaction between formal scientists and informal/citizen ones, also through the availability of low-budget underwater photography and video-capture hardware on the market. An example of a national citizen science campaign is Spot the Alien Fish (www.aliensmalta.eu) one, targeting fish NIS in the Maltese Islands, whilst a number of additional citizen science campaigns operate on.

Within hotspots, a two-pronged monitoring approach is recommended, namely:

(i) Rapid Assessment Survey, as optimised for NIS monitoring within hotspots in Minchin (2007) and in UNEP/MAP (2014). These surveys are conducted by a team of marine species experts spending a specified time period (ideally, this is standardised to ensure uniformity, with a duration of 30 minutes considered to be a feasible one for each individual survey) at the survey site (preferably through SCUBA diving, but possibly even through snorkelling in very shallow areas) and identifying species by observation of artificial substrates such as jetties and wharves, pontoons, long-standing buoys and other artificial structures such as fish-farm cages. A site master records the scientists, findings and abundance of species at each site. Samples of specimens may also be taken back to the lab, where species identification is confirmed, through ex situ analyses involving dissection, microscopic examination and liaison with reputable taxonomists of a pan-Mediterranean profile. This is especially feasible for taxonomically challenging groups such as sponges, hydroids, serpulids, bryozoans and ascidians. In order to further assist in taxonomic identification efforts within the targeted taxa, samples of recorded species should be preserved in absolute, non-denatured ethanol for subsequent molecular analyses. The basic equipment necessary to conduct this monitoring survey includes underwater photographic and/or video cameras, preferably supplemented by the provisions of high levels of artificial light (e.g. through the provision of strobes or basic flash) and underwater data recording facilities, which might include an underwater slate and pencil, or a laminated notebook, per SCUBA diver.

(ii) Scraping technique. This is to be deployed along vertical transects running from the surface of the monitored artificial structure hosting the fouling assemblage down to the foot of the same structure, with sampling stations being placed at a minimum of three different depths along the same transect. The scraping protocol was developed within CIESM's PORTAL programme (Galil, 2008), which in turn was based on the CRIMP methods first described by Hewitt & Martin (1996) and later by Hewitt & Martin (2001). It involves the collection of the fouling community enclosed within a quadrat of standard dimensions (commonly, 50cm x 50cm) through scraping by means of appropriate utensils (e.g. hammer and chisel), within a fine-mesh bag, followed by ex situ, laboratory analyses and identification. Once on land, the collected samples should be preserved by placing the fine-mesh bag directly in a five-litre bucket where its contents are left to soak in non-denatured ethanol (at least 70%) prior to laboratory examination. Different preservatives other than ethanol might need to be deployed for taxa such as ascidians, for which a formaldehyde: seawater mixture is preferred. Caution should be applied when handling formaldehyde given its highly corrosive and carcinogenic nature.

Figure 1 illustrates the standard 50cmx50cm quadrat normally deployed during scraping exercises within fouling communities.

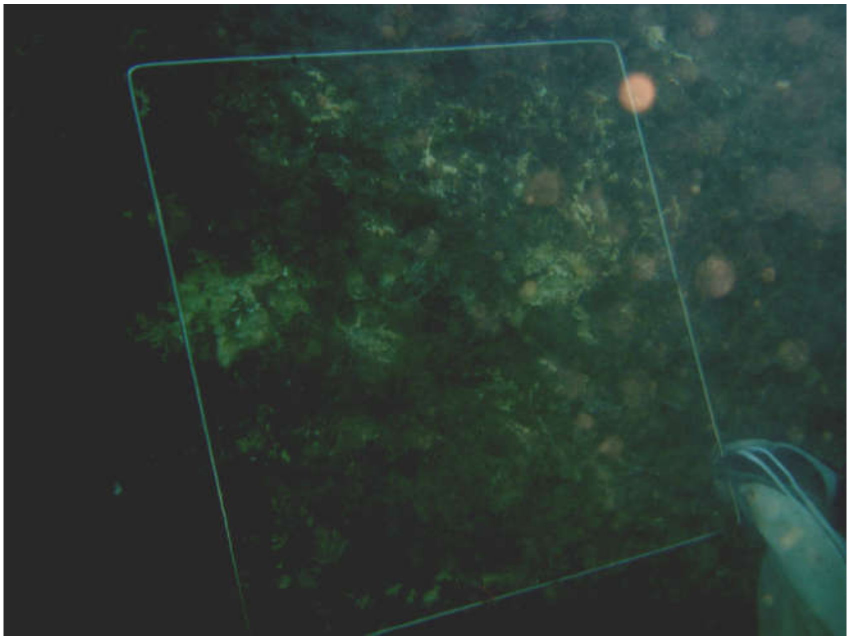

Figure 1 – 50cmx50cm quadrat deployed during scraping exercises within fouling communities (credits for photo: A. Deidun).

Within MPAs, the monitoring protocol for NIS have been developed by the IUCN and is elucidated in Otero (2013). Linear transects having an individual length of 100m, perpendicular to the shoreline and representative of the habitats, depth ranges and substrates within the MPAs are identified. Three replicate and comparable transects at each MPA sampling station are deployed, with a minimum distance of 10m between each transect. Ideally, the linear transect is laid out in the field through the use of a measuring tape of adequate length, which is secured on the seabed at both ends through the use of extra weights.

31. The location of each transect is identified by GPS coordinates for latitude and longitude to ensure faithful reproduceability in future occasions of the conducted monitoring. Non-indigenous species encountered up to five meters on either side of transect are recorded, counted and geo-referenced. Figure 2 illustrates the field conduction of the prescribed monitoring protocol within MPAs.

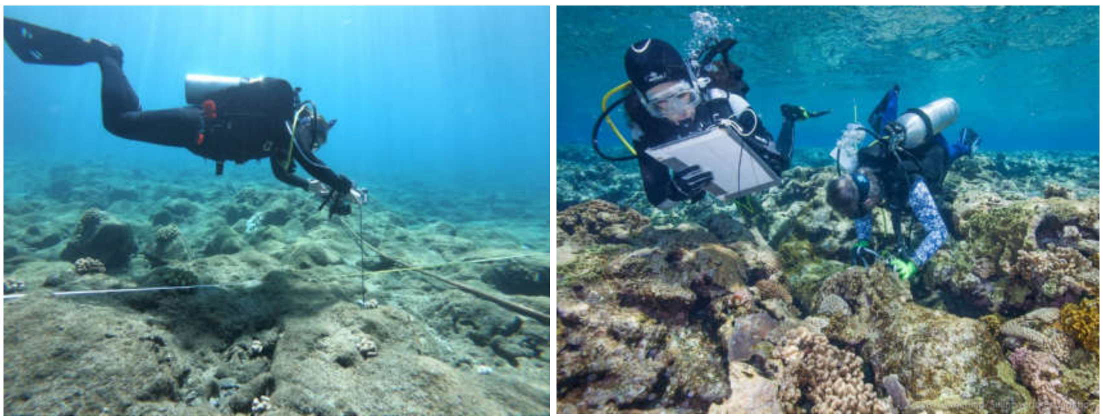

Figure 2 – Field conduction of the proposed monitoring protocol within MPAs (credits for photos: http://blog.owuscholarship.org/).

32. The water depth at which different NIS species are recorded during RAS or at which scraping samples are collected should be recorded. SCUBA divers must thus be equipped with water depth gauges to be able to achieve this requisite. Voucher specimens of first records should be retained within catalogued collections for reference purposes.

33. Additional, complementary data which should be collected for **both hotspots and MPA**s on a non-mandatory basis include:

(a) Semi-quantitative estimates of abundance of both (i.e. native and non-native) community components, through the deployment of different techniques for different taxonomic groups. For instance,

(i) for fish, direct counting for a fixed (e.g. 10-15 minutes at each site) span of time within a visual census could be deployed;

(ii) for benthic macroalgae, direct counting of clusters of the same species, followed by an estimation of the Braun-Blanquet cover index for a standard number of clusters (e.g. 3) of the same macroalgal species could be performed. A similar approach would be useful for quantifying sessile, encrusting invertebrates present in the area. Alternatively, the CARLIT index, adopted within the Water Framework Directive (WFD) and the MSFD, could be quantified;

(iii) individuals sessile and slow-moving non-encrusting invertebrates (e.g. gastropods) can be counted directly over a pre-determined time span (e.g. 10-15 minutes) or within a pre-determined spatial area (e.g. 5mx5m benthic area).

(b) Values for salient water biogeochemical parameters, including water column temperature, salinity and dissolved oxygen content, should be recorded, where possible.

Collection of ancillary socio-economic metrics, through:

(c) Preliminary observations of tangible impacts of the recorded NIS on native species, also through semi-quantitative (and probably arbitrary) indices of impact intensity on native species, potentially including broad impact categories ranging from 'High' to 'Low';

(d) Assessment and identification of potential introduction pathways for each recorded NIS.

Assessment of potential introduction pathways should take into consideration ongoing developments from the pathway assessment exercise by the IUCN-Species Survival Commission-Invasive Species Specialist Group on pathway terminology, classification and analysis of pathway data (http://www.cbd.int/doc/meetings/cop/cop-12/information/cop-12-inf-10-en.pdf).

34. The salient features of every proposed NIS monitoring protocol for both invasion hotspots and MPAs are summarised in Table 1.

Table 1 - Summary table of salient features of the proposed NIS monitoring protocols for invasions hotspots and MPAs.

Monitored marine area typology	Monitoring parameter	Recommended monitoring methodology	Recommended equipment to be deployed during monitoring	Advantages of monitoring protocol	Limitations of monitoring protocol
NIS hotspots	Number/diversity of broader NIS community	Rapid Assessment Survey (RAS)	• Underwater photographic and/or video camera • Underwater slates or notebooks	Rapid and easy to apply	Requires taxonomic experts in the field; might overlook some cryptic NIS through non-observation; provides only semi-quantitative

					measures of abundance
	Number, abundance and density of native and non-native fouling community	Scraping technique	• Quadrat (e.g. 50cmx50cm) • Chisel and hammer • Fine-mesh bag • Five-litre buckets • Preservative (e.g. non-denatured ethanol)	Exhaustively records all species (both NIS and non-NIS) occurring in an area; provides abundance and density (quantitative data)	Destructive technique
MPAs	Number and abundance of NIS	Linear transect and visual census technique	• Underwater photographic and/or video camera • Measuring tape • Extra weight for securing both ends of measuring tape Underwater slates or notebooks	Rapid and easy to apply; allows analyses of trends in NIS abundance if conducted regularly in the same area	Requires taxonomic experts in the field; might overlook some cryptic NIS through non-observation; provides only semi-quantitative measures of abundance

2.4 Data analyses and interpretation

35. A positive or negative trend in [B] illustrates respectively an increase and a decrease in the total number of non-indigenous species in an area, which is a good trend indicator of non-indigenous species. One also needs to calculate [A] however as it is possible to have both a negative trend in [B], indicating a decrease in the total number of non-indigenous species, and a positive trend in [A] at the same time, indicating that management in the area is not sufficient yet. A positive trend in [A] ([A]>0) indicates that ―new species are introduced into the area and one should therefore investigate how and with which pathway they are introduced. If this concerns a pathway introduced by anthropogenic activities, one may focus management on that pathway. If the new non-indigenous species arrive by their natural distribution capacities, one may focus on back tracking the location of origin and focus management on that location.

36. Consequently, for all monitored stations, [A] at T_n = [A] at T_{n-1} = [A] at T_{n-2} = 0 and [B] at T_n = [B] at T_{n-1} = [B] at T_{n-2}, should indicate that no new non-indigenous species were introduced in the last three years, and that the number of non-indigenous species is decreased to a level where only settled (for at least three years) non-indigenous species are present.

3. Data handling policies

37. NIS and ancillary data collected on a national basis should be validated by an expert panel prior to it being submitted to a pan-Mediterranean, geo-referenced repository which can referenced by different user typologies (e.g. MPA managers, government environmental agencies, NGOs, research institutes). The MAMIAS database is a good candidate for such a repository, given its pan-Mediterranean nature, but unless this database is re-activated and its public access reinstated, alternative, relevant repositories should be availed of, including the EASIN, CIESM and GBIF ones. Protocols detailing how the NIS databases held within the selected final repository can be

supplemented by citizen science reports being submitted by the public should be elucidated at a subsequent stage.

38. Field workers engaged in the deployment of the monitoring protocols must be confident they are recording most of the NIS species occurring in a particular area, in order to ensure a good quality of the data being recorded. UNEP/MAP (2014) states that the minimum threshold of the total NIS in an area which need to be recorded is that of 90% and that different statistical techniques exist for assessing progress towards achieving this. Further guidance to NIS monitoring practitioners should be provided in future on how to quantify statistically the fraction of total NIS occurring in an area which have been sampled.

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F. Guidelines for monitoring marine benthic habitats in the Mediterranean Sea

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General premise

1. The Contracting Parties to the Barcelona Convention have adopted the Ecosystem Approach (EcAp) in 2008 with the Decision IG. 17/6, aimed at reaching "A healthy Mediterranean with marine and coastal ecosystems that are productive and biologically diverse for the benefit of present and future generations" (UNEP/MAP, 2008). This process (EcAp) aims to achieve the Good Environmental Status (GES) through informed management decisions, based on integrated quantitative assessment and monitoring of the marine and coastal environment of the Mediterranean, in order to manage human activities sustainably.
2. In 2016, during the 19th Meeting of the Contracting Parties to the Barcelona Convention (COP 19, Athens, Greece, 9-12 February 2016), an Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP) has also been adopted by the Mediterranean region. The resulting document describes the strategy, objectives and products that the Contracting Parties have to deliver over the second period of the implementation of the EcAp (2016-2021) in the framework of the Mediterranean Action Plan (UNEP/MAP, 2008). The main goal of IMAP is to build and implement a regional integrated monitoring system gathering reliable quantitative and updated data on the status of marine and coastal Mediterranean environment. A list of agreed 27 Common Indicators (CIs), articulated on 11 Ecological Objectives (EO) in synergy with the European Union's Marine Strategy Framework Directive (2008/56/EC), and GES targets of the IMAP have been set in the Decision IG.22/7. In the context of the IMAP, a Common Indicator is defined as "an indicator that summarizes data into a simple, standardized, and communicable figure and is ideally applicable in the whole Mediterranean basin, or at least on the level of sub-regions, and is monitored by all Contracting Parties. A common indicator is able to give an indication of the degree of threat or change in the marine ecosystem and can deliver valuable information to decision makers".
3. During the initial phase of the IMAP implementation (2016-2019), the Contracting Parties to the Barcelona Convention were asked to develop or update their national monitoring programmes in order to provide all the data needed to assess whether the GES defined through the EcAp process has been achieved or maintained. Monitoring programmes at the national level are shared to create a compatible, shared Mediterranean pool of data, usable by each Contracting Party to product common indicator assessment reports in an integrated manner, which ensures comparability across the Mediterranean region.

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

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

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

Purpose and aims

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

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

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

<u>Common Indicator 2: Condition of the habitat's typical species and communities.</u>

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

9. The main aim of these guidelines is to provide guidance to managers and decision makers (e.g., environmental authority representatives, researchers, Marine Protected Area - MPA representatives) on field methodologies for long-term monitoring of marine benthic habitatsin 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 from human activity.,. These indications should help environmental practitioners in deciding what kind of method to choose at regional and national level to answer the Common Indicators 1 and 2.

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

(1) Guidelines for monitoring marine vegetation

(2) Guidelines for monitoring coralligenous and other calcareous bioconstructions

(3) Guidelines for monitoring dark habitats.

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

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

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

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

1. Guidelines for standardisation of mapping and monitoring methods of marine Magnoliophyta in the Mediterranean (UNEP/MAP-RAC/SPA, 2015a)[3]
2. Methods for inventorying and monitoring coralligenous and rhodoliths assemblages (UNEP/MAP-RAC/SPA, 2015b)[4]
3. Draft guidelines for inventorying and monitoring of dark habitats (UNEP/MAP-SPA/RAC, 2017)[5].

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

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

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

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

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

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

1. Guidelines for monitoring marine vegetation in Mediterranean

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Introduction

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 17000 $ 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

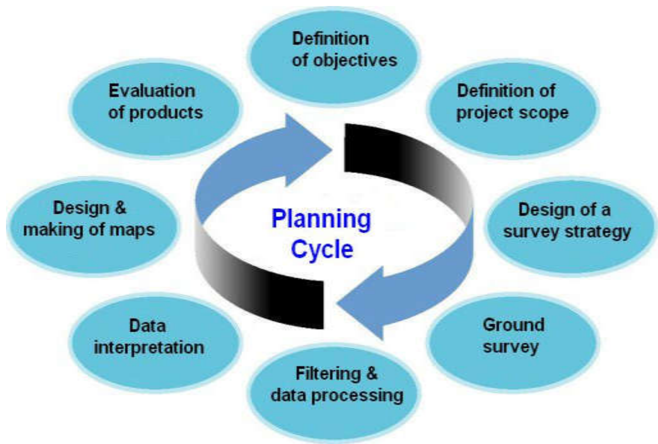

Figure 1: Planning cycle for a habitats' mapping programme (according to the MESH project, 2008).

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

Resolution

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

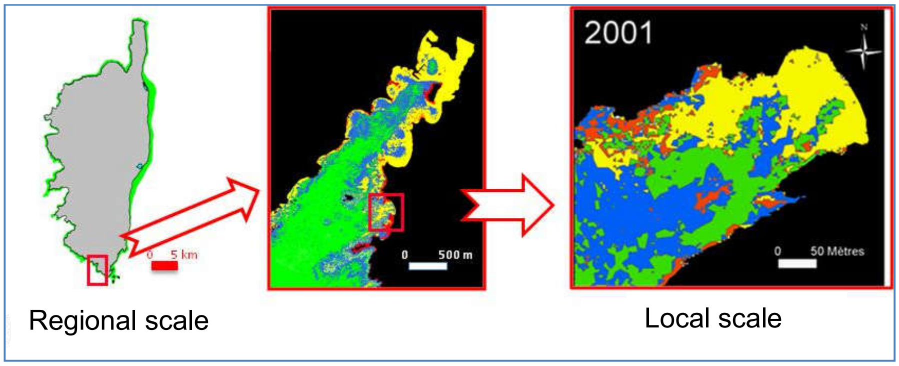

Figure 2: Resolution of a map from regional study to local study (from UNEP/MAP-RAC/SPA, 2015).

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 (Pergent et al., 1995a). Recently, some global maps showing the distribution of *Posidonia oceanica* meadows in the Mediterranean have been produced (Giakoumi et al., 2013; Telesca et al., 2015) (Fig. 3). These maps, however, are still incomplete being the available information highly heterogeneous due to the high variability in the mapping and monitoring efforts across the Mediterranean basin. This is especially true for the southern and the eastern coasts of the Mediterranean, where data are scarce, often patchy and can be difficultly found in literature. In data-poor regions, availability of high-quality mapping information on benthic habitat distribution is practically inexistent, due to limited resources. However, these low-resolution global maps can be very useful for an overall knowledge of the bottom areas covered by the plant, and to evaluate where surveys must be enforced in the future to collect missing data. Also, those maps are important to highlight specific areas subjected to a declining trend, where monitoring and management actions must be implemented to reverse the observed trend and to ensure proper conservation.

