MONITORING CLIMATE-RELATED RESPONSES IN MEDITERRANEAN MARINE PROTECTED AREAS AND BEYOND:

6 STANDARD PROTOCOLS

MPA ENGAGE

DRAFT VERSION

MAY 2020

Project co-financed by the European Regional Development Fund

IDENTIFICATION

Project Number	5MED18_3.2_M23_007	Acronym	MPA-ENGAGE
Full title	MPA Engage: Engaging Mediterranean key actors in Ecosystem Approach to manage Marine Protected Areas to face Climate change		
Axis	3.2: To maintain biodiversity and natural ecosystems through strengthening the management and networking of protected areas		
Partner Responsible	CSIC		
Contact Person	Joaquim Garrabou – garrabou@icm.csic.es		

Authors	Azzurro E., Bensoussan N., Cerrano C., Cebrian E, Guala I, Grech D, Palma, M. Garrabou J.
Version	DRAFT 20 May 2020
Citation	Azzurro E., Bensoussan N., Cerrano C., Cebrian E, Guala I, Grech D, Palma, M., Garrabou J (2020) Monitoring Climate-related responses in Mediterranean Marine Protected Areas and beyond: 6 standard protocols. Draft version May 2020 – MPA-Engage project.

Project co-financed by the European Regional Development Fund

TABLE 1: Information about the name of each protocol, in which webinar was presented, the location of the document and the person responsible for each protocol.

PROTOCOL	WEBINAR	LOCATION	CONTACT
LEK-1: Exploring Local Ecological Knowledge to construct historical changes	Webinar 1	Document "MPA-ADAPT: Five standard protocols"	**Ernesto Azzurro** IRBIM-CNR Ancona and SZN Naples (Italy) eazzurr@gmail.com
LEK-2: Exploring Local Ecological Knowledge for periodical monitoring	Webinar 1	Document "MPA-ADAPT: Five standard protocols"	**Ernesto Azzurro** IRBIM-CNR Ancona and SZN Naples (Italy) eazzurr@gmail.com
LEK-3: Exploring Local Ecological Knowledge for mass mortalities	Webinar 1	**This document**	**Ernesto Azzurro** IRBIM-CNR Ancona and SZN Naples (Italy) eazzurr@gmail.com
Monitoring temperature conditions	Webinar 2	Document "MPA-ADAPT: Five standard protocols"	**Nathaniel Bensoussan** ICM-CSIC (Spain) nbensoussan@gmail.com
Monitoring of mass mortalities	Webinar 2	Document "MPA-ADAPT: Five standard protocols"	**Joaquim Garrabou** ICM-CSIC (Spain) garrabou@icm.csic.es
Fish visual census of climate change indicators	Webinar 3	Document "MPA-ADAPT: Five standard protocols"	**Ernesto Azzurro** IRBIM-CNR Ancona and SZN Naples (Italy) eazzurr@gmail.com
BARD: Benthic alien species rapid detection	Webinar 3	**This document**	**Emma Cebrian** University of Girona (Spain) emma.cebrian@udg.edu

URCH: Sea urchins populations in Marine Protected Areas	Webinar 3	**This document**	**Joaquim Garrabou** ICM-CSIC (Spain) garrabou@icm.csic.es
POFA: *Posidonia oceanica* fast assessment on meadows conservation status face to climate change	Webinar 4	**This document**	**Ivan Guala** IMC, Oristano (Italy) guala@fondazioneimc.it
FAP: Fast assessment *Pinna nobilis*	Webinar 4	**This document**	**Daniele Grech** IMC, Oristano (Italy) d.grech@fondazioneimc.it **Carlo Cerrano** Polytechnic University of Marche, Ancona (Italy) c.cerrano@univpm.it
SFM: Photogrammetry as monitoring tool for benthic habitats structure and dynamics	Webinar 5	**This document**	**Carlo Cerrano** Polytechnic University of Marche, Ancona (Italy) c.cerrano@univpm.it **Marco Palma** UBICA srl (Italy)

INDEX

1. LEK-3: MASS MORTALITIES (WEBINAR 1)

1.1. RATIONALE AND OBJECTIVES

Mass mortalities of marine organisms are a matter of increasing concern, often linked to/or determined by specific climatic conditions. These events may involve a different number of species and habitats and often occur before the eyes of people such as fishers and divers. The following protocol can be used to access their knowledge, documenting these events.