16. On the contrary, when smaller areas have to be mapped, a much higher precision and resolution level is required and is easily achievable thanks to the high-resolution mapping techniques available to date. However, obtaining detailed maps is time consuming and costly, thus practically impossible when time or resources are limited (Giakoumi et al., 2013). The minimum surface area can be lower or equal to 1 m^2 in local scale studies (Pergent et al., 1995a). These detailed maps provide 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.

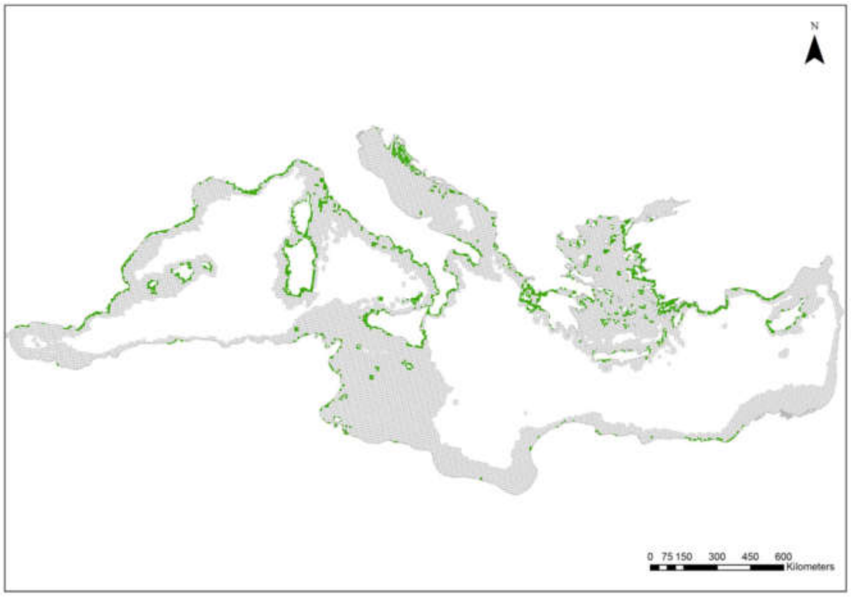

Figure 3: Distribution of *Posidonia oceanica* meadows in the Mediterranean Sea (green areas) (from Giakoumi et al., 2013).

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 to distinguish seagrass species Possibility to collect data even during bad weather conditions	Complex acquisition and processing procedures requiring the presence of specialists Necessary to validate the observations with field data Difficulty in habitat identification in the case of very patchy populations	Mumby and Edwards (2002); Mumby et al. (2004); Dekker et al. (2006); Gagnon et al. (2008);

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Aerial images	From 0 to 10-15 m	Adapted to small areas (10 km²), but it can be used for areas over 100 km²	From 0.3 m	Over 10 km²/hour	• Very high resolution • Manual, direct and easy interpretation of the images • Availability of libraries with chronological series of images (often free) • Good identification of boundaries between populations	• Same limits as for satellite images • Difficulty in geometrical corrections and strong deformations if verticality is not respected or if image covers a small area (low altitude view) • Difficulty in obtaining 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 m^2 to 100 m² for permanent square	From 0.1 m	0.01 km²/hour	• Very high resolution and detail in the information collected • Possibility to identify small structures (patches) and to localize population boundaries • Ground-truthing of the remote sensing data • 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 significantly improved in the last decade, the data collected is still not sufficient for medium to small coastal dynamics. 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 (Ciraoloet 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 (Clabautet 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 (Clabautet 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

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

[1]CASI: Compact Airborne Spectrographic Imager

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 DGPS receivers and submerged hydrophones. Each of the hydrophones receives the acoustic impulses emitted periodically by a synchronized pinger installed on-board the underwater platform and records their times of arrival. Knowing the moment of emission of these signals and the sound propagation speed in the water, the distances between the pinger and the 4 buoys is directly calculated. The buoys communicate via radio with a central station (typically on-board a support vessel) where the position of the underwater target is computed and displayed. The depth is also indicated by the pressure sensor (Alcocer et al., 2006). To optimize meadows mapping operations, the pinger can be also fixed on a submarine scooter driven by a diver. The maximum distance of the pinger in relationship to the centre of the polygon formed by the 4 buoys can be approx. 1500 m (UNEP/MAP-RAC/SPA, 2015).

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 angiosperms (e.g. *Zostera noltei*)

INFRALITTORAL

MB1.5 Infralittoral rock

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

[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.

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).

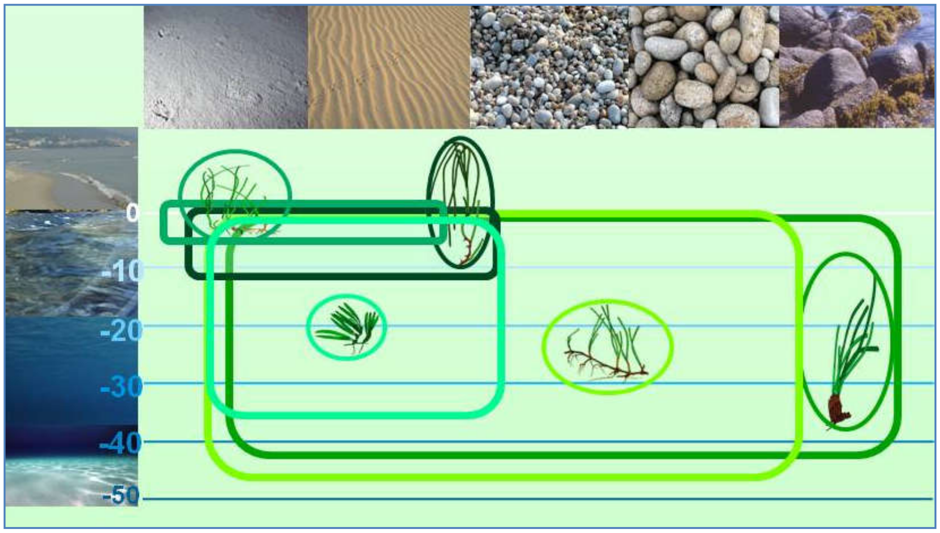

Figure 5: Distribution of seagrass species depending on the nature of the substrate and the depth in the Mediterranean (from UNEP/MAP-RAC/SPA, 2015).

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).

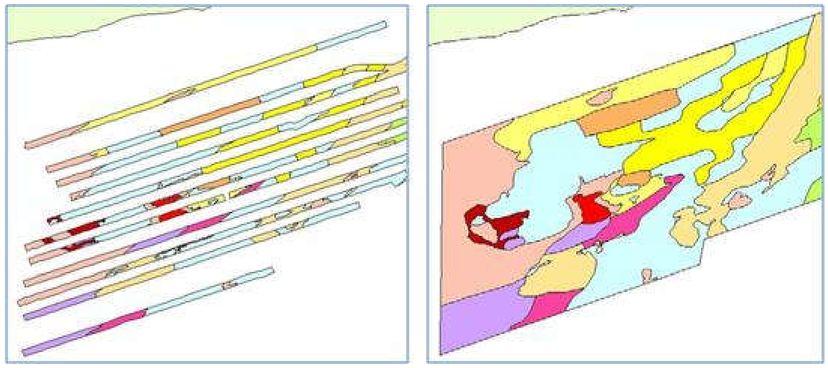

Figure 6: Example of partial coverage survey (left) and the output of the final map produced through interpolation (right). The area surveyed is about 20 km wide (from UNEP/MAP-RAC/SPA, 2015).

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).

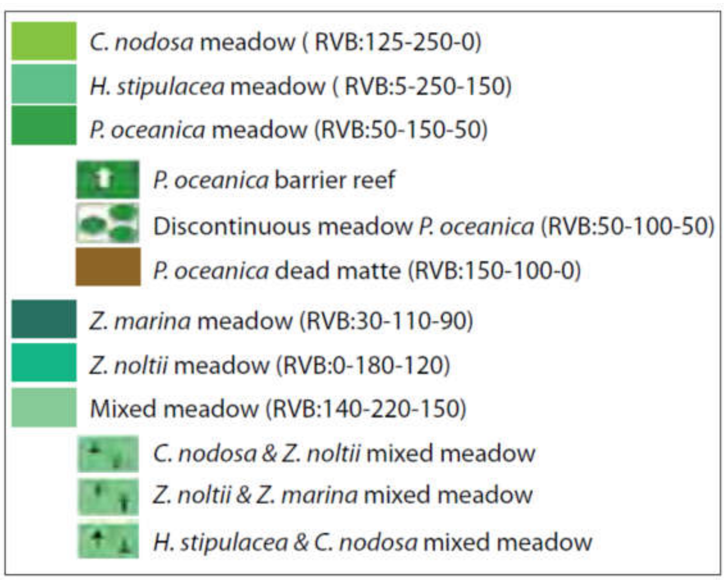

Figure 7: symbols and colours used for the graphic representation of the main seagrass assemblages. RVB: values in red, green and blue for each type of meadow (from UNEP/MAP-RAC/SPA, 2015).

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

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 2 (list of endangered or threatened species) of the Protocol concerning Specially Protected Areas and Biological Diversity (Decision of the 16th Ordinary meeting of the Contracting Parties, Marrakech, 3-5 November 2009; UNEP/MAP, 2009)
- Three species (*C. nodosa, P. oceanica*, and *Z. marina*) are listed in the Annex 1 (strictly protected flora species) of the Bern Convention concerning the Mediterranean geographical region
- Seagrass meadows 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 by scuba diving (e.g., lower limit type, cover, and rhizome baring); iii) direct sampling of plants (e.g., phenological descriptors). All methods requiring the direct sampling of plants for subsequent laboratory analyses are destructive,

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

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 5 replicated counts per station. An adequate number of stations must be localised randomly within the meadow, and usually in correspondence of the meadow upper limit, the meadow lower limit and at intermediate depths, in a number of 2 to 3 sampling stations per depth. To assess the overall ecological condition of the meadow, samples of shoots can be performed only at the intermediate meadow depth, which is usually at about 15 m depth, where the meadow is expected to find the optimal conditions for its development (Buia et al., 2004) and during late spring or early summer season (Gobert et al., 2009).

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 scales for its classification have been adapted with the creation of five classes (bad, poor, moderate, good, and high; Annex 1). This scale provides a tool to classify the ecological status of the meadow that can be used in the frame of the IMAP under the EcAp. Evaluating depth and typology of both the upper and the lower limits of the meadow and monitoring over time their positions with permanent marks (i.e., *balises*) are commonly adopted procedures to assess the evolution of the meadow in term of stability, improvement or regression that is linked to water transparency, hydrodynamic regimes, sedimentary balance and human activities along the coastline (Fig. 8). The classification scale of the lower limit depth (Annex 1) is another valid tool, although this scale could require some adaptations according to the specific geographical area and the morphodynamics setting of the site. For instance, in many *P. oceanica* meadows in the Ligurian Sea (NW Mediterranean) the lower limit rarely reaches depths greater than 20-25 m, due to natural constrains (e.g., substrate typology, seafloor topography). In all these cases, meadows would be classified from moderate to bad ecological status using the lower limit depth, even without or with very few human pressures.

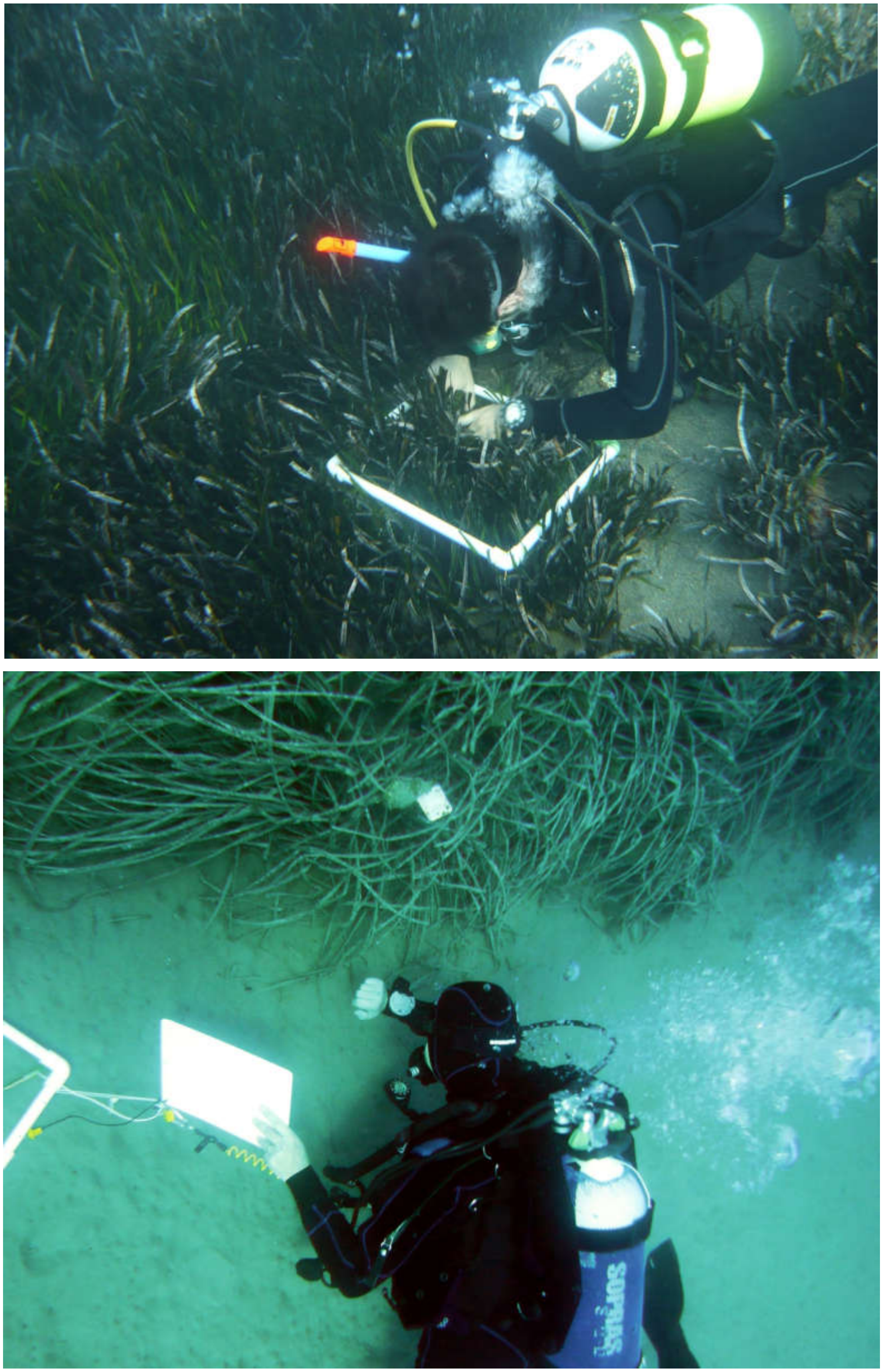

Figure 8: In situ measurement of *Posidonia oceanica* shoot density using the standard square frame of 40 cm × 40 cm (upper image) and monitoring over time of the meadow lower limit position with permanent marks (lower image).

Table 4: Synthesis of main descriptors used in seagrass monitoring 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 necessitating periodical monitoring or observations at the same season for all sites Fixed markers might disappear if site is strongly frequented	Pergent et al. (1995); Montefalcone (2009)

Descriptor	Method	Expected response/factors	Destr.	Target species	Advantages	Limits	References
Bathymetric position of meadow lower limit (in m)	A detailed mapping of seagrass extension limit seaward (Cf. Part “a” of this document) or placing fixed markers (e.g., permanent blocks, acoustic system)	Shift of the lower limit landward at shallower depths Turbidity	No	All	Easily measured (also by scuba diving) Classification scale available for Po	For Cn, Hs and Zn, strong seasonal variability necessitating periodical monitoring or observations at the same season for all sites Beyond 30 m depth, acquisition is difficult and costly (limited diving time, need for experienced divers, numerous dives requested) Fixed markers (balises) might disappear (e.g., 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	Easy to measure Surface areas can be measured on maps	Dead matte areas are natural components intrinsic to some types of meadows (e.g., striped meadows) and do not reflect systematically human influence	Boudouresque et al. (2006)

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

Descriptor	Method	Expected response/factors	Destr.	Target species	Advantages	Limits	References
Percentage of plagiotropic rhizomes	Counting of plagiotropic rhizomes in a given surface area (e.g., 40 cm × 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/ No (Zm)	All	For Po lepidochronolo-gy 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	Easy to measure in situ Not destructive and low-cost Independent from season		Boudoresque et al. (2006)

Descriptor	Method	Expected response/factors	Destr.	Target species	Advantages	Limits	References
Physiological (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)

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)
Descriptor	**Method**	**Expected response/factors**	**Destr.**	**Target species**	**Advantages**	**Limits**	**References**
Community							
Epiphytes biomass (in mg dry weight · $shoots^{-1}$ or % dry weight · $shoots^{-1}$) and epiphytes cover (in %) of leaves	Measure of biomass (µg · shoots-1) after scraping, drying and weighing Measure of nitrogen content (in % dry weight) Measure using simple CHN analyser Estimate the epiphytes cover on leaves under a binocular Indirect estimation of biomass from epiphytes cover	Increase Nutriments enhancement from rivers, high touristic frequentation	Yes	All	Easy to measure Low-cost (biomass and cover) Classification scale available for Po Early-warning indicator	Time-consuming Strong seasonal and spatial variability Specific analytical equipment (nitrogen content) necessary 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 to compare different meadows in the Mediterranean and also to evaluating the plant's vitality and the quality of the environment in which it grows. Other monitoring systems, 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	x		
Temperature	x		x
Salinity	x		
Lower limit	Depth	Depth, type and cartography	Depth, type and cartography
Upper limit	Depth	Depth, type and cartography	Cartography
Density	12 measurements along each transect	Measurement at each of the 11 markers	Measurement at each of the 11 markers
% Plagiotropic rhizomes		Measurement at each of the 11 markers	Measurement at each of 11 markers
Baring of rhizomes		Measurement at each of the 11 markers	Measurement at each of the 11 markers
Cover	12 measures along transect	At each marker using video (50 m)	Measurement at each of the 11 markers
Phenological analysis	12 measures along transect	20 shoots	20 shoots
Lepidochronological analysis		10 shoots	10 shoots
State of the apex		20 shoots	20 shoots
Biomass (g DW)	Leaves		
Necromass	Rhizome and scales		
Granulometry of sediments		1 measurement	1 measurement
% organic material in sediment		1 measurement	1 measurement
Trace-metal content			Ag and Hg

Data processing and interpretation

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 (Table 6):

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

74. Most of the ecological indices integrate different ecological levels (Table 6). The POSWARE index is based on 6 descriptors working at the population and individual 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 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 indexes 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 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 on a single site. As a general comment, those indices based on a high number of descriptors imply excessive costs in terms of acquisition time and the budget required (Fernandez-Torquemada et al., 2008).

Table 6: Descriptors used in the synthetic ecological indices mostly adopted in the regional/national monitoring programs to evaluate environmental quality based on the "seagrass" biological quality element. 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 – Classification scales of the ecological status available in literature for some descriptors of *Posidonia oceanica* meadow

Meadow (population level)

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

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

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

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

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

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

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

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

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

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

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

Plant (individual level)

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

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

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

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

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

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

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%

<u>Cell (physiological level): environment contamination</u>

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

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

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

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

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

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

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

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

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

Table of contents

Introduction

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 caespitose*, *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 (pictures by Simone Musumeci, Monica Montefalcone).