1.2. TARGET SPECIES

There are basically no target species, since ALL the reported mass mortalities are our focus. Nevertheless, the interviewer should be aware of the most common mass mortalities occurring in the Mediterranean region:

- Groupers (particularly *Epinephelus marginatus*)
- Moray eel (*Muraena helena*)
- Gorgonians (*Paramuricea clavata, Eunicella singularis, E. cavolini*..)
- Sea urchins (*Paracentrotus lividus*)
- Noble pen shell (*Pinna nobilis*)
- *Oysters* (*Spondylus* spp)
- Mortalities of any other marine organism is also our focus
-

1.3. MATERIALS

- Print copies of QuestionnaireLEK3.pdf to carry out the interview

- Excel file DATALEK3.xls where to input the recorded data

- "Guide" on the most common species affected by mass mortalities

1.4. INTERVIEWS

Interviewers should be practitioners skilled in species identification and with a good knowledge of local fishery and/or underwater environments. Respondents can be either professional fishers, recreational fishers or scuba divers. Fishers and divers can be interviewed at any time and in any place, taking into account the general rules provided in this kind of practice: *i*) be humble; *ii*) to behave like a facilitator and not an expert; *iii*) to show a genuine interest towards what respondents say but *iv*) to keep the interview on track and v) to critically review the received information.

1.5. THE LEK-3 QUESTIONNAIRE

QuestionnaireLEK3.pdf to be printed and used during the interview with local fishers and/or divers

LEK_3 THE MASS MORTALITIES PROTOCOL

Mass mortalities have been reported for species such as groupers (particularly Epinephelus marginatus), Moray eel (Muraena helena), Gorgonians (Paramuricea clavata, Eunicella singularis, E. cavolini..), Sea urchins (Paracentrotus lividus), Noble pen shell (Pinna nobilis), Oysters (Spondylus spp) etc... **mass mortalities of these and any other marine organism are our focus.**

INTERVIEW NUMBER................................ DATE... COMPILER...

Location/MPA... Country.................

CODE or NAME INTERVIEWED......................Age.........................Sex..............

PROFESSIONAL FISHER □ RECREATIONAL FISHER □

Set nets □ Traps □ Purse seine □ Trawl □ Longlines □ Angling □ Spearfishing □ Others ....

PROFESSIONAL DIVER □ RECREATIONAL DIVER □

FISHING or DIVING EXPERIENCE SINCE (year)...........

Question 1: Have you ever noticed mortalities of marine organisms (yes/no)? ....If yes, of what species?

Name ofspecies or taxon (check the corret identification) ....

Location of mass mortality ....

Radius of the affected area estimated in Km2..

Mortality started on (date year/month/and day if available)...

Mortality ended on (date year/month/and day if available)..

Total number of affected or dead specimens observed..

Estimated % of affected individuals ....

Estimated % of dead individuals..

NOTES and description of the mortality, syntomps and any other relevant info:

Question 2: According to your experience, what are the reasons of this mortality? (multiple choice)

Warm temperatures □ Fishing □ Pollution □
Cold temperatures □ Pathogens □ I don't know □

Others (explain) □ ....