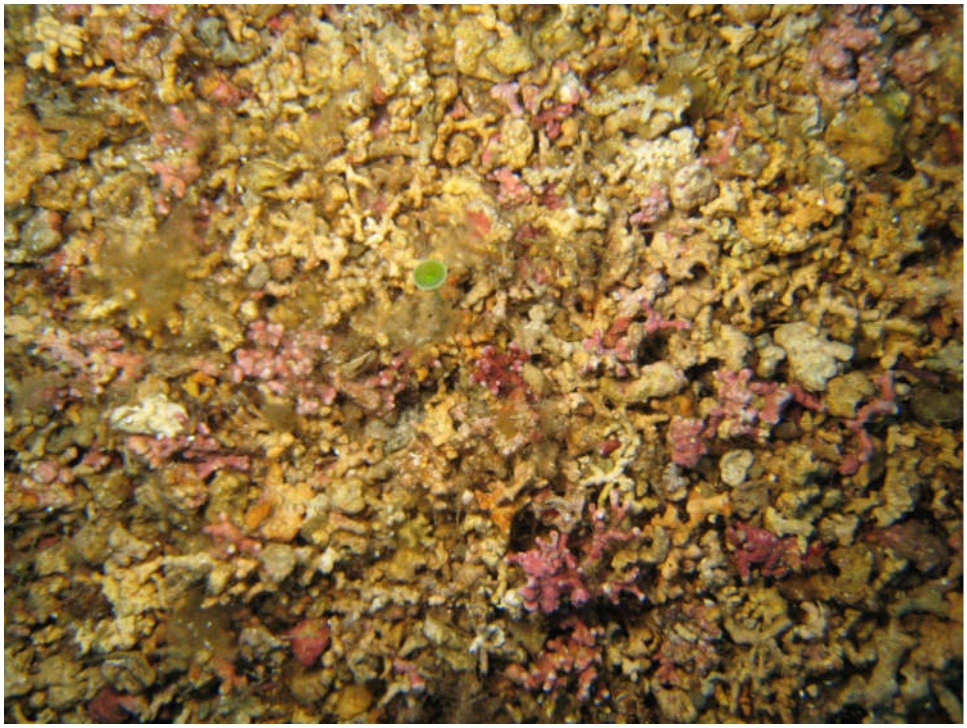

Figure 2: Rhodoliths habitat (picture from UNEP/MAP-RAC/SPA, 2015).

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 growing on the circalittoral rocks (cliffs or outcrops), and coralligenous developing over circalittoral soft/detritic bottoms creating biogenic platforms (Piazzi et al., 2019b). Coralligenous structure results from the dynamic equilibrium between bioconstruction, mainly made by encrusting calcified Rhodophyta belonging to Corallinales and Peyssonneliales (such as the genera *Lithophyllum*, *Lithothamnion*, *Mesophyllum*, *Neogoniolithon*, and *Peyssonnelia*), with an accessory contribution by serpulid polychaetes, bryozoans and scleractinian corals, and destruction processes (by borers and physical abrasion), which create a morphologically complex habitat where highly diverse benthic assemblages develop (Ballesteros, 2006). Light represents the main factor limiting bioconstruction, and coralligenous reefs are able to develop in dim light conditions (<3% of the surface irradiance), from about 20 m down to 120 m depth. Also, the upper mesophotic zone (where the light is still present, from 40 m to 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 monitoring emphasized. Coralligenous has also been included in the European Red List of marine habitats, where it is classified as "data deficient" (Gubbay et al., 2016), thus demonstrating the urgent need for thorough investigations and accurate monitoring plans. In the same year, the Marine Strategy Framework Directive (MSFD, 2008/56/EC) included "seafloor integrity" as one of the descriptors to be evaluated for assessing the Good Environmental State of the marine environment. Biogenic structures, such as 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.

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

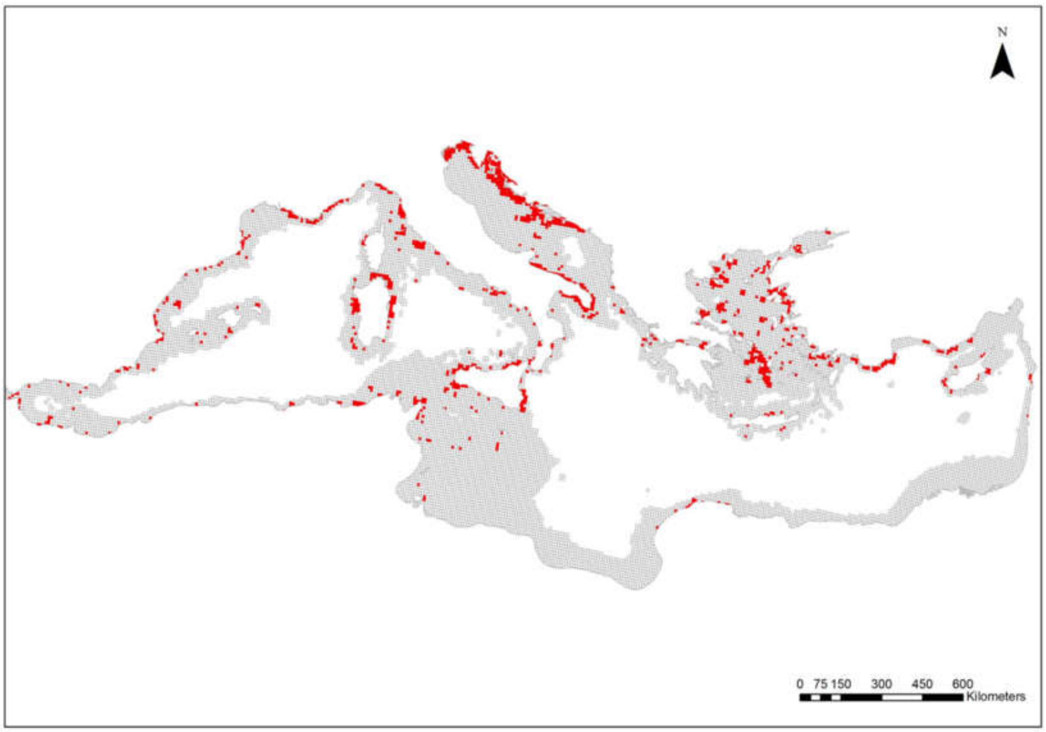

Figure 3: Distribution of coralligenous habitats in the Mediterranean Sea (red areas) (from Giakoumi et al., 2013).

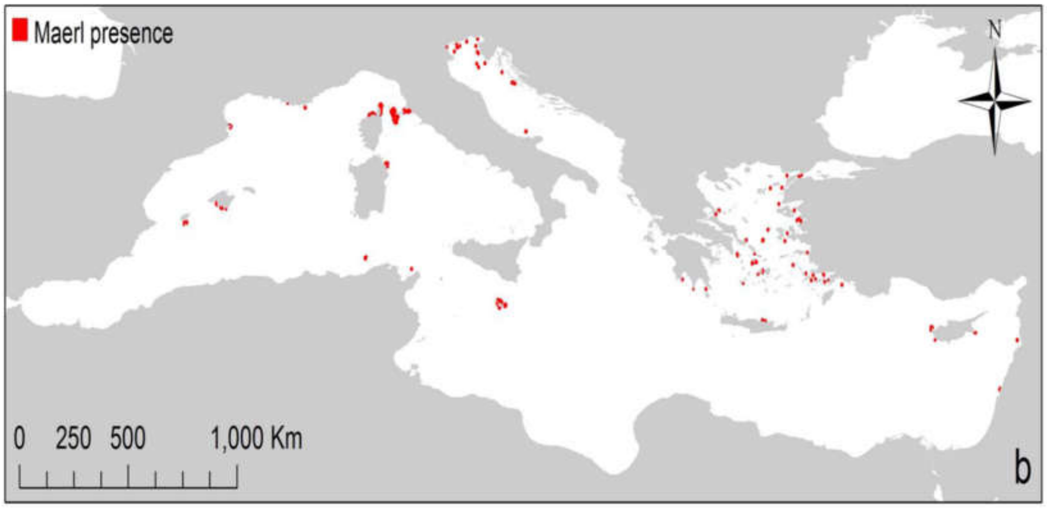

Figure 4: Distribution of maërl habitats in the Mediterranean Sea (red areas) (from Martin et al., 2014).

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 used and 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. 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 (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 $\geq 0.16\ m^2$ is recommended because it has the advantage of preserving the original substratum stratification. The use of dredges for sampling rhodoliths should be discouraged, in order to minimize the impact of the investigation.

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 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 rhodoliths habitats. All the acoustic mapping techniques are intrinsically affected by uncertainties due to manual classification of the different acoustic signatures of substrate types on sonograms. Errors in sonograms interpretation may arise when two substrate types are not easily distinguished by the observer. Interpretation of remote sensing data requires extensive field calibration and the ground-truthing process remains essential. As the interpretation of sonograms is 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 rhodoliths populations, a predictive modelling was carried out to produce two continuous maps of these two habitats across the Mediterranean Sea (Martin et al., 2014). For coralligenous, bathymetry, slope of the seafloor and nutrient input were the three main contributors to the model. Predicted areas with suitable conditions for the occurrence of coralligenous habitat have been reported 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 rhodoliths habitats. When available, the depth range, the surface area mapped, the spatial resolution, the efficiency (expressed as area mapped in km^2 per hour), the main advantages or the limits of each tool are indicated, with some bibliographical references.

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

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Side scan sonar	8 m to over 120 m (until the lower limit of the coralligenous habitat)	From intermediate to large areas (50-100 km²)	From 1 m	1 to 4 km²/hour	Wide bathymetric range Realistic representation of the seafloor Good identification of the nature of the bottom and of assemblages (rhodoliths) with location of edges Quick execution 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 km^2)	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 rhodoliths 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 bathymetry for 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 often 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 rhodoliths habitats. On the resulting maps the habitat distributional range and its total extent (expressed in square meters or hectares) can be defined. These maps could be also compared with previous historical available data from literature to evaluate any changes experienced by benthic habitats over a period of time (Giakoumi et al., 2013). Using the overlay vector methods on GIS, a diachronic analysis can be done, where temporal changes are 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 rhodoliths habitats will then reflect the Good Environmental Status (GES) pursued by the Contracting Parties to the Barcelona Convention under the Ecosystem Approach (EcAp) and under the Marine Strategy Framework Directive (MSFD).

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

- Two maërl forming species, *Phymatolithon calcareum* and *Lithothamnion corallioides* are protected under the EU Habitats Directive (92/43/ EEC) in the Annex V
- Coralligenous reefs and rhodoliths seabeds are listed among the "special habitats types" needing rigorous protection by the Protocol 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 rhodoliths seabeds, 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 medium time 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 characterisation of the typical species and communities occurring within each habitat. Monitoring of these two habitats basically relies on underwater diving, although this technique gives rise to many constraints due to the conditions of the environment in which these habitats develop (great depths, weak luminosity, low temperatures, presence of currents, etc.): it can only be done by confirmed and expert scientific divers (for safety) and over a limited underwater

time (Bianchi et al., 2004b; Tetzaff and Thorsen, 2005). Adoption of new investigation tools (e.g., ROVs) allows for a less precise assessment but over larger spatial 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 the habitats, especially for coralligenous. The surveys method depends greatly on the scale of the work and the spatial resolution requested (Tab. 2). The complementarities of these techniques must be taken into account when planning an operational strategy (Cánovas Molina et al., 2016b).

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 photographs recorded will be analysed in laboratory (also with the help of taxonomists) to list the main conspicuous species/taxa or morphological groups recognisable on images and to evaluate their abundance (coverage or surface area in cm^2). Photographs can be then archived to create temporal datasets.

48. At shallower depths (up to about 40 m, and according to local rules for scientific diving), direct underwater visual surveys by scuba diving are strongly suggested. 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 the coralligenous assemblages, semi-quantitative evaluations often give sufficient information (Bianchi et al., 2004b); thus, it is possible to estimate the abundance (usually expressed as % cover) by 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, 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 or abundance of a given species, assessing its vitality seems a particularly interesting parameter. The presence of broken individuals (especially of the branching colonies occurring in the intermediate and upper layers 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 the habitat (Harmelin, 1990; Gatti et al., 2012).

nthesis of the main methods used to characterise coralligenous and rhodoliths habitats in the Mediterranean, as the first necessary step n Indicator 2_Condition of the habitat's typical species and communities. When available, the depth range, the surface area surveyed he efficiency (expressed as area surveyed in km^2 per hour), the main advantages or the limits of each tool are indicated, with some bib

	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits
	From 2 m to over 120 m	Small-Intermediate areas of about 1 km^2	From 1 m to 10 m	0.025 to 0.01 km²/hour	Non-destructive method Possibility of taking pictures Wide bathymetric range Good identification of facies and associations Possibility of semi-quantitative/quantitative evaluation	Need of specialists in taxonomy High cost, major means out at sea Difficulty of observation and access according to the complexity of the habitat (multilayer assemblages) Quantitative assessments only on conspicuous species/taxa
	0 m up to 40 m, according to local rules for scientific diving	Small areas (less than 250 m^2)	From 1 m	0.0001 to 0.001 km²/hour	Non-destructive Very good precision for the identification (taxonomy) and characterisation of the habitat (also its 3-D) Low cost, easy to implement Possibility to collect samples Data already available after dive	Need of specialists in taxonomy Small area inventoried Very time-consuming underwater Limited operational depth Highly qualified divers required Subjectivity of the observer Quantitative assessments only on conspicuous species/taxa
y	0 m up to 40 m, according to local rules for scientific diving	Small areas (less than 10 m^2)	From 1 m	0.0001 to 0.001 km²/hour	Very good precision for the identification (taxonomy) and characterisation of the habitat All species taken into account *A posteriori* identification Low cost, easy to implement	Destructive method Very small area inventoried Sampling material needed Limited operational depth Highly qualified divers required Very time-consuming underwater Analysis of samples in laboratory very time-consuming

	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits
y	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 Quantitative assessments only on conspicuous species/taxa 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 depth Highly qualified divers required Tools to collect photos/video necessary Limited number of species/taxa observed Only 2-D observation allowed
s	0 m to about 120 m (until the lower limit of the rhodoliths habitat)	Intermediate areas (a few km^2)	From 1 to 10 m	0.025 to 0.01 km^2/hour	Very good precision for the identification (taxonomy) and characterisation of the habitat All species taken into account *A posteriori* identification Low cost, easy to implement	Destructive method Small area inventoried Sampling material needed Samples analysis in laboratory very time-consuming

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

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

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

53. Differently from seagrass, the descriptors used to monitor coralligenous assemblages vary greatly from one team to another and from one region to another, as well as their measuring protocol (Piazzi et al., 2019a and references therein). A first 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

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

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).

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.

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.

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.

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).

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.

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.

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

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.

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.

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).

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)
- Sedimentationlevel, estimated considering the percentage of hard bottom covered by sediments: 0%, <30% (low), 30-60% (medium), >60% (high).

Protocol for monitoring rhodoliths habitat

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.

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, towed cameras, or more usually sampling from vessels using blind grabs, dredges or box corers are often favoured because of the greater homogeneity of these populations (Tab. 4). Monitoring should address all the variables already described for the first descriptive characterisation of the habitat, with the addition of the full quantitative description of the rhodoliths community, through periodical surveys. A decrease in rhodoliths beds extent, live/dead rhodoliths ratio, live rhodoliths percentage cover, associated with change in the composition of the macrobenthic community (calcareous algal engineers and associated taxa) may reveal potential negative impacts acting on rhodoliths beds. All possible variations in growth form, shape, and internal structure of rhodoliths have been simplified in a scheme with three major categories as focal points along a continuum: compact and nodular pralines, larger and vacuolar 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 no information on rhodoliths composition owing to the absence of conspicuous, easy-to-detect species. Moreover, since most coralline species belong to a few genera only, the use of taxonomic ranks higher than species is not useful.

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

Underwater visual observation	
Advantages	Low cost, results immediately available, non-destructive method, reference samples, taxonomical precision, information on the distribution of species
Limits	Work limited as regards to depth, small area inventoried
Use	Exploratory studies, monitoring of assemblages, bionomic studies
Blind sampling (dredges, grabs or box corers)	
Advantages	Low cost, easy to implement, taxonomical precision, reference samples, analysis of substratum (granulometry, calcimetry, % of organic matter), large depth-range investigated
Limits	Low precision of observation, several replicates needed, limited area inventoried, destructive method
Use	Localised studies integrating a taxonomical element, validation of acoustic methods

ROV and towed 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

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

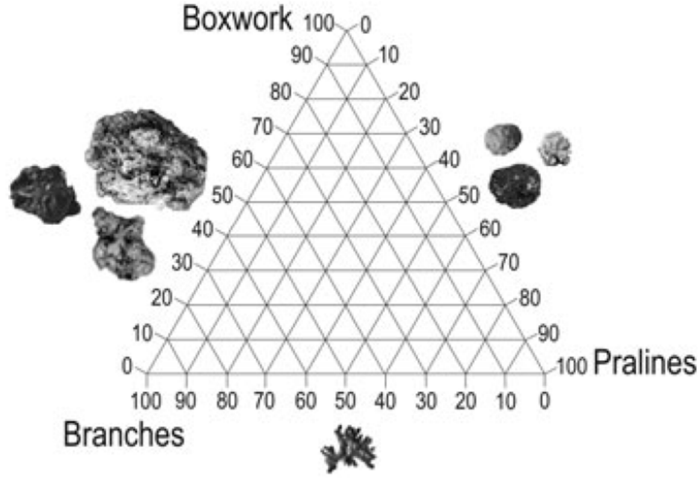

Figure 5: ternary diagram for the description of the rhodoliths bed tridimensionality. The percentage cover of each rhodoliths morphotype, relative to the total rhodoliths cover, can be plotted on the correspondent axis. The three main rhodoliths morphotypes (box work rhodoliths, pralines and unattached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

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.

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 mudand builder organisms (i.e., Corallinales, bryozoans, scleractinians) for CAI.

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.

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.

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.

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.

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

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.

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

Coralligenous

Builders

Algal builders

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

Animal builders

Foraminifera
Miniacina miniacea Pallas, 1766

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

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

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

Bioeroders

Sponges

Clionidae (Cliona, Pione)

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

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

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

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

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

Algae

Green algae
Flabellia petiolata (Turra) Nizamuddin, 1987
Halimeda tuna (J. Ellis & Solander) J.V. Lamouroux, 1816
Palmophyllum crassum (Naccari) Rabenhorst, 1868
Caulerpa cylindracea Sonder, 1845
Caulerpa taxifolia (M. Vahl) C. Agardh, 1817*
Codium bursa (Olivi) C. Agardh, 1817**
Codium fragile (Suringar) Hariot, 1889*
Codium vermilara (Olivi) Chiaje, 1829**

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

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

<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

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

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

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

Algae
Red algae (calcareous)
#*Lithophyllum racemus* (Lamarck) Foslie, 1901
#*Lithothamnion corallioides* (P.L. Crouan & H.M. Crouan) P.L. Crouan & H.M. Crouan, 1867
#*Lithothamnion valens* Foslie, 1909
#*Peyssonnelia 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
<u>Brown algae</u>
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
<u>Sponges</u>
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
<u>Cnidaria</u>
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
<u>Polychaetes</u>
Aphrodita aculeata Linnaeus, 1758
Sabella pavonina Savigny, 1822
Sabella spallanzanii Gmelin, 1791
<u>Bryozoans</u>
Cellaria fistulosa Linnaeus, 1758
Hornera frondiculata (Lamarck, 1816)
Pentapora fascialis Pallas, 1766
Turbicellepora spp.
<u>Tunicates</u>
Aplidium spp.
Ascidia mentula Müller, 1776
Diazona violacea Savigny, 1816
Halocynthia papillosa Linnaeus, 1767
Microcosmus spp.
Phallusia mammillata Cuvier, 1815
Polycarpa spp.
Pseudodistoma crucigaster Gaill, 1972
Pyura dura Heller, 1877
Rhopalaea neapolitana Philippi, 1843
Synoicum blochmanni Heiden, 1894
<u>Echinodermata</u>
Astropecten irregularis Pennant, 1777
Chaetaster longipes (Bruzelius, 1805)
Echinaster (Echinaster) sepositus Retzius, 1783
Hacelia attenuata Gray, 1840
Holothuria (Panningothuria) forskali Delle Chiaje, 1823
Leptometra phalangium Müller, 1841
Luidia ciliaris Philippi, 1837
Ophiocomina nigra Abildgaard in O.F. Müller, 1789
Parastichopus regalis Cuvier, 1817
Spatangus purpureus O.F. Müller 1776
Sphaerechinus granularis Lamarck, 1816
Stylocidaris affinis Philippi, 1845
<u>Pisces</u>
Mustelus spp.
Pagellus acarne (Risso, 1827)
Pagellus erythrinus (Linnaeus, 1758)
Raja undulata Lacepède, 1802
Scyliorhinus canicula (Linnaeus, 1758)
Squatina spp.
Trachinus radiatus Cuvier, 1829

3. Guidelines for monitoring dark habitats in Mediterranean

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Introduction

1. Dark habitats[1] are environments where the luminosity is extremely weak (deep mesophotic zone), or even absent (aphotic zone) distributed throughout the Mediterranean basin from the sea surface (i.e., caves) to the deep-sea realm. The bathymetric extension of this lightless zone depends to a great extent on the turbidity of the water and corresponds to benthic and pelagic habitats starting from the deep circalittoral. Caves, which show peculiar environmental conditions that favour the installation of organisms typical of dark habitats, are also taken into account. Dark habitats are dependent on very diverse geomorphologic structures, e.g. underwater caves, submarine canyons, seamounts, slopes, isolated rocks, abyssal plains, brine anoxic lakes, and chemo-synthetic features such as cold seeps and hydrothermal springs. Dark habitats are considered as sensitive habitats in the Mediterranean Sea requiring protection (Habitat Directive 92/43), supporting peculiar assemblages that constitute veritable reservoirs of biodiversity that, therefore, must be protected and need further attention. Thus, dark habitats were considered under the Action Plan for their conservation adopted in the 18th Ordinary Meeting of the Contracting Parties to the Barcelona Convention (Turkey, December 2013). Among the objectives of the Action Plan (UNEP/MAP-RAC/SPA, 2015) there was the need to improve knowledge about dark populations (e.g., location, specific richness, functioning, and typology) through national and regional programs aimed at establishing a shared knowledge of dark habitats, of their distribution around the Mediterranean in the form of a geo-referenced information system (GIS), and of their condition to implement specific management interventions at the basin scale.

2. In this context, the need of practical guidelines aimed at harmonising existing methods for dark habitats monitoring and for subsequent comparison of results obtained by different countries has been highlighted. In the framework of the Ecosystem Approach implementation, The Specially Protected Areas Regional Activity Centre (SPA/RAC) has been asked to improve the existing inventory tools and to propose a standardization of the mapping and monitoring techniques for dark habitats in the context of the IMAP common indicators and in order to ease the task of the Countries when implementing their monitoring programmes. Thus, the main methods used in the Mediterranean for inventory and monitoring of dark habitats have been recently summarised in the "Draft guidelines for inventorying and monitoring of dark habitats (UNEP/MAP-SPA/RAC, 2017)" and the "Guidelines for inventorying and monitoring of dark habitats in the Mediterranean Sea" (SPA/RAC-UN Environment/MAP OCEANA, 2017). These guidelines are the base for the updating and harmonization process undertaken in this document.

3. The updated guidelines aim to establish common methods for inventorying and monitoring Mediterranean deep-sea habitats and marine caves, in order to settle the basis for a regional-based assessment. Furthermore, they aim at reviewing the known distribution and main characteristics of these ecosystems. Although the Dark Habitats Action Plan covers entirely dark caves[2], inventorying and monitoring initiatives focusing on marine caves should consider the cave habitat as a whole. Therefore, this updated document presents methodologies that cover both semi-dark and dark caves. Notwithstanding the increased scientific knowledge on dark habitats during the last decades, there is still a significant gap today. The number of human activities and pressures impacting marine habitats has considerably increased throughout the Mediterranean Sea, including deep-sea habitats (e.g., destructive fishing practices such as bottom trawling, oil and gas exploration, deep-sea mining); thus, there is an urgent need for establishing a regional monitoring system. Nevertheless, the development of comprehensive inventorying initiatives and monitoring tools becomes extremely challenging due to: (1) the scarcity of information on the current state of these habitats (distribution, density of key species, etc.), (2) the high cost and difficulties for accessing, and (3) the lack of historical data and long-time series. In this context, MPAs and Fishing Restricted Areas (FRAs) may be considered as essential tools for the conservation and monitoring of dark

[1] Dark habitats are those where either no sunlight arrives or where the light that does arrive is insufficient for the development of plant communities. They include both shallow marine caves and deep habitats (usually at depths below 120-200 m).
[2] <0.01% of the light at the sea surface level, according to Harmelin et al. (1985).

habitats. However, to date there is an obvious gap in the protection and monitoring of deep-sea habitats as they are mainly located in offshore areas where information remains limited. This issue should be addressed by CPs at the earliest convenience in order to put in place control systems aiming at the implementation of Ecosystem Approach (EcAp) procedures, and particularly the implementation of the IMAP at the regional and national level.

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

Marine caves

5. Marine caves support well diversified and unique biological communities (Pérès and Picard, 1949; Pérès 1967; Riedl 1966; Harmelin et al., 1985), harbouring a variety of sciaphilic communities, usually distributed according to the following zonation scheme: (a) a (pre-)coralligenous[1] algae-dominated community at the entrance zone, (b) a semi-dark zone dominated by sessile filter-feeding invertebrates (mainly sponges and anthozoans), and (c) a dark zone at the end or at the confined areas of the cave, which is sparsely colonized by sponges, serpulid polychaetes, bryozoans and brachiopods (Pérès, 1967). Nevertheless, there is a lamentable dearth of information on the gradients of physical-chemical parameters acting on the marine cave biota (Gili et al., 1986; Morri et al., 1994a; Bianchi et al., 1998). A general description of the semi-dark and dark cave communities, which are considered in the present document, can be found bellow.

- *Semi-dark cave communities*

6. Hard substrates in semi-dark caves are typically dominated by sessile invertebrates (sponges, anthozoans, and bryozoans) (see Appendix I). The most frequently recorded sponge species are *Agelas oroides*, *Petrosia ficiformis* (often discoloured), *Spirastrella cunctatrix*, *Chondrosia reniformis* (often discoloured), *Phorbas tenacior*, and *Axinella damicornis* (Fig. 1). The sponge *Aplysina cavernicola* has been also described as a characteristic species of the semi-dark community in the north-western Mediterranean basin (Vacelet, 1959). Sponges of the class Homoscleromorpha (e.g., *Oscarella* spp. and *Plakina* spp.) may also significantly contribute to the local sponge assemblages.

7. Three anthozoan facies have been recorded in semi-dark caves (mostly on ceilings) (Pérès, 1967; Zibrowius, 1978): (i) facies of the scleractinian species *Leptopsammia pruvoti*, *Madracis pharensis* (particularly abundant in the eastern basin), *Hoplangia durotrix*, *Polycyathus muellerae*, *Caryophyllia inornata*, and *Astroides calycularis* (in the southern areas of the central and western Mediterranean Sea) (Fig. 1); (ii) facies of *Corallium rubrum*, which is more common in the north-western Mediterranean Sea but can be found only in deep waters (below 50 m depth) in the north-eastern basin (Fig. 1); and (iii) facies of *Parazoanthus axinellae*, which is more common close to the cave entrance or in semi-dark tunnels with high hydrodynamic regime (more common in the Adriatic Sea) (Fig. 1). Facies of erect bryozoans (e.g., *Adeonella* spp. and *Reteporella* spp.) often develop in semi-dark caves (Pérès, 1967; Ros et al., 1985) (Fig. 1).

- *Dark cave communities*

8. The shift from semi-dark to dark cave communities is evidenced through a sharp decrease in biotic coverage, biomass, three-dimensional biotic complexity, species richness, and the

[1]Coralligenous and semi-dark cave communities have been integrated into the Action Plan for the conservation of the coralligenous and other calcareous bio-concretions in the Mediterranean Sea (UNEP/MAP-RAC/SPA, 2008).

appearance of a black mineral coating of Mn-Fe oxides on the substrate (Pérès, 1967; Harmelin et al., 1985). This community is usually sparsely colonized by sponges, serpulids, bryozoans and brachiopods (Pérès, 1967) (see Appendix I). Common sponge species are *Petrosia ficiformis* (usually discoloured), *Petrobiona massiliana* (mainly in Western Mediterranean caves), *Chondrosia reniformis* (usually discoloured), *Diplastrella bistellata*, *Penares euastrum*, *P. helleri*, *Jaspis johnstoni*, *Haliclona mucosa*, and *Lycopodina hypogea*.

9. Serpulid polychaetes are among the dominant taxa in these caves, with the typical species being *Serpula cavernicola* and *Spiraserpula massiliensis* (Zibrowius, 1971; Bianchi and Sanfilippo, 2003; Sanfilippo and Mòllica, 2000). In some caves, the species *Protula tubularia* forms aggregates that constitute the basis for the creation of bioconstructions; these "biostalactites" are constructed by invertebrates (serpulids, sponges, and bryozoans), foraminiferans and carbonate-forming microorganisms (Sanfilippo et al., 2015).

10. Encrusting bryozoans (e.g. *Onychocella marioni*) can also produce nodular constructions in the transitional zone between semi-dark and dark cave communities (Harmelin, 1985). Brachiopods (e.g., *Joania cordata*, *Argyrotheca cuneata*, and *Novocrania anomala*) are common in dark cave habitats (Logan et al., 2004). The species *N. anomala* is frequently found in high numbers, cemented on cave walls and roofs (Logan et al., 2004). A number of deep-sea species belonging to various taxonomic groups (e.g., sponges, anthozoans, and bryozoans) have been recorded in sublittoral dark caves, regardless of depth (Zibrowius, 1978; Harmelin et al., 1985; Vacelet et al., 1994).

11. Several motile species often find shelter in dark caves, such as the mysids *Hemimysis margalefi* and *H. speluncola*, the decapods *Stenopus spinosus*, *Palinurus elephas*, and *Plesionika narval* (more common in southern and eastern Mediterranean areas) and the fish species *Apogon imberbis* and *Grammonus ater* (Pérès, 1967; Ros et al., 1985, Bussotti et al., 2002).

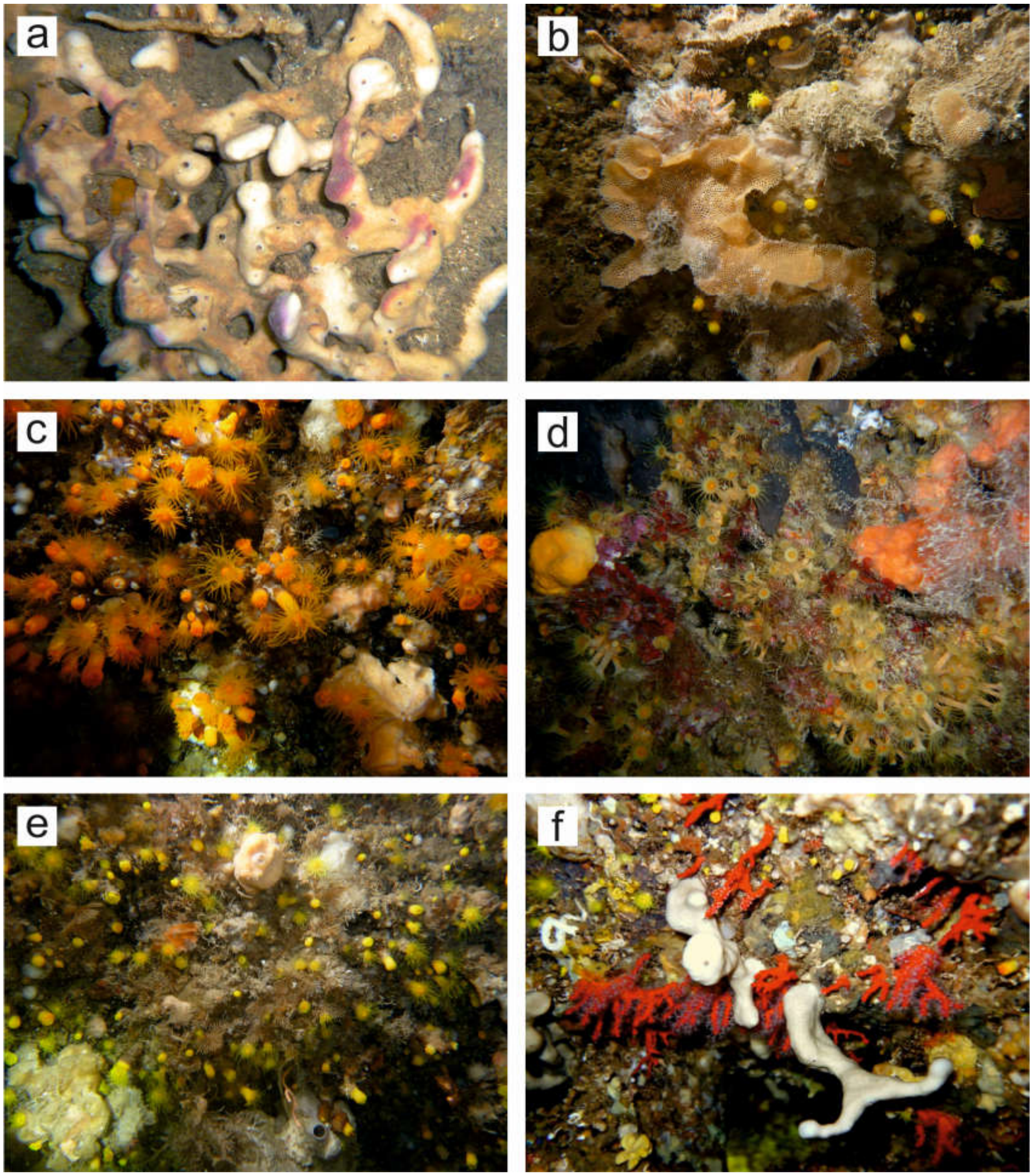

Figure 1: facies with *Petrosia ficiformis* (a), *Reteporella grimaldii* and other bryozoans (b), *Astroides calycularis* (c), *Parazoanthus axinellae* (d), *Leptopsammia pruvoti* (e), and *Corallium rubrum* (f) in semi-dark marine caves. Pictures by Monica Montefalcone (a-e) and Vasilis Gerovasileiou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

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

Western Mediterranean Sea

13. A total of 1046 marine caves have been recorded in the western Mediterranean basin (Giakoumi et al., 2013). The rocky coasts of the Tyrrhenian Sea and the Algero-Provençal Basin have been extensively studied for their cave biodiversity, with 822 and 650 taxa recorded from these two areas respectively (Gerovasileiou and Voultsiadou, 2014). The first and some of the most influential studies on the diversity and structure of marine cave communities were carried out in the French, Italian and Catalan coasts (e.g., Pérès and Picard, 1949; Riedl, 1966; Harmelin et al., 1985; Ros et al., 1985; Bianchi and Morri, 1994; Bianchi et al., 1996). A synthesis of the existing

knowledge on Italian marine caves, accumulated in fifty years of research, was compiled by Cicogna et al. (2003). The fully submerged caves of Figuier, Jarre, Riou, Trémies and Triperie in the karstic coasts of Marseille-Cassis area are among the species-richest Mediterranean caves, while the famous Trois Pépés cave has been characterised as a unique “deep-sea mesocosm” in the sublittoral zone, supporting deep-sea faunal elements in its inner dark sectors (Vacelet et al., 1994; Harmelin, 1997). Submarine caves in the region of Palinuro (Tyrrhenian Sea) have been found to host sulphur springs that support trophic webs based on chemosynthesis (Bianchi et al., 1994; Morri et al., 1994b; Southward et al., 1996), presenting analogies with deep-water chemosynthetic ecosystems. The submarine cave of Bergeggi (Ligurian Sea, Italy) provides the longest series of data on the status of benthic communities, being studied regularly since 1986 (Parravicini et al., 2010; Montefalcone et al., 2018).

14. The number of species reported from marine caves decreases towards the insular and southern sectors of the western Mediterranean basin, according to differences in temperature and trophic conditions (Uriz et al., 1993) and to a notable decrease in research effort (Gerovasileiou and Voultsiadou, 2014). For instance, the Alboran Sea is one of the least studied areas regarding its marine cave fauna (but see Navarro-Barranco et al., 2014, 2016). Nevertheless, recent research expeditions in the framework of the MedKeyHabitats project have provided baseline information for the previously understudied Alboran coasts of Morocco (PNUE/PAM-CAR/ASP, 2016).

Ionian Sea and central Mediterranean

15. The western coasts of the Ionian Sea are among the best-studied Mediterranean areas regarding their marine cave biodiversity, with almost 700 taxa reported in this area (Gerovasileiou and Voultsiadou, 2014). To date 375 marine caves are known from the Ionian Sea and the Tunisian Plateau/Gulf of Sidra (Giakoumi et al., 2013). Most of the regional inventories, mapping initiatives and biodiversity studies have taken place in the Salento Peninsula (e.g., Onorato et al., 1999; Bussotti et al., 2002, 2006; Denitto et al., 2007; Belmonte et al., 2009; Bussotti and Guidetti, 2009) and in Sicily (e.g., Rosso et al., 2013, 2014; Sanfilippo et al. 2015). Marine caves in this area were recently studied and evaluated for their ecological status.

Adriatic Sea

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

Aegean Sea and Levantine Sea

17. The coasts of the eastern Mediterranean basin host approximately one third (738) of the marine caves recorded in the Mediterranean Sea, mostly across the complex coastline of the Greek Islands in the Aegean Sea (Giakoumi et al., 2013). A total of 520 taxa have been found in caves of the Aegean and the Levantine seas (324 and 157, respectively) (Gerovasileiou et al., 2015). Lesvos Island in the North Aegean Sea hosts two of the best-studied marine caves with regard to their diversity (approximately 200 taxa recorded in each cave), community structure and function (Gerovasileiou and Voultsiadou, 2016; Sanfilippo et al., 2017). Several caves scattered across the Aegean ecoregion were recently studied for their biodiversity (e.g., Rastorgueff et al., 2014; Gerovasileiou et al., 2015), community structure and ecological quality. One of the most well-known

insular areas concerning their marine cave formations is encompassed within the National Marine Park of Alonissos and Northern Sporades, hosting numerous cave habitats, critical for the survival of the endangered Mediterranean monk seal *Monachus monachus* (Dendrinos et al., 2007). The coasts of Lebanon host most of the studied Levantine caves (e.g., Bitar and Zibrowius, 1997; Logan et al., 2002; Pérez et al., 2004; Vacelet et al., 2007; Morri et al., 2009). Forty-six non-indigenous species have been recorded in 80% of the marine caves and tunnels known to exist in the Levantine Sea, mostly at their entrance and semi-dark zones (Gerovasileiou et al., 2016b), indicating a potential new threat for cave communities that should be further monitored.