Final evaluation of the interview: VERY TRUSTHWORTHY □ ACCEPTABLE □ DOUBTFUL □

If relevant pictures are obtained, save it with the name: Nameofspecies_Ninterviews_location.jpg
If more than one mortality event is reported, use a new page to fill in the info (this also apply to more than one event for the same species).
Please explain to the interviewed the aims of the interview and ask his/her oral consensus to use these data for scientific purposes. **Oral consensus received** □

NOTES:

NOTES:

- Ask for pictures or accurate description to correctly identify the species and save the picture with the name: Nameofspecies_Ninterviews_location.jpg
- If more than one mortality event is reported, use a new page to fill in the info (this also apply to more than one event for the same species)
- Please explain to the interviewed the aims of the interview and ask his/her oral consensus to use these data for scientific purposes. **Oral consensus received** □

1.6. MONITORING PERIOD AND SAMPLING SIZE

Interviews can be done at any time. It is suggested to have at least 20 interviewed expert/area

1.7. DATABASE, REPORTING AND DATA POLICY

Input data to DataLEK3.xlsx following the example given within the same excel file. Collected data can be used for your needs, but they can also be shared with the LEK-Team, by sending them to: ernesto.azzurro@cnr.it

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	**Interview**					**Observer**													
2	Interview N	Date	Compiler	LocationMPA	Country	Name Interviewed	Age	Sex (M or F)	Prof fisher	Recr fisher	Set nets	Traps	Purse seine	Trawl	Longlines	Angling	Spearfising	Prof Diver	Recr Diver
3	Example_1_2020	2020 March	nesto Azzu	Tavolara MPA	IT	Nino P	61	M	x		x	x							

	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN
1	**Mortality observed**										**Reasons**								**QUALITY interv.**		
2	Noted mortality (Yes/NO)	**Species**	Location	Radius estimated (Km)	Start mortality	End mortality	Tot Number observed	Estimated % affected	Estimated % dead	NOTES	Warm T	Cold T	Fishing	Pathogens	Pollution	Others explain	I don't know	Pictures(yes/no)	**Very trustful**	**Acceptable**	**Doubtful**
3	x	Epinephelus marginatus	ra and surrou	10	2017 May 5	2017 June 5	5	50	30	d with floatin	x			x				no	x		

Option for regional studies and scientific publications of the collected data will be discussed with the all data providers.

2. BARD: BENTHIC ALIEN SPECIES RAPID DETECTION (WEBINAR 3)

2.1. RATIONALE AND OBJECTIVES

Benthic invasive species have been reported as the second most common cause of species extinctions. Their ecological impacts can propagate along the food web and affect ecosystem functioning, having important socio-economic and health impacts with important loss of ecosystem services.

Managing invasive alien species is one of the greatest challenges for the conservation of marine native biodiversity. International institutions have explicitly recognized the need to control and eradicate biological invasions and have set relevant targets (e.g. the Aichi Target 9 set by the Convention on Biological Diversity). Guidance to decision-makers on how to prioritize management actions for the control of marine invasive species clearly indicates that early detection is key for the implementation of more effective measures to control invasive species (Giakoumi et al. 2019).

MPA managers can substantially contribute to the early detection of new invasive species and reduce management response-time for the implementation of other control measures by means of early detection protocols.

2.2. OBJECTIVES

Determine the abundance of benthic invasive species in rocky benthic habitats within the MPAs.

2.3. TARGET HABITAT AND SPECIES

Infralitoral and circalitoral rocky habitats.

The target species are alien rocky benthic species that have been identified in other areas in the Mediterranean.

2.4. MATERIALS

- A portable GPS (on board)

- Underwater camera with housing and external light system (e.g. electronic strobes)