Deep-sea habitats

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

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

✓ Assemblages of underwater canyons

✓ Assemblages associated with seamounts

✓ Engineering benthic invertebrate assemblages

- Black coral and gorgonian forests on hard substrata
- Beds with *Isidella elongata* and beds with pennatulaceans on detritic substrata
- Associations of sponges on both types of substrata

✓ Deep-sea chemo-synthetic assemblages

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

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

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

22. The best known are Cold-Water Coral (CWC) reefs, mainly formed by *Desmophyllum pertusum (*ex *Lophelia pertusa*) and *Madrepora oculata* (Orejas and Jiménez, 2019). They usually occur in rocky substrates (e.g., seamounts, canyons or escarpments) although they could also be found in highly silted areas. Their bathymetric range is usually between about 200 m and down to more than 1000 m. They have been found both in the western and eastern central Mediterranean Sea, in areas such as the Cabliers, Chella and Avempace seamounts in the Alboran Sea (Pardo et al., 2011; de la Torriente et al., 2014; Lo Iacono et al. 2014), in canyons in the Gulf of Lion and the surrounding

area such as Cassidaigne and Creus (Bourcier and Zibrowius, 1973; Orejas et al., 2009; Fourt and Goujard, 2012; Gori et al. 2013), in the estern Ligurian Sea (Fanelli et al., 2017), in the southern Catalan canyons (e.g., La Fonera canyon; Lastras et al., 2016; Taviani et al., 2019), south of Sardinia in the Nora Canyon (Taviani et al., 2017), in the Gulf of Naples (Taviani et al., 2017), offshore Santa Maria di Leuca in the Northern Ionian Sea (Taviani et al., 2005a, 2005b; Mastrototaro et al., 2010; Savini et al., 2014; Vertino et al., 2010; D'Onghia et al., 2012), south of Malta and other sites in the Strait of Sicily (Schembri et al., 2007; Freiwald et al., 2009; Taviani et al., 2009, 2011a; Evans et al., 2016), next to the Jabuka-Pomo depression (Županović, 1969), in the Bari canyon and off Apulia in the south-western Adriatic Sea (Freiwald et al., 2009; Angeletti et al., 2014; D'Onghia et al., 2015), in the Montenegrin canyons (Angeletti et al., 2014, 2015a), in the Adriatic Sea, trough off Thassos in northern Aegean Sea (Vafidis et al., 1997), in the Marmara Sea (Taviani et al., 2011a), in the deep waters of the Hellenic Arc in the south of the Aegean/Levantine basin (Fink et al., 2015), among others.

23. Other stony corals that form important marine habitats are the tree corals (*Dendrophyllia* spp.). *D. cornigera* can form dense aggregations in deep seabeds, although in the Mediterranean Sea it is rare to find places with dense populations (Pardo et al., 2011; Bo et al., 2014a). Its bathymetric range can vary from shallow water to depths of more than 600 m. It has been found mainly in the western basin, on seamounts in the Alboran Sea (Pardo et al., 2011; de la Torriente et al., 2014), in submarine canyons in the Gulf of Lion and Corsica (Orejas et al., 2009; Gori et al. 2013; Fourt et al., 2014a), in the Balearic Archipelago continental shelf and slope (Orejas et al., 2014), on seamounts in the Tyrrhenian Sea (Bo et al., 2011), at mesophotic depths in the Ligurian Sea (Bo et al., 2014a), in some areas of the central Mediterranean Sea (Würtz and Rovere, 2015), including the banks of the Ionian Sea (Amendolara Bank, Tursi et al., 2004; Bo et al., 2014a), and in the southern Adriatic Sea (Freiwald et al., 2009; Angeletti et al., 2015a). *D. ramea* is more common in shallower waters, especially at mesophotic depths. Recently, however, *D. ramea* communities have been found in deep waters in the eastern Mediterranean Sea, such as the deep seabeds of Cyprus (Orejas et al., 2017) and the submarine canyons off Lebanon (R. Aguilar, pers. obs.). Both species can occur on rocky and soft seabeds. Furthermore, in the northern part of the Sicilian coast, between 80 and 120 m depth, a huge population of *D. ramea* with several colonies was recently discovered. Many colonies showed severe injure caused by lost fishing gear (Salvati et al., submitted). Probably this species showed a more diffuse abundance and distribution in the past.

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

25. There are also solitary corals that sometimes create important aggregations. This is the case of the pan-Mediterranean *Desmophyllum dianthus*, a solitary coral with a pseudocolonial habit found in both canyons and deep seabeds, alone or even participating in the formation of reefs with *Desmophyllum pertusum* and *Madrepora oculata* (Galil and Zibrowius, 1998; Montagna et al., 2006; Freiwald et al., 2009; Taviani et al., 2011b, 2016a, 2017; de la Torriente et al., 2014; Fourt et al., 2014a).

26. Species of the genus *Caryophyllia* settle on rocky and detritic bottoms and may become important. For example, *Caryophyllia (Caryophyllia) calveri* is one of the most common solitary coral species in deep rocky bottoms, being capable of forming dense communities, sometimes along with other scleractinians such as *Javania cailleti*, *Stenocyathus vermiformis* and other *Caryophyllia* spp. It has been found in seamounts, escarpments or rocky bottoms (Galil and Zibrowius, 1998; Mastrototaro et al., 2010; Aguilar et al., 2013, 2014). In the case of soft bottoms, mainly in detritic sands, beginning in the deep circalittoral sand and extending to depths down to 400-500 m,

Caryophyllia (Caryophyllia) smithii can cover significant areas (de la Torriente et al., 2014), similar to *Flabellum* spp. in the Atlantic (Baker et al., 2012; Serrano et al., 2016).

- *Habitats dominated or structured by black corals*

27. Antipatharians, or black corals, are represented in the Mediterranean by just a few species, although this number may increase with the new deep-sea explorations. They are found on hard bottoms, although they can withstand some sedimentation and may occur on rocky bottoms slightly covered by sediments. They can also occur on seamounts, in canyons or on deep sea environments where hard substrates are present. The species that reach the highest densities are *Antipathella subpinnata*, *Leiopathes glaberrima*, and (in some occasions) *Parantipathes larix* that can form monospecific assemblages (e.g., Bo et al., 2009, 2015, 2019a, 2019b; Ingrassia et al., 2016). *Antipathes dichotoma* can also occur with high densities, but many times are part of other black coral communities alongside gorgonians. They have a wide bathymetric distribution with some species occurring also in the upper mesophotic zone at relatively shallow depths (about 60 m) (Bo et al., 2009, 2019b), and others extending to the superficial bathyal zone and reaching depths of over 2000 m. It is known that some *Leiopathes* sp. inhabit depths down to 4000 m outside the Mediterranean Sea (Molodtsova, 2011). Dense aggregations have been found on seamounts in the Alboran (de la Torriente et al., 2014), the Balearic Archipelago (Grinyó, 2016), the Ligurian Sea (Bo et al., 2014a, 2019a), and the Tyrrhenian Seas (Bo et al., 2011, 2012; Fourt et al., 2014a; Ingrassia et al., 2016), in south-western Sardinia (Bo et al., 2015; Cau et al., 2016a), on the escarpments in the south of Malta (Deidun et al., 2015; Evans et al., 2016), in the Ionian Sea (Mytilineou et al., 2014) and in the eastern Adriatic Sea (Angeletti et al. 2014; Taviani et al., 2016a). Sporadic occurrences have been also reported from the Malta Escarpment and offshore Rhodes (Taviani et al., 2011b; Angeletti et al., 2015b).

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

29. Recently other black coral species have also been observed forming dense aggregations. Some examples are *Parantipathes larix* found in some areas of the Alboran Sea (Pardo et al., 2011) and in deep waters off the Tuscan and Pontin archipelago in the Tyrrhenian Sea (Bo et al., 2014b, Ingrassia et al., 2016), also in Corsica and Provence region (Fourt et al., 2014a), and *Phanopathes rigida*, newly reported on seamounts between 180-400 m from the south of the Alboran Sea in the Cabliers Bank (Bo et al., 2019b). *Parantipathes larix* has a wide bathymetric distribution, from 120 m down to over 2000 m (Opresko and Försterra, 2004; Fabri et al., 2011; Bo et al., 2012b).

- *Habitats dominated by gorgonians*

30. Deep Mediterranean gorgonian assemblages (Alcyonacea, excluding Alcyoniina) can be highly diverse and present a wide geographic and bathymetric distribution (Gori et al., 2017, 2019). Most are species that attach to a hard substrate, although some can withstand high levels of sedimentation and a few species can occur in soft bottoms, both detritic and muddy (Mastrototaro et al., 2017). Some of the assemblages that reach high densities are those formed by the Atlanto-Mediterranean gorgonian *Callogorgia verticillata*. Dense forests have been found that can begin in the deep mesophotic zone and extend to a depth of more than 1000 m (de la Torriente et al., 2014; Angeletti et al., 2015a; Evans et al., 2016; Gori et al., 2017, 2019). These forests may be monospecific or may be formed by several gorgonian species (e.g., *Bebryce mollis*, *Swiftia pallida*), antipatharians (e.g., *L. glaberrima* and *A. dichotoma*) or scleractinian white corals (e.g., *Desmophyllum pertusum*, *Dendrophyllia* spp). A frequent association of this species is with the whip

coral (*Viminella flagellum*), especially in the deep circalittoral and upper bathyal zones (Giusti et al., 2012; Lo Iacono et al., 2012; Chimienti et al., 2019), where it is more common.

31. Another species that commonly occurs on hard substrates of the continental slope is *Acanthogorgia hirsuta* that can occur as isolated colonies (Grinyó et al., 2016) or forming dense assemblages (Aguilar et al., 2013; Fourt et al., 2014b), sometimes with other gorgonians such as *Placogorgia* spp., on the slopes of seamounts or on the gently inclining edges of escarpments (de la Torriente et al., 2014; Enrichetti et al., 2019). It is also a species observed as part of the Alcyonacea that grow among coral rubbles or with other communities of deep-seabed corals and gorgonians, usually below 250-300 m.

32. *Eunicella cavolini* and *E. verrucosa* are the only species of the genus *Eunicella* that can be found on rocky bottoms from littoral to great depths. *E. cavolini* was observed down to 280 m in the Nice canyon (Fourt and Chevaldonné, pers. obs.); however, they are more common on the tops of seamounts, forming monospecific assemblages or mixed with *Paramuricea clavata* (Aguilar et al., 2013; De la Torriente et al., 2014). The latter is not usually found beyond 140-150 m, but becomes very abundant on the summits of seamounts, like the Palos, the Chella Banks (Aguilar et al., 2013), or in heads of some canyons (Pérez-Portela et al., 2016), such as Cassidaigne canyon where it occurs at a depth around 200 m (Fourt et al., 2014a). It shares this characteristic with *E. cavolini*, which has been found on rocky bottoms in the heads of canyons in the Balearic Sea (Grinyó et al., 2016) and the Gulf of Lion (Fourt and Goujard, 2012).

33. There is a wide range of small gorgonians that can form dense thickets (Angiolillo et al., 2014; Grinyó et al., 2016) or co-occur alongside larger species such as *C. verticillata*, antipatharians or alongside cold-water coral reef building species (Evans et al., 2016; Chimienti et al., 2019). Among these species can be found *Bebryce mollis*, *Swiftia pallida*, *Paramuricea macrospina* and *Villogorgia bebrycoides*, which can occur on unstable substrata and coarse detritic bottoms, from the shelf edge (or even the deep circalittoral zone) to depths of 600-700 m (Bo et al., 2011, 2012b, 2015; Giusti et al., 2012; Aguilar et al., 2013; Angeletti et al., 2014; Grinyó et al., 2015; Evans et al., 2016; Taviani et al., 2017).

34. *Swiftia pallida* forms important single species thickets in the upper bathyal zone, usually between 200 and 700 m, although it may have a greater bathymetric range. It is widely distributed throughout the Mediterranean Sea, having been found on seamounts of the Alboran Sea (de la Torriente et al., 2014) to places as far away as the canyons off Lebanon (R. Aguilar, pers. obs.) and Israel (Zvi Ben Avraham, pers. obs.). It can occur on rocky and deep detritic bottoms, tolerating a certain level of sedimentation.

35. *Muriceides lepida* and *Placogorgia massiliensis*, on the other hand, occur as accompanying species in the assemblages described above, although they can also be the dominant species in some escarpments or in combination with sponge aggregations or other benthic communities (Maldonado et al., 2015; Evans et al., 2016). Both can be found in the western and central Mediterranean Sea in zones ranging from a depth of 300 m to over 1000 m (Sartoretto and Zibrowius, 2018; Chimienti et al., 2019).

36. The case of *Dendrobrachia bonsai* is similar, although it is a species associated with greater depths (usually below 400-500 m). It has been found forming thickets in deep rocky bottoms or as the predominant species in areas of escarpments and canyons with a steep inclination (Sartoretto, 2012; de la Torriente et al., 2014; Evans et al., 2016).

37. In the case of *Nicella granifera*, so far this has only been found in the western Mediterranean Sea, in seamounts between the Alboran and the Balearic Seas (Aguilar et al., 2013). It has a deep bathymetric distribution, usually below 400 m.

38. Finally, the red coral (*Corallium rubrum*) shows a wide bathymetric range that stretches from shallow-water caves in the infralittoral zone to depths greater than 1000 m in the bathyal zone (Rossi et al., 2008; Taviani et al., 2010; Knittweis et al., 2016), with a peak at mesophotic depths (Cattaneo et al., 2016). Although it may form single-species forests on rocky bottoms or be the predominant species on escarpments and in caves (Cau et al., 2016b), it has also been found as part of mixed forests associated with white corals, antipatharians or large gorgonians (Freiwald et al., 2009; Constatini et al., 2010; Evans et al., 2016).

39. On soft bottoms, the most characteristic community is that of the bamboo corals (*Isidella elongata*). It is a species that is almost exclusive to the Mediterranean Sea and that usually

appears in muddy bottoms below depths of 400 m. It has been found on seamounts in the Alboran and Balearic Seas (Aguilar et al., 2013; de la Torriente et al., 2014; Mastrototaro et al., 2017), deep seabeds in the Spanish slope (Cartes et al., 2013), in front of the canyons in the Gulf of Lion (Fabri et al., 2014), over the Carloforte Shoal at 190 m depth (Bo et al., 2015), in the bathyal plain of Malta (R. Aguilar, pers. obs.), and in the Ionian Sea (Mytilineou et al., 2014), among other places.

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

- *Habitats dominated by pennatulaceans*

41. Since these are species that bury part of the colony in the substrate, they require soft bottoms, either sandy or muddy, between the infralittoral zone and the bathyal zone. They can therefore appear in all kinds of soft bottoms on seamounts and in canyons, on bathyal plains and shelf edges (Chimienti et al., 2019). Species of the genera *Pennatula* and *Pteroeides* can form mixed communities that become numerous on the shelf edges and the beginning of the slope (e.g., Chella Bank) (Gili and Pagès, 1987; Aguilar et al., 2013; de la Torriente et al., 2014). The species may vary according to the depth, with *Pennatula rubra* being more frequent in shallower areas, while *P. phosphorea* occupies deeper seabeds, at depths reaching the muddy areas of the bathyal zone. Their distribution is pan-Mediterranean.

42. *Virgularia mirabilis* and *Veretillum cynomorium* are also species with a wide bathymetric and geographical distribution. Found all over the Mediterranean Sea on seamount slopes, the shelf edges, plains, and in canyons (Gili and Pagès, 1987; Aguilar et al., 2013), they occupy muddy-sandy bottoms, from the infralittoral to the bathyal zones, sometimes also mixing with other pennatulaceans or forming monospecific communities.

43. *Funiculina quadrangularis* also shares characteristics with other pennatulaceans, but it is a species typical of deep soft bottoms, found throughout the Mediterranean Sea, at depths ranging from the circalittoral to the bathyal zone. It forms dense forests in shelf areas, gently sloping areas in canyons, and muddy-sandy interstices on seamounts (Morri et al., 1991; Fabri et al., 2014; de la Torriente et al., 2014). It may appear in mixed communities with other pennatulaceans, bamboo corals, or other soft-bottom species, such as various bryozoans and sponges.

44. Recently, another pennatulacean whose distribution was believed to be exclusively Atlantic has been discovered in several areas of the Mediterranean Sea (Balearic Sea, Central Mediterranean and Ionian Sea). This is *Protoptilum carpenteri* (Mastrototaro et al., 2015, 2017; R. Aguilar, pers. obs.), which has a preference for the same substrate and looks very similar to *Funiculina quadrangularis*, which has sometimes led to it going unnoticed.

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

- *Habitats with other anthozoans*

46. Other groups of anthozoans, such as Alcyoniidae, sea anemones (Actinaria) and cerianthids also give rise to communities' characteristic of dark habitats. These include newly discovered or rediscovered species, such as *Chironephthya mediterranea* (López-González et al., 2015) and *Nidalia studeri* (López-González et al., 2012), which create dense aggregations in the lower circalittoral and bathyal zones, between 150 m and 400 m. They can be found on hard bottoms, and on gravel and coarse sediments of seamounts, slope edges and submarine canyons. Their known geographical distribution stretches from the western to the central Mediterranean Sea, although a wider distribution has not been ruled out.

47. Equally important are species such as *Alcyonium palmatum* and *Paralcyonium spinulosum* (Templado et al., 1993; Fava and Ponti, 2007; Bo et al., 2011; Marin et al., 2011b, 2014; UNEP/MAP-RAC/SPA, 2013), since their plasticity in the occupation of both soft and hard bottoms allows them colonising large areas of the Mediterranean basin, in both shallow and dark habitats, usually found on seamounts' summits. It is not uncommon for them to associate with other anthozoans.

48. With regard to anemones, at present only *Actinauge richardii* can be considered as a dark habitat species, which forms communities of importance. Habitual in sedimentary bottoms, preferably sandy, between the circalittoral and the bathyal zones, it is found in large numbers on the gentle slopes of seamounts in the western Mediterranean or in bathyal plains in the central Mediterranean Sea (R. Aguilar, pers. obs.).

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

- *Sponge grounds with demosponges*

50. Various demosponges give rise to dense aggregations, on some occasions as the dominant species and on others in combination with corals and gorgonians. *Poecillastra compressa* and *Pachastrella monilifera* appear to have the most extensive geographical distribution within the Mediterranean basin and an important role in deep ecosystems (Bo et al., 2012a; Calcinai et al., 2013; Angeletti et al., 2014; Taviani et al., 2016a), while those of the genus *Phakellia* are more common in the western basin (Aguilar et al., 2013; de la Torriente et al., 2014). They may begin to appear in the lower circalittoral, but their presence is more common in the bathyal zone.

51. The eastern Mediterranean is home to large Dictyoceratida of the genera *Spongia*, *Ircinia*, *Sarcotragus*, *Scalarispongia*, as well as *Agelasida* (i.e., *Agelas oroides*), which are common in shallow areas developing on the heads of canyons, shelf edges and in the upper bathyal zones (R. Aguilar, pers. obs.).

52. Both Axinellida and Haplosclerida can also show similar behaviour, becoming abundant in the deep circalittoral and upper bathyal zones, especially on seamounts and other rocky bottoms (Bo et al., 2011, 2012b; Aguilar et al., 2013).

53. Desma-bearing demosponges or Tetractinellida (ex Lithistida), can form large aggregations, even reef formations, in deep zones of the bathyal, like the one of *Leiodermatium pfeifferae* found in a seamount at depths of more than 700 m near the Balearic Islands (Maldonado et al., 2015) and on Mejean bank between 380 and 455 m (Fourt and Chevaldonné, pers. obs.). It is not known whether other "stone sponges" present in the Mediterranean, such as *Leiodermatium lynceus* or *Neophrissospongia nolitangere*, and which give rise to similar formations in the Atlantic, could also do the same in the Mediterranean Sea.

54. In soft bottoms, the presence of sponge aggregations is limited to a few species, such as *Thenea muricata*, which is common in muddy bottoms of the bathyal zone throughout the Mediterranean Sea (Pansini and Musso, 1991; de la Torriente et al., 2014; Fourt et al., 2014a; Evans et al., 2016), sometimes with the presence of the carnivorous sponge *Cladorhiza abyssicola*, while *Rhizaxinella pyrifera* is more common in sandy-detritic bottoms (Bo et al., 2012a), but can also be found in cold seeps on mud volcanoes (Olu-Le Roy et al., 2004).

- *Sponge grounds with hexactinellids*

55. The large glass sponge *Asconema setubalense* is the most important in the formation of these aggregations of sponges in the Aboran Sea, western Mediterranean (Boury-Esnault et al., 2015; Aguilar et al., 2013), mainly on rocky bottoms on seamounts at depths below 200 m but has not been found beyond this area.

56. With a much wider distribution in the Mediterranean, reaching the eastern basin, *Tetrodictyum reiswegi* (Aguilar et al., 2014; Boury-Esnault et al., 2015, 2017) is smaller than the previously mentioned sponge and usually less numerous, although it can form aggregations on hard bottoms on seamounts, escarpments, and in canyons, at depths of 200-2500 m.

57. It is not known whether other species of hexactinellids that inhabit the Mediterranean Sea can form aggregations similar to those that they create in the Atlantic, as in the cases of the genera *Aphrocallistes* or *Farrea* (Boury-Esnault et al., 2017). Another sponge, *Pheronema carpenteri*, can also give rise to important formations of scattered individuals, but in this case on muddy bottoms. In the Mediterranean Sea it has been found from the Alboran to the Tyrrhenian Sea at depths between 350 m and more than 2000 m (Boury-Esnault et al., 2015).

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

- *Habitats dominated by crustaceans*

59. There are two groups of crustaceans that give rise to deep sea habitats in the Mediterranean Sea: the cirripeds and the Ampeliscidae. In the case of cirripeds, the Balanomorpha *Pachylasma gigantea* is the predominant species, even contributing to deep-sea coral habitats (Schembri et al., 2007; Angeletti et al., 2011; Deidun et al., 2015), also in association with *Errina aspera* (Salvati et al., 2010), although *Megabalanus* spp. may also create a number of communities of some importance, usually together with molluscs and corals (R. Aguilar, pers. obs.). In the case of the Ampeliscidae, their tubes cover vast extensions of sedimentary bottoms. There are several dozens of species of the genera *Ampelisca*, *Haploops* and *Byblis* and they have been found on slope edges, on the gentle slopes of escarpments and in canyons and even on seamounts and hydrothermal fields (Bellan-Santini, 1982; Dauvin and Bellan-Santini, 1990; Marín et al., 2014; Esposito et al., 2015; R. Aguilar, pers. obs.), at depths that range from the edge of shelf or on the seamount summits to down to more than 700 m.

- *Habitats dominated by bryozoans*

60. The bryozoans usually form mixed aggregations with other benthic invertebrate species, but in some cases, they may be dominant, as in the case of large and arborescent species of the genera *Reteporella*, *Hornera*, *Pentapora*, *Myriapora*, and *Adeonella*. All of them attach to rocky substrates, but also to gravel or coarse sediment, and their distribution covers the entire Mediterranean basin. Although these species are common in shallow bottoms, they may extend to deeper areas (Bellan-Santini et al., 2002), including escarpments, deep rocky bottoms and seamount summits (Aguilar et al., 2010; de la Torriente et al., 2014). In soft bottoms, down to 350-400 m depths, some stalked species such as *Kinetoskias* sp. (Harmelin and D'Hondt, 1993; Aguilar et al., 2013, Maldonado et al., 2015), or species from the Candidae family (R. Aguilar, pers. obs.), may begin to appear. These bryozoans living on muddy bottoms have been found in the western and central Mediterranean basin (Mastrototaro et al., 2017).

- *Habitats dominated by polychaetes*

61. Many polychaetes form associations with species such as anthozoans, sponges, bryozoans, and brachiopods on rocky substrates of escarpments and mountains, in canyons and caves, but may also occur in single-species aggregates or as a dominating species on soft bottoms. Sabellids and serpulids are among the most widely distributed tube polychaetes. They have been found forming dense aggregates in deep sedimentary bottoms around Alboran Island, as in the case of *Sabella pavonina* (Gofas et al., 2014); they may create small reefs together with corals, as for *Serpula vermicularis* in the Bari Canyon (Sanfilippo et al., 2013), or they can be found in great numbers occupying extensive areas in detritic beds on the slopes of seamounts, the continental slope or submarine canyons heads, as in the case of *Filograna implexa* (Würtz and Rovere, 2015) that can also collaborate in deep-sea coral reef forming (D'Onghia et al., 2015), such as the eunicidan *Eunice norvegica* (Taviani et al., 2017).

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

63. The siboglinids, meanwhile, generate important aggregations in mud volcanoes, hypersaline lakes and other structures with chemo-synthetic communities, such as the Amsterdam mud volcano, between the Anaximenes and Anaxagoras marine ranges in the eastern Mediterranean basin (Shank et al., 2011).

- *Habitats dominated by molluscs*

64. The main aggregations, concretions and mollusc reefs in deep bottoms are those formed by oysters of the Gryphidae family. *Neopycnodonte cochlear* can be found in the photic zone, but it also creates beds in the deep-sea, whether on rocky or detritic bottoms, on escarpments and seamounts, and in canyons (de la Torriente et al., 2014; Fabri et al., 2014). *N. zibrowii* is found only on rocky bottoms, also belonging to escarpments, seamounts and canyons, but its distribution is usually at greater depths, from 350 m down to more than 1000 m (Beuck et al., 2016; Taviani et al., 2017). The large limid *Acesta excavata* contributes to hard bottom communities in the Gulf of Naples associated with *N. zibrowii* and the stony corals *M. oculata*, *Desmophyllum pertusum*, *D. dianthus*, and *Javania cailleti* (Taviani et al., 2016b, 2019).

65. There are also other species of molluscs, such as Spondylus gussoni and Asperarca nodulosa, which can occur in large numbers, sometimes co-occurring with deep-sea corals (Foubert et al., 2008; Rosso et al., 2010; Taviani et al., 2017). Their facies may be dominant in some seabeds or be part of other deep-sea dwelling communities, on the rocky bottoms of escarpments and canyons, together with brachiopods or other bivalves.

- *Other habitats*

66. Brachiopods such as *Megerlia truncata*, *Terebratulina retusa*, *Argyrotheca* spp., *Megathyris detruncata*, *Novocrania anomala*, form part of many marine habitats and microhabitats on rocky bottoms, including underwater canyons and stony coral bathyal habitats (Madurell et al., 2012; Angeletti et al., 2015a; Taviani et al., 2017). However, there is another species that forms important facies in soft bottoms, with a wide bathymetric range, although the higher concentrations are usually found in detritic areas on the edge of the shelf and the beginning of the continental slope, which is *Gryphus vitreus* (EC, 2006; Madurell et al., 2012; Aguilar et al., 2014).

67. In other cases, the dominant species are the Ascidiacea such as *Diazona violacea* and *Dicopia antirrhinum* (UNEP/MAP-RAC/SPA, 2013; Mechò et al., 2014) and/or different species of solitary ascidians belonging to the families Molgulidae, Ascidiidae, Pyuridae, and Styelidae (Templado et al., 2012). These aggregations may occur on seamounts or in slope areas, on detritic muddy bottoms (Pérès and Picard, 1964) or rocky bottoms heavily covered by sediments.

68. Worthy of note within the non-sessile species are the communities formed by echinoderms that play a key role in the structuring of soft and hard bottoms. The habitats formed by large aggregations of crinoids (*Leptometra* spp.) are recognised as sensitive because of the abundance of associated species and their importance for some commercial species (Colloca et al., 2004). However, *Leptometra phalangium* is not exclusively restricted to soft bottoms, but can also occur in equal numbers on rocky bottoms (Marín et al., 2011a, b) or even on coral reefs (Pardo et al., 2011; R. Aguilar, pers. obs.). It is also important to note the occurrence of this type of aggregation on soft bottoms involving urchins, such as *Gracilechinus acutus* and *Cidaris cidaris* (Templado et al., 2012; Mastrototaro et al., 2017; R. Aguilar, pers. obs.), holothurians such as *Mesothuria intestinalis* and *Penilpidia ludwigi* (Pagès et al., 2007; Cartes et al., 2009), ophiuroids such as *Amphiura* spp., and also on some rocky bottoms and reefs, with an abundance of specimens of *Ophiothrix* spp. and *Holothuria forskali* (Templado et al., 2012).

69. Equally important are the Archaean communities and microbial mats (Pachiadaki et al., 2010; Pachiadaki and Kormas, 2013; Giovannelli et al., 2016), together with their associated chemo-symbiotic molluscs (e.g., Lucinidae, Vesicomyidae, Mytilidae, Thyasiriidae) or polychaetes (*Lamellibrachia* sp., *Siboglinum* sp.), and ghost shrimps (*Calliax* sp.), which inhabit areas rich in sulphur and methane (Taviani, 2014). Most sites refer to cold seepage and occur in the eastern Mediterranean basin, at the Napoli mud volcano in the abyssal plain between Crete and North Africa (revised by Olu-Le Roy et al., 2004; Taviani, 2011), or in the Osiris and Isis volcanoes in the fluid seepage area in the Nile deep-sea fan (Dupré et al., 2007; Southward et al., 2011), and the Eratosthenes seamount south of Cyprus (Taviani, 2014), but they are also known in the Gela Basin pockmark field to the south of Sicily (Taviani et al., 2013), and in the Jabuka-Pomo area in the Adriatic (Taviani, 2014). Hydrothermal communities are rarer and documented on submarine volcanic apparatuses in the Tyrrhenian and Aegean Seas (Taviani, 2014). These chemo-synthetic communities usually occur at great depths, down to more than 2000 m.

- *Thanatocoenoses*

70. The fossil or subfossil remains of many marine species generate thanatocoenoses (assemblages of dead organisms or fossils), which provide habitats of great importance in dark habitats. These can have very diverse origins but continue to constitute biogenic structures that act as reefs or three-dimensional formations, and which also provide substrate for the settlement of multiple species. Among these formations are the thanatocoenoses dominated by ancient remains and reefs of coral, molluscs, brachiopods, polychaetes and sponges. These bottoms are found on seamounts, bathyal plateaus, escarpments, and in canyons. They include the compacted seabeds of old aggregations of *Gryphus vitreus* (R. Aguilar, pers. obs.), reefs and rubble of *Madrepora oculata*, *Desmophyllum pertusum*, *D. dianthus*, *Dendrophyllia cornigera*, oysters (*Neopycnodonte zibrowii*) (Županović, 1969; Taviani and Colantoni, 1979; Zibrowius and Taviani, 2005; Taviani et al., 2005b; Rosso et al., 2010; Bo et al., 2014c; Fourt et al., 2014b), beds of *Modiolus modiolus* shells (Aguilar et al., 2013; Gofas et al., 2014), subfossil reefs of polychaetes such as *Spirobranchus triqueter* (Domínguez-Carrió et al., 2014), fossilised structures of old sponge aggregations such as *Leiodermatium* sp. (R. Aguilar, pers. obs.), concentrations of hexactinellid spicules, bryozoan remains (Di Geronimo et al., 2001), and even accumulations of algae and plants such as rhizomes and leaves of *Posidonia oceanica* transported from superficial areas to deep-sea bottoms.

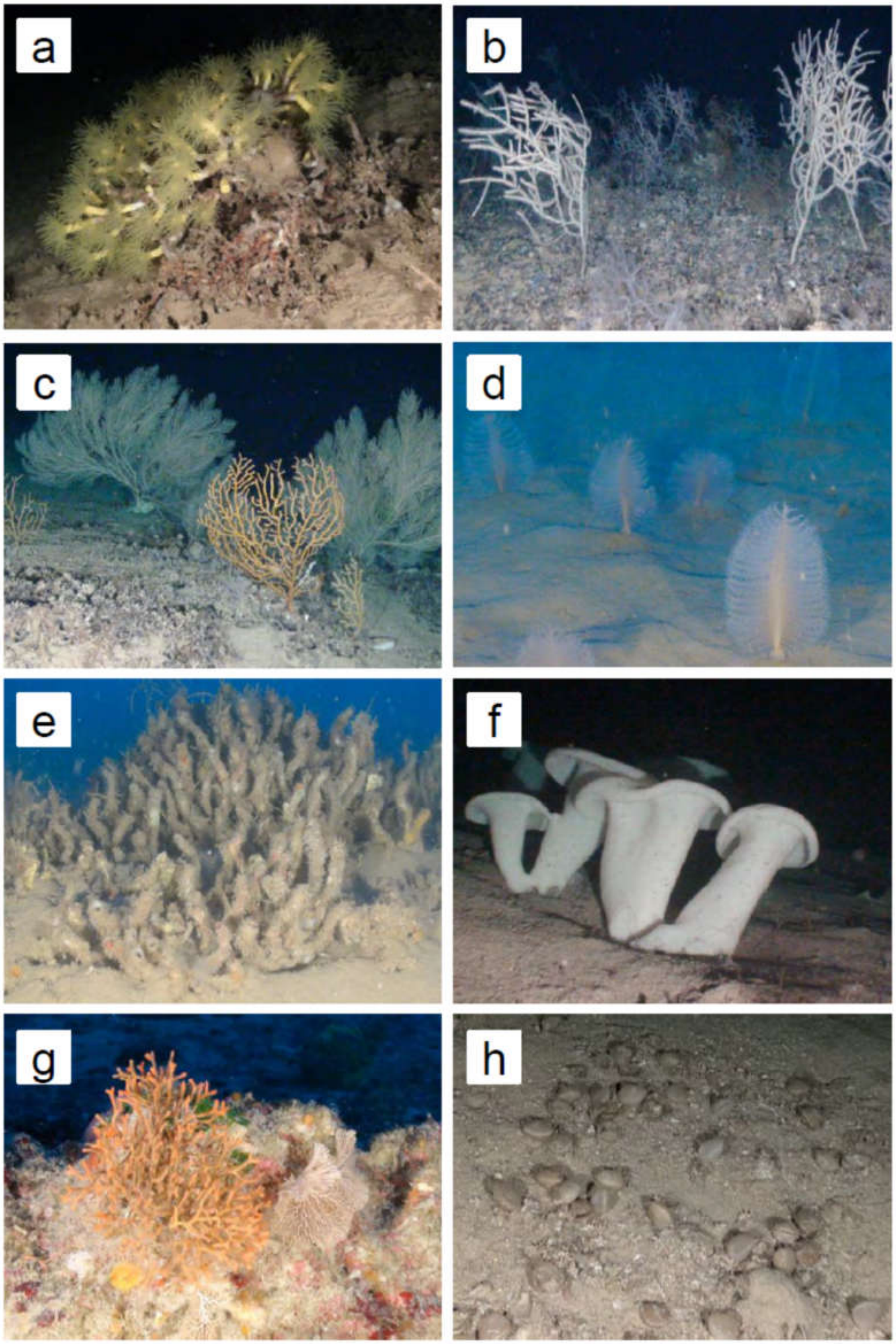

Figure 2: Characteristic species of deep-sea habitats. *Dendrophyllia cornigera*, Catifas Bank (a); *Antipathes dichotoma* and *Leiopathes glaberrima*, Malta (b); *Callogorgia verticillata* and *Placogorgia* sp., Ses Olives Seamount (c); *Pennatula rubra*, Lebanon (d); reef of vermetids, Lebanon (e);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

Monitoring methods

a) COMMON INDICATOR 1: Habitat distributional range and extent

Approach

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

72. Three main steps can be identified for mapping dark habitats:

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

Resolution

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

<u>Marine caves</u>

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

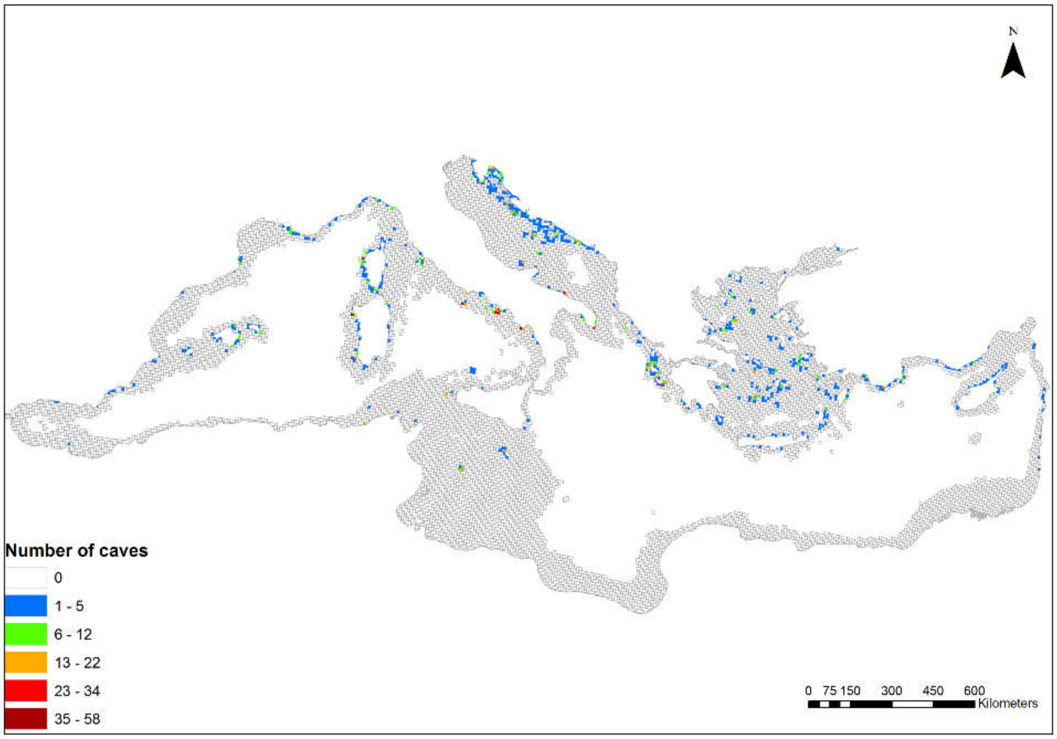

Figure 3: Distribution of marine caves in the Mediterranean Sea; different colours represent the number of caves in 10 km × 10 km cells (from Giakoumi et al., 2013).

<u>Deep-sea habitats</u>

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

76. In the Mediterranean, 518 large canyons have been identified (Harris and Whiteway, 2011) (Fig. 5), along with around 242 underwater mountains or seamount-like structures (Würtz and Rovere, 2015) (Fig. 6) and there are some twenty sites where deep-water chemo-synthetic assemblages have been confirmed (Taviani, 2014) (Fig. 7). However, there are still many other canyons, underwater structures and sites involving the release of gas that have not yet been studied, which is certain to change these figures. Also, 80% of the Mediterranean seabeds are at a depth of more than 200 m and could therefore potentially be home to dark habitats.

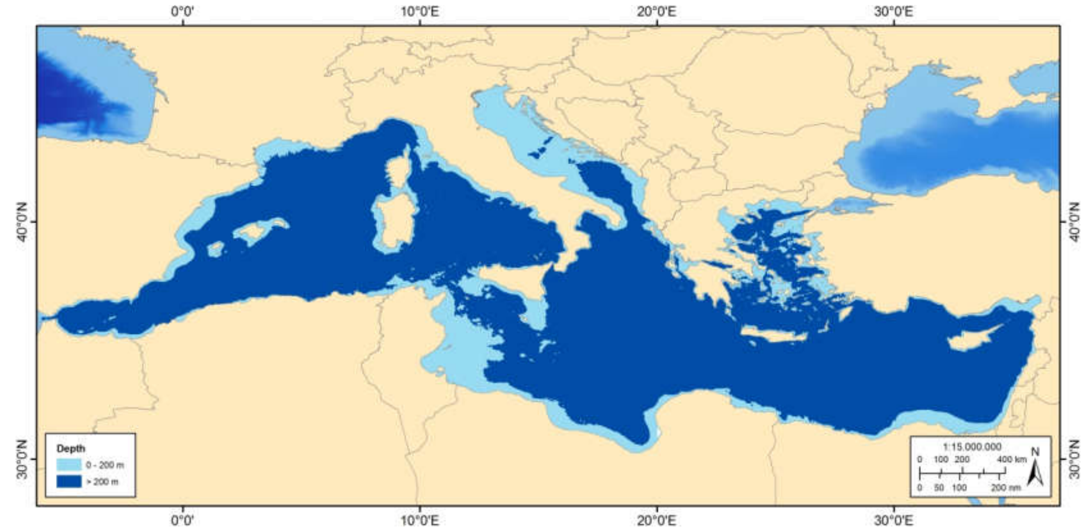

Figure 4: Deep-sea areas in the Mediterranean Sea below 200 m depth (from UNEP/MAP-SPA/RAC, 2017).

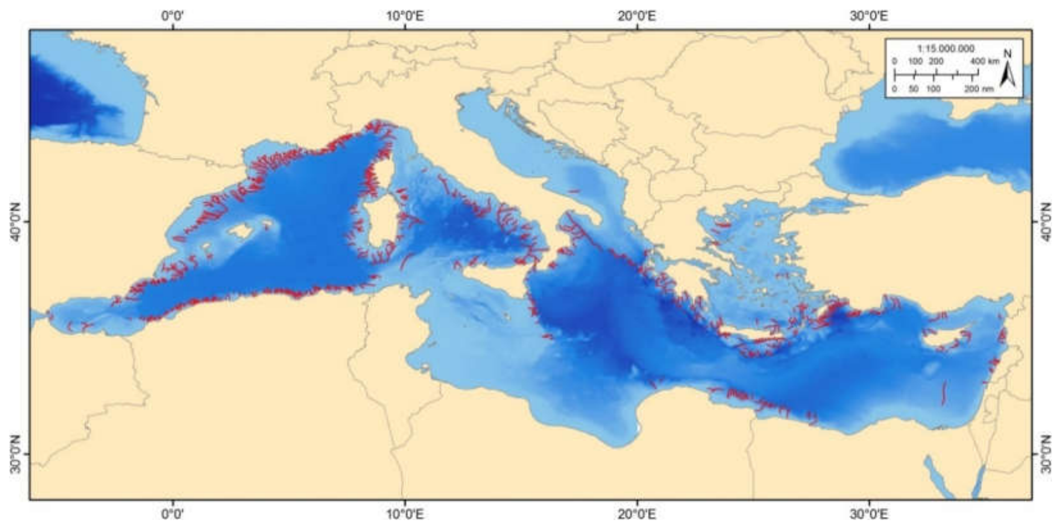

Figure 5: Distribution of Mediterranean submarine canyons (from UNEP/MAP-SPA/RAC, 2017).

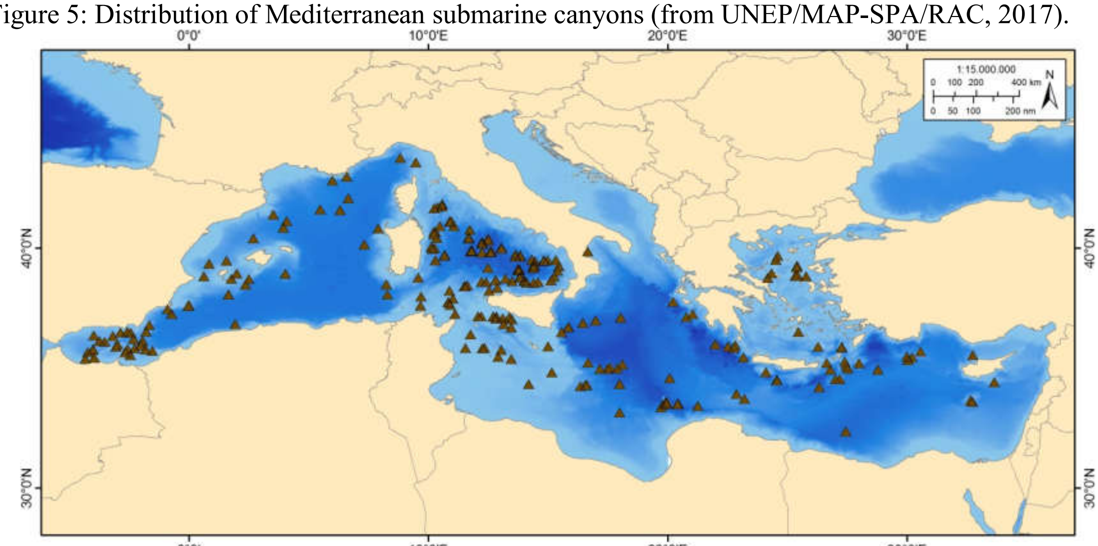

Figure 6: Distribution of Mediterranean seamounts (from UNEP/MAP-SPA/RAC, 2017).

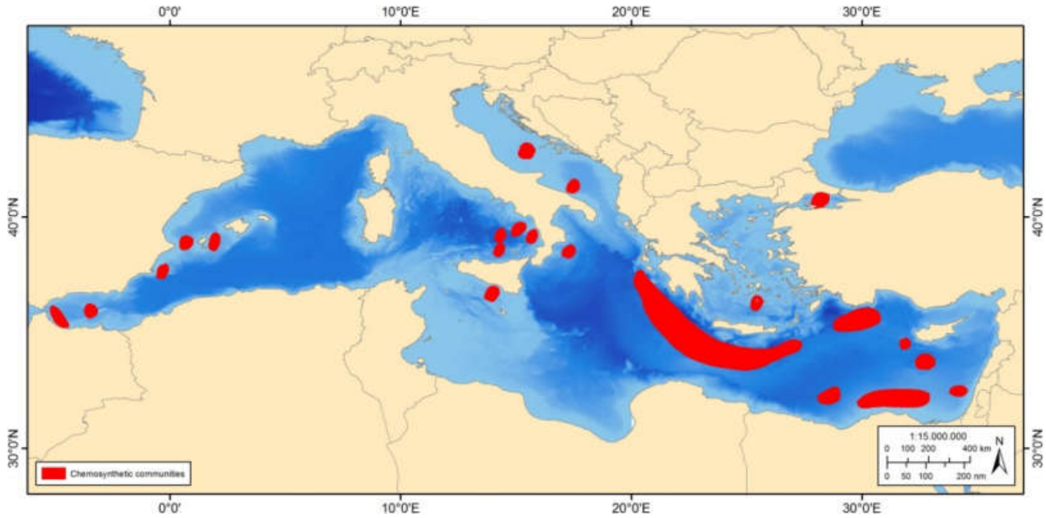

Figure 7: Identified areas with chemo-synthetic assemblages (from UNEP/MAP-SPA/RAC, 2017).

Methods

<u>Marine caves</u>

77. Inventorying of marine cave communities requires two steps:

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

Underwater diving

78. For marine caves up to 40 m depths (and according to local rules for scientific diving) diving is necessary for the exploration, mapping and inventorying, except for shallow caves of the semi- submerged type, which can be often spotted and accessed at the sea surface level. To a certain level, basic information on the location, depth and morphology of marine caves could be derived from local diving and fishing communities, prior to any cave mapping initiative. Diving in marine caves, even in the shallower ones, is logistically challenging and requires the adoption of appropriate safety measures under the precautionary approach, even for experienced divers. The cave bottom is often covered by silty sediment, which could easily be stirred up by divers reducing visibility and making it difficult – or impossible – to locate the cave entrance. Therefore, a dive reel with calibrated line (e.g., distance markers every 1 m) is necessary along with standard scuba equipment (e.g., dive computer, lights, magnetic compass, slate) (Barbieri, 2014). Additional equipment is needed for taking distance measurements (e.g., tape measure, portable echosounder, compass and waterproof range finder for semi-submerged caves).

79. Topography plays a crucial role in structuring marine cave communities and, thus, recording of basic topographic features is important for cave inventories, as well as for the design of appropriate sampling schemes and monitoring protocols. Good knowledge of the cave's topography prior to underwater fieldwork is important for safety reasons (Rastorgueff et al., 2015). The most striking topographic features to be considered during marine cave inventorying are: i) depth; ii) orientation and dimensions of the cave entrance(s); iii) cave morphology (e.g., blind cave or tunnel); iv) submersion level (e.g., semi-submerged or submerged cave); v) maximum and minimum water depth inside the cave; and vi) total length of the cave. Definitions for these topographic attributes are available in the World Register of marine Cave Species (WoRCS) thematic species database of the World Register of Marine Species (Gerovasileiou et al., 2016a). Unique abiotic and biotic features, such as micro-habitats that could support distinct communities and rare species (e.g., sulphur springs, freshwater springs, bioconstructions, etc.) should be also recorded. A useful protocol for inventorying semi-submerged caves has been provided by Dendrinos et al. (2007); however, in areas supporting the Mediterranean monk seal (*Monachus monachus*) populations, such initiatives should

be undertaken during periods with low in-cave seal activity (e.g., late spring or early summer) to minimize potential disturbance.

80. Most of the Mediterranean marine caves studied are semi-submerged or shallow and very few exceed the maximum depth of 30 m, probably due to the logistic constraints in underwater work. The inventorying of deeper and complex cave formations requires highly specialized skills and diving equipment (e.g., Close Circuit Underwater Breathing Apparatus – CCUBA), inducing a greater extent of risks than conventional scuba diving. The exploration of deep-sea caves and overhangs requires the use of ROVs, even though several limitations linked with the possibility to penetrate into these confined habitats (Fairfield et al., 2007; Stipanov et al., 2008).

Deep-sea habitats

Acoustic and video surveys

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

82. Definition of distributional range and extent of deep-sea habitats requires "traditional" habitat mapping techniques, similar to those used for deep coralligenous reefs (Tab. 1). Being the deep-sea habitats distributed in deep waters (down to 120 m depth), the use of bathyscaphes, submarines, landers, etc., provide visual and georeferenced information on the geological formations and benthic communities on these seabeds. Acoustic techniques (e.g., side scan sonar, multi-beam echosounder) or underwater video recordings (ROV) are usually recommended. Sonar provides topobathymetric images of the seafloor through the emission and reception of ultrasounds; it creates a three-dimensional map that allows the identification of potential sites with deep habitats, especially reefs and aggregations of corals and sponges. The use of remote sensing allows characterising extensive areas for the assessment of the overall spatial patterns of deep-sea habitats. From maps obtained through remote sensing surveys, the presence/absence of the habitat, its distributional range and the total habitat extent can be easily obtained. Acoustic methods are presently the most convenient technique for mapping deep-sea habitats, associated with ground-truthing by ROV and, sometimes, box-coring. The simultaneous use of two or more methods makes it possible to optimize the results being the information obtained complementary. The strategy to be adopted will thus depend on the aim of the study and the area concerned, means and time available. Multi-beam sonar, side scan sonar, and sub-bottom profilers like TOPAS (Topographic parametric sonar) provide an important overview of the seabed, making it possible to identify and locate the presence of specific geomorphologic features such as seamounts, canyons, mud volcanoes, pockmarks, carbonated mounds, reefs, etc.

83. For all remote sensing techniques, distinguishing habitats from each other and from the surrounding seabed depends on the resolution of the sampling method, higher resolution will provide better data to distinguish habitats, but covers smaller areas and is more expensive to collect and process than lower resolution data. All the acoustic mapping techniques are intrinsically affected by uncertainties due to manual classification of the different acoustic signatures of substrate types on sonograms. Errors in sonograms interpretation may arise when two substrate types are not easily distinguished by the observer. Interpretation of remote sensing data requires extensive field calibration and the ground-truthing process remains essential. As the interpretation of sonograms is also 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). These methods allow a good discrimination between soft sediments and rocky reefs. Human eye, however, always remains the final judge.

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

Sampling methods

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

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

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

Survey tool	Depth range	Surface area	Resolution	Efficiency	Advantages	Limits	References
Multi-beam echosounder	Down to 4000 m	From small (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 Realistic representation of the seafloor Quick execution Non-destructive Very big mass of data	Less precise imaging (nature of the bottom) than side scan sonar Acquisition of field data necessary to validate sonograms High cost of instruments and research vessels High resolution maps not usually available	Palmiotto and Loreto (2019)
Remote Operating Vehicle (ROV), bathyscaphes, or submarines	Down to 4000 m	Small-intermediate areas (a few 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 Difficult to handle at great depths High cost of instruments and research vessels	Enrichetti et al. (2019); Rogers (2019)

Data interpretation

86. Once the field survey 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. Acoustic data must be always integrated by a great number of samplings or video recordings by ROVs for ground-truthing, especially given the wide distribution and complexity of deep-sea habitats.

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

a. Processing, analysis, interpretation and classification of field biological data, to be integrated with acoustic data 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.

88. During the processing analysis and classification step, the updated list of benthic marine habitat types for the Mediterranean region[12] should be consulted (UNEP/MAP-SPA/RAC, 2019) to recognize any specific dark habitat type (e.g., marine cave, circalittoral rock, bathyal sand) and its main characteristic associations and facies. A complete description of these habitats and the criteria for their identification are also available in Bellan-Santini et al. (2002). Dark habitats that must be reported on maps are the following (UNEP/MAP-SPA/RAC, 2019):

LITTORAL

MA1.5 Littoral rock

MA1.52 Mediolittoral caves

MA1.521 Association with encrusting Corallinales or other Rodophyta

INFRALITTORAL

MB1.5 Infralittoral rock

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

CIRCALITTORAL

MC1.5 Circalittoral rock

MC1.53 Semi-dark caves and overhangs

MC1.53a Walls and tunnels

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

MC1.532a Facies with Hydrozoa

[12] 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.

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

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

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

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

MC1.537a Facies with Ascidiacea

MC1.53b Ceilings

See MC1.53a for examples of facies

MC1.53c Detritic bottom

See MC3.51 for examples of associations and facies

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

MC1.531d Facies with Heteroscleromorpha sponges

OFFSHORE CIRCALITTORAL

MD1.5 Offshore circalittoral rock

MD1.51 Offshore circalittoral rock invertebrate-dominated

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

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

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

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

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

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

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

MD1.518 Facies with Polychaeta

MD1.519 Facies with Bivalvia

MD1.51A Facies with Brachiopoda

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

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

See MD1.51 for examples of facies

MD1.53 Deep offshore circalittoral banks

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

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

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

MD2.5 Offshore circalittoral biogenic habitat

MD2.51 Offshore reefs

MD2.511 Facies with Vermetidae and/or Serpulidae

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

See MD1.51 for examples of facies

MD3.5 Offshore circalittoral coarse sediment

MD3.51 Offshore circalittoral detritic bottoms

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

ME2.512 Facies with Brachiopoda

MD3.513 Facies with Polychaeta

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

MD3.515 Facies with Ophiuroidea

MD3.516 Facies with Echinoidea

MD4.5 Offshore circalittoral mixed sediment

MD4.51 Offshore circalittoral detritic bottoms

See MD3.51 for examples of facies

MD5.5 Offshore circalittoral sand

MD5.51 Offshore circalittoral sand

See MD3.51 for examples of facies

MD6.5 Offshore circalittoral mud

MD6.51 Offshore terrigenous sticky muds

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

MD6.512 Facies with Polychaeta

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

MD6.514 Facies with Brachiopoda

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

UPPER BATHYAL

ME1.5 Upper bathyal rock

ME1.51 Upper bathyal rock invertebrate-dominated

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

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

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

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

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

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

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

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

ME1.519 Facies with Brachiopoda

ME1.52 Caves and ducts in total darkness

ME2.5Upper bathyal biogenic habitat

ME2.51 Upper bathyal reefs

ME2.511 Facies with small sponges (sponge ground)

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

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

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

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

ME2.516 Facies with Brachiopoda

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

See ME1.51 for examples of facies

ME3.5 Upper bathyal coarse sediment

ME3.51 Upper bathyal coarse sediment

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

ME4.5 Upper bathyal mixed sediment

ME4.51 Upper bathyal mixed sediment

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

ME4.512 Facies with Brachiopoda

ME5.5 Upper bathyal sand

ME5.51Upper bathyal detritic sand

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

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

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

ME5.514 Facies with Echinoidea

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

ME5.516 Facies with Brachiopoda

ME5.517 Facies with Bryozoa

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

ME6.5 Upper bathyal muds

ME6.51 Upper bathyal muds

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

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

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

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

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

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

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

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

ME6.519 Facies with Brachiopoda

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

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

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

LOWER BATHYAL

MF1.5 Lower bathyal rock

MF1.51 Lower bathyal rock

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

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

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

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

MF2.5 Lower bathyal biogenic habitat

MF2.51 Lower bathyal reefs

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

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

See MF1.51 for examples of facies

MF6.5 Lower bathyal muds

MF6.51 Sandy muds

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

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

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

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

MF6.515 Facies with bioturbations

ABYSSAL

MG1.5 Abyssal rock

MG1.51 Abyssal rock

MG1.511 Facies with small sponges

MG1.512 Facies with Alcyonacea

MG1.513 Facies with Polychaeta

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

MG6.5 Abyssal muds

MG6.51 Abyssal muds

MG6.511 Facies with small sponges

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

MG6.513 Facies with Polychaeta

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

MG6.515 Facies with bioturbations

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

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

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

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

visualization of entirely and semi-submerged marine caves with a simple, non-dendritic morphology, has been developed and described by Gerovasileiou et al. (2013), using handheld echosounder. The method can be applied by two divers in 1-2 dives and enables the automatic production of 3D depictions of cave morphology using the accompanying "cavetopo" software. A GPS device is necessary for geo-referencing the location of the access point to the surveyed marine cave at the sea surface level. Recently, in the framework of the Grotte-3D Project, three submerged caves in Parc National des Calanques (France) were depicted in high-resolution 3D models using photogrammetry (Chemisky et al., 2015).

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

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

Approach

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

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

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

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, where clear scientific competences are needed because acquired data must be interpreted. Duration of the monitoring, in order to be useful, must be mediumtime at least.

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

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

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

100. 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).

101. The lack or scarcity of quantitative data and long-time-series from marine caves and deep-sea habitats in most of the Mediterranean areas is a major impediment to evaluate changes in their ecological status. There is evidence of alterations through time in caves of the north-western Mediterranean Sea, suggesting that there might be an unregarded decrease in quality at a broader scale (Parravicini et al., 2010; Rastorgueff et al., 2015; Gubbay et al., 2016; Nepote et al., 2017; Montefalcone et al., 2018). The most important pressures affecting marine cave communities are: mechanical damage of fragile species caused by unregulated diving activities, physical damage and siltation due to coastal and marine infrastructure activities, marine pollution (e.g., sewage plant outflow, marine litter), extractive human activities (e.g., red coral harvesting), water temperature rise, and potentially non-indigenous species (Chevaldonné and Lejeusne, 2003; Guarnieri et al., 2012; Giakoumi et al., 2013; Gerovasileiou et al., 2016b). Main threats to deep-sea habitats include climate change-related pressures (e.g., ocean warming, changes in primary production, hypoxia, and ocean acidification) and deep-water fishing, including bottom trawling (Rogers, 2019). Increased temperatures can lower oxygen thresholds and reduce the tolerance of species to acidification, while, in turn, hypoxia and acidification can reduce thermal tolerance. Physical disturbances caused by

bottom trawling, deep-sea mining, and oil and gas extraction can increase physiological stress due to climate change factors.

Methods

Monitoring marine cave communities

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

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

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

105. A list of the main conspicuous species/taxa or morphological groups recognisable underwater, or on images, is then produced. A list of species that are frequently reported in Mediterranean marine caves is presented in Appendix 1. This species list is not exhaustive but includes species reported from a considerable number of semi-dark and dark caves at the Mediterranean scale according to data from the Mediterranean marine cave biodiversity database (Gerovasileiou and Voultsiadou, 2012, 2014). Most of the present knowledge concerns the biota associated with the rocky walls and vaults of caves, while less information is available about the infauna in cave floor sediments (Bianchi and Morri, 2003). Marine caves are characterised by a high

degree of natural heterogeneity and their communities present qualitative and quantitative differences in species composition across different Mediterranean eco-regions (Gerovasileiou and Voultsiadou, 2012). For instance, species that have been traditionally considered cave characteristic in the western basin (e.g., *Corallium rubrum*) may be rare or even absent in the eastern basin and vice versa. Thus, the list is annotated with comments on the distribution of certain taxa. Advanced image processing softwares dedicated to marine biological research integrate methods and tools for the following accurate extraction of species coverage (%) or abundance (cm^2) from photo-quadrates (e.g., Teixidó et al., 2011; Trygonis and Sini, 2012). Monitoring of marine cave communities and sessile invertebrates with slow growth rates could be also benefited from methods quantifying 3D features, using photogrammetry (e.g., Chemisky et al., 2015).

106. Visual census methods can be also applied for studying the structure of mobile cave fauna; specifically, a modified transect visual census method (Harmelin-Vivien et al., 1985) adapted to cave habitats has been developed and applied in several Mediterranean caves for the study of fish assemblages (Bussotti et al., 2002, 2006; Bussotti and Guidetti, 2009), as well as for decapods crustaceans (Denitto et al., 2009). The number of species and individuals observed at 5 minutes interval must be recorded on the slate.

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

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

109. An ecosystem-based index (CavEBQI) for the evaluation of the ecological quality of marine cave ecosystems has been recently developed and tested in the western Mediterranean basin (Rastorgueff et al., 2015). According to this approach, the following features could be indicative of high quality status: high spatial coverage of suspension feeders with a three-dimensional form (e.g., *Corallium rubrum*) and large filter feeders (e.g., the sponges *Petrosia ficiformis* and *Agelas oroides*) along with the presence of mysid swarms and several species of omnivorous and carnivorous fish and decapods. In the framework of a recent evaluation of ecological quality status in 21 western Mediterranean caves using the CavEBQI index, 14 caves were found in favourable status (good/high ecological quality) and no cave was found to be of bad ecological quality (Rastorgueff et al., 2015). However, a comparison of data obtained in 1986 and 2004 from the Bergeggi cave (Ligurian Sea, Italy) revealed a decrease in ecological quality attributed to summer heat waves (Parravicini et al., 2010; Rastorgueff et al., 2015; Montefalcone et al., 2018). Piccola del Ciolo cave, which is one of the most studied Mediterranean marine caves, was evaluated to be of high ecological quality using CavEBQI index (Rastorgueff et al., 2015).

110. A fill-in form that could be used as a basis for recording (a) basic topographic features, (b) characteristic species from different functional components of the ecosystem-based approach by Rastorgueff et al. (2015), (c) protected species, and (d) pressures and threats is shown in Figure 8.

Figure 8: Modified example of fill-in sheet developed in the context of monitoring studies by V. Gerovasileiou (HCMR). The form was based on the approach for the evaluation of the ecological quality of marine cave habitats developed by Rastorgueff et al. (2015). In addition to the species data included in the form, photo-quadrates covering a total surface of about 1-4 m^2 should be acquired for the study of sessile communities.

Area:	**Date:**		**Observer:**	
Latitude:	**Longitude:**			
Submersion level: Submerged / Semi-submerged	**Cave morphology:** Blind cave / Tunnel No. of entrances:			
Total length of cave:	**Maximum water depth:**		**Minimum water depth:**	
Entrance A – Max depth (m):	Height (m):	Width (m):	Orientation:	
Entrance B – Max depth (m):	Height (m):	Width (m):	Orientation:	
Other topographic features: Internal beach / Air pockets / Speleothems /				
Micro-habitats:				
Detritivorous / omnivorous species (number of species and individuals observed at 5 min interval)				
Herbstia condyliata	1–2	3–4	5–10	>10
Galathea strigosa	1–2	3–4	5–10	>10
Scyllarus arctus	1–2	3–4	5–10	>10
	1–2	3–4	5–10	>10
	1–2	3–4	5–10	>10
	1–2	3–4	5–10	>10
	1–2	3–4	5–10	>10
	1–2	3–4	5–10	>10
Mysids	0	few		swarm
Fish species observed/ cave zone (CE: entrance, SD: semi-dark zone, DZ: dark zone)	**Decapods species observed / cave zone** (CE: entrance, SD: semi-dark zone, DZ: dark zone)			
/	/			
/	/			
/	/			
/	/			
/	/			
/	/			
/	/			
/	/			
Cerianthus membranaceus **(number of individuals)**	0	1-2		>2
Arachnanthus oligopodus **(number of individuals)**	0	1-2		>2
Other typical and/or protected species	**Threats and pressures**			
	Broken bryozoans			
	Air bubbles			
	Marine litter			
	Non-indigenous species			
	Other comments			

Monitoring deep-sea habitats

111. Following the preliminary definition of the distributional range and extent of deep-sea habitats (the previous CI1), assessment of the condition of these habitats starts with an overall

characterisation of the typical species and communities occurring within each habitat. Methodologies to monitor the condition of deep-sea dark habitats include a wide array of technologies and equipment (see Tab. 1). Selection of the methods for monitoring depends on the habitat type (and selected target species) to be addressed. Large sessile epibenthic species on hard substrates are preferably monitored using optical, non-destructive methods, such as ROVs. Living specimens can be collected by ROV arm. Endobenthic communities are sampled using standardized grabs or corers. The use of ROVs, bathyscaphes, or submarines provide visual and georeferenced information on the benthic communities on these habitats. Data about the presence of species, distribution patterns, estimates of densities, biological associations, etc., can be obtained. In the case of the ROVs and submarines, these allow the completion of video transects and the selective collection of samples, which greatly facilitates the identification of key species in the habitat formation, as well as the species associated with them. High quality photographs and video recorded will then be analysed in laboratory (also with the help of taxonomists) to list the main conspicuous species/taxa or morphological groups recognisable on images and to evaluate their abundance (coverage or surface area in cm^2). Photographs can be archived to create temporal datasets. A selection of target species should be defined per sub-region (or bioregion) to allow for the consistent assessment of their state/condition. Long-lived species and species with high structuring or functional value for the community should preferably be included; however, the list should also contain small and short-lived species if they characteristically occur in the habitat under natural conditions, as they can also be functionally very important for the community. This list should be updated every six years.

112. Although destructive methods are not desirable for long-term regular monitoring (UNEP/MAP-RAC/SPA, 2008), they become indispensable for a high-resolution characterisation of deep-sea communities on soft bottoms. A variety of sampling gears has been used to collect sediment samples from vessels to identify the type of substrate, the granulometry, the organic matter content, and for the study of deep-sea organisms (Danovaro et al., 2010). Common devices are grabs, gravity cores, piston cores, box cores, and multiple corers, used in a number of randomly selected points within a study area. The use of grabs allows more extensive sampling in large areas, also providing information on species of infauna and on small organisms that it is not possible to detect/identify with other methods. Sometimes benthic trawling has been recommended as appropriate for sampling benthic habitats; however, despite they can provide useful data, these methods are forbidden for assessment of highly sensitive habitats to the impact of physical damage such as rocky reefs, and must be avoided on soft bottom communities dominated by long-lived species (e.g., large sponges, gorgonians, bamboo corals).

113. Deep-sea macrofauna has been sampled in the western Mediterranean by different methods, depending on the depth considered and the research teams (Danovaro et al., 2010 and references therein). Commercial trawls can be used, having horizontal mouth openings of 20-25 m and 3-5 m of vertical opening, with a 40 mm stretched mesh in the codend liner, which are trawled over the seafloor at about 3 knots. The otter semiballoon trawl gear (OTSB: 8 m horizontal spread and 0.8 vertical mouth opening) has been also used in the Mediterranean Sea. This sampling device was subsequently transformed into the otter trawl Maireta System (OTMS: 12 m horizontal spread and 1.4 m vertical opening approximately). The OTMS is equipped with SCANMAR sensors that provide information on bottom contact time and vertical and horizontal opening of the trawl's mouth down to 1500 m depth, allowing calculation of sampled area. Furthermore, the Agassiz benthic trawl has been commonly used to sample the deep western and eastern Mediterranean benthos since the late 1980s. A modified Agassiz trawl (2.3 m wide and 0.9 m high), a 14.76 m Marinovich-type deep-water trawl (codend mesh 6 mm) with a 0.5 mm plankton net secured on top, and different types and sizes of box corers have also been used. A 0.062 m^2 box corer with an effective penetration of 40 cm (Ocean Instruments model 700 AL) has been used in the Levantine Sea. The samples are typically preserved in 10% buffered formalin aboard the vessel. In the laboratory, samples are washed and sieved through 250 µm mesh (Danovaro et al., 2010).

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

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

Protocol for monitoring deep rocky reefs habitats down to 120 m depth

116. Although no standardized protocols exist to date for monitoring deep-sea habitats, the protocol recently proposed for monitoring mesophotic coralligenous reefs (down to 40 m depth) (Enrichetti et al., 2019) can be applied and adapted for monitoring deep-sea rocky habitats in the offshore circalittoral and the bathyal zones. The proposed protocol (all details can be found in Cánovas-Molina et al., 2016; Enrichetti et al., 2019) suggests a standard sampling design conceived to gather various quantitative components, such as the occurrence and extent of the rocky habitat, the siltation level, and the abundance, condition and population structure of habitat-forming megabenthic species (i.e., animal forests), as well as presence and typology of marine litter, through ROVs surveys.

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

118. From each transect the following parameters are measured from videos:

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

- The percentage of colonies with signs of epibiosis, necrosis and directly entangled in lost fishing gears are calculated individually for all structuring anthozoans
- Marine litter is identified and counted. The final density (as n° of items m^{-2}) is computed considering the entire transect (100 m^2).

119. 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)
- Sedimentationlevel, estimated considering the percentage of hard bottom covered by sediments: 0%, <30% (low), 30-60% (medium), >60% (high).

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

Final remarks

121. Inventorying and monitoring dark habitats in the Mediterranean constitute a unique challenge given the ecological importance of their communities and the threats that hang over their continued existence. Long neglected due to their remote location and the limited means to investigate these areas, today these habitats must be the subject of priority programs. There is a huge necessity to improve knowledge of dark habitats and their distribution in the Mediterranean Sea, in order to establish international cooperation networks and also to facilitate sharing of experiences among Mediterranean countries. The existing scientific information on the distribution, biodiversity, functioning and connectivity of dark habitats on seamounts, in canyons, caves and escarpments must be continuously improved. Nevertheless, there are still obvious gaps of knowledge with regard to the distribution and diversity of dark habitats from the eastern and the southern parts of the Mediterranean Sea. The available scientific databases must be updated and integrated setting up collaborative tools and/or platforms to help scientists in exchanging data and experience. The assessment of associated ecosystem services should be also undertaken. Common monitoring protocols have to be defined, shared, and applied at the Mediterranean scale. The process of designation of new protected areas, aiming at the conservation of deep-sea habitats, must be enforced, as well as the existing regulatory measures, particularly to avoid the impact of destructive fishing practices over identified deep-sea sensitive habitats, vulnerable marine ecosystems or essential fish habitats (spawning and nursery grounds).

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

Foraminiferans
Miniacina miniacea (Pallas, 1766)

Sponges
Aaptos aaptos (Schmidt, 1864)
Acanthella acuta Schmidt, 1862
Agelas oroides (Schmidt, 1864) – more abundant in the Eastern Mediterranean
Aplysilla rosea (Barrois, 1876)
Aplysina cavernicola (Vacelet, 1959)
Axinella damicornis (Esper, 1794)
Axinella verrucosa (Esper, 1794)
Chondrosia reniformis Nardo, 1847 – often discoloured
Clathrina coriacea (Montagu, 1814)
Clathrina clathrus (Schmidt, 1864)
Cliona viridis (Schmidt, 1862)
Cliona schmidti (Ridley, 1881)
Cliona celata Grant, 1826
Crambe crambe (Schmidt, 1862)
Dendroxea lenis (Topsent, 1892)
Diplastrella bistellata (Schmidt, 1862)
Dysidea avara (Schmidt, 1862)
Dysidea fragilis (Montagu, 1814)
Erylus discophorus (Schmidt, 1862)
Fasciospongia cavernosa (Schmidt, 1862)
Geodia cydonium (Linnaeus, 1767)
Haliclona (Halichoclona) fulva (Topsent, 1893)
Haliclona (Reniera) cratera (Schmidt, 1862)
Haliclona (Rhizoniera) sarai (Pulitzer-Finali, 1969)
Haliclona (Soestella) mucosa (Griessinger, 1971)
Hemimycale columella (Bowerbank, 1874)
Ircinia dendroides (Schmidt, 1862)
Ircinia oros (Schmidt, 1864)
Ircinia variabilis (Schmidt, 1862)
Jaspis johnstoni (Schmidt, 1862)
Lycopodina hypogea (Vacelet & Boury-Esnault, 1996)
Myrmekioderma spelaeum (Pulitzer-Finali, 1983)
Oscarella spp.
Penares euastrum (Schmidt, 1868)
Penares helleri (Schmidt, 1864)
Petrobiona massiliana Vacelet & Lévi, 1958 – more common in the Western Mediterranean
Petrosia (Petrosia) ficiformis (Poiret, 1789) – often discoloured
Phorbas tenacior (Topsent, 1925)
Plakina spp.
Pleraplysilla spinifera (Schulze, 1879)
Scalarispongia scalaris (Schmidt, 1862)
Spirastrella cunctatrix Schmidt, 1868
Spongia (Spongia) officinalis Linnaeus, 1759 *
Spongia (Spongia) virgultosa (Schmidt, 1868)
Terpios gelatinosus (Bowerbank, 1866)

Cnidarians
Arachnanthus oligopodus (Cerfontaine, 1891)
Astroides calycularis (Pallas, 1766) * – in southern areas of the Western Mediterranean
Caryophyllia (Caryophyllia) inornata (Duncan, 1878)
Cerianthus membranaceus (Gmelin, 1791)
Corallium rubrum (Linnaeus, 1758) *
Eudendrium racemosum (Cavolini, 1785)
Eunicella cavolini (Koch, 1887) – more common in the Western Mediterranean
Halecium spp.
Hoplangia durotrix Gosse 1860
Leptopsammia pruvoti Lacaze-Duthiers 1897
Madracis pharensis (Heller, 1868) – more abundant in the Eastern Mediterranean
Obelia dichotoma (Linnaeus, 1758)
Paramuricea clavate (Risso, 1826) * – more common in the Western Mediterranean
Parazoanthus axinellae (Schmidt, 1862) – more common in the Adriatic and the Western Mediterranean
Phyllangia americana mouchezii (Lacaze-Duthiers, 1897)
Polycyathus muellerae (Abel, 1959)

Decapods
Athanas nitescens (Leach, 1813)
Dromia personata (Linnaeus, 1758)
Eualus occultus (Lebour, 1936)
Galathea strigosa (Linnaeus, 1761)
Herbstia condyliata (Fabricius, 1787)
Lysmata seticaudata (Risso, 1816)
Palaemon serratus (Pennant, 1777)
Palinurus elephas (Fabricius, 1787)
Plesionika narval (Fabricius, 1787) – more common in the Eastern Mediterranean
Scyllarides latus (Latreille, 1803)
Scyllarus arctus (Linnaeus, 1758)
Stenopus spinosus Risso, 1826

Mysids
Harmelinel lamariannae Ledoyer, 1989
Hemimysis lamornae mediterranea Bacescu, 1936
Hemimysis margalefi Alcaraz, Riera & Gili, 1986
Hemimysis speluncola Ledoyer, 1963 *
Siriella jaltensis Czerniavsky, 1868

Polychaetes
Filograna implexa Berkeley, 1835
Filogranula annulata (O. G. Costa, 1861)
Filogranula calyculata (O.G. Costa, 1861)
Filogranula gracilis Langerhans, 1884
Hermodice carunculata (Pallas, 1766)
Hydroides pseudouncinata Zibrowius, 1968 [original]
Janita fimbriata (Delle Chiaje, 1822)
Josephella marenzelleri Caullery & Mesnil, 1896
Metavermilia multicristata (Philippi, 1844)
Protula tubularia (Montagu, 1803)
Semivermilia crenata (O. G. Costa, 1861)
Serpula cavernicola Fassari & Mollica, 1991
Serpula concharum Langerhans, 1880
Serpula lobiancoi Rioja, 1917

Serpula vermicularis Linnaeus, 1767
Spiraserpula massiliensis (Zibrowius, 1968)
Spirobranchus polytrema (Philippi, 1844)
Vermiliopsis labiata (O. G. Costa, 1861)
Vermiliopsis infundibulum (Philippi, 1844)
Vermiliopsis monodiscus Zibrowius, 1968

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

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

Brachiopods
Argyrotheca cistellula (Wood, 1841)
Argyrotheca cuneata (Risso, 1826)
Joania cordata (Risso, 1826)
Megathiris detruncata (Gmelin, 1791)
Novocrania anomala (O.F. Müller, 1776)
Tethyrhynchia mediterranea Logan & Zibrowius, 1994

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

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

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