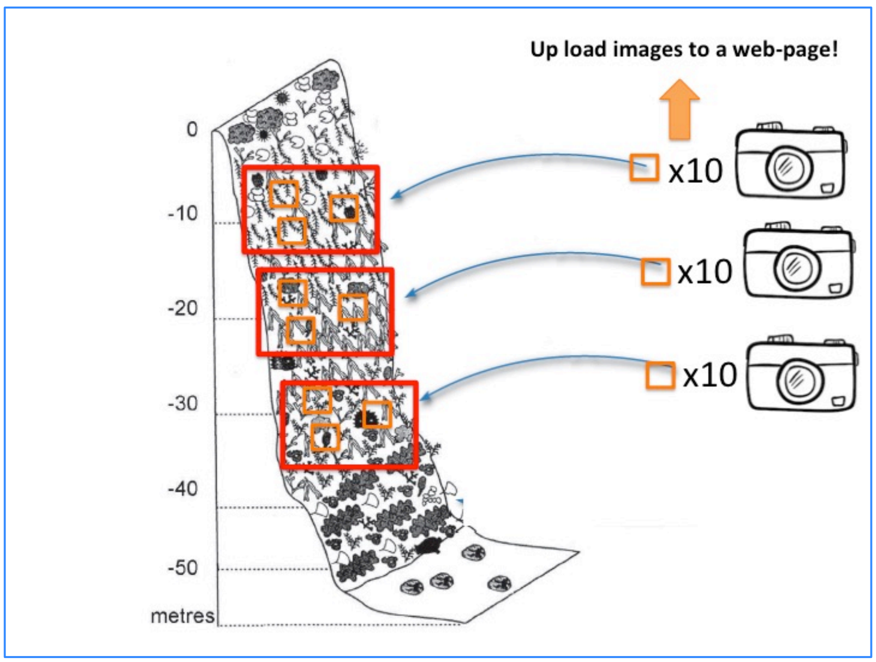
- 25 x 25 quadrat

- Slate with pencil

- We will provide a plastic board, bearing identification drawings of the main benthic exotic species identified in the Mediterranean Sea by now, but of course, during your sampling, you can discover any new species introduced in the Mediterranean Sea. Close up pictures of any strange or new species will be very helpful for the identification.

2.5. METHODS

In the selected sites, dive along a linear transect perpendicular to the shore. When you reach 30m depth take 10 photographs of 25 x 25 cm at random within an area of 100 m². Repeat the same procedure at 20 and 10 meters depth. During the dive you can take photographs or notes on the presence of conspicuous alien benthic species in other depths ranges (see figure).



The photographs will be analyzed in the lab to assess the presence and abundance of different alien benthic species as well as main benthic species/categories found in the monitoring sites. The list of main benthic species/categories to be considered will be provided along with appropriate identification guidelines.

2.6. DETERMINATION OF FIELD WORKERS AND SPECIALIZATION

Fieldwork can be carried out by trained professional scuba divers, working in couple.

2.7. PERIOD OF MONITORING

Sampling should be run twice a year in summer and winter to detect the presence of alien species of seasonal occurrence. If that is not possible due to logistical or financial constraints, the monitoring should be run at least once a year, preferably in summer, and at the same time each year. Perennial algae species display their greatest growth over summer making them easier to detect at this time.

2.8. SAMPLING SITES- STUDY SITES LOCATION AND DEPTHS

Select a minimum of 3 sites within the MPA separated by a minimal distance of about 0,2-0,5 km when possible. It is preferable to select sites with steep slopes to reach easily the 30 m depth. Depending on the habitat diversity and features in the MPA, the number of sampling sites should be increased to ensure the detection of alien benthic species.

2.9. DATABASE AND REPORTING

A specific procedure for the photo analysis is provided (see Annex 1). The pictures along with a corresponding Excel file for each depth and monitoring site will be uploaded to a new section on the T-MEDNet platform (to be implemented).

3. URCH: SEA URCHINS POPULATIONS IN MARINE PROTECTED AREAS (WEBINAR 3)

3.1. RATIONALE AND OBJECTIVES

Macroalgal forests play a crucial role in the structure and functioning of rocky benthic ecosystems worldwide. In the Mediterranean these habitats are well represented and display a high diversity of species. In these habitats thrive *Cystoseira* spp. like species which are strictly protected under Annex I of the Bern Convention; while the Barcelona Convention's Mediterranean sea action plan identifies the conservation of all but one species (*C. compressa*) as a priority. *Cystoseira* spp. species are also under surveillance as vulnerable by the IUCN and the MedPAN network.

The human activities caused the degradation of macroalgal forests mainly overfishing, contamination and habitat destruction and more recently the climate change. The degradation of the macroalgal forests results in habitat ranging from loss of perennial algal canopies, communities dominated by annual macroalgal species to "barrens" meaning a complete loss of macroalgal cover. Along this gradient there is a loss of biodiversity and productivity.

Marine Protected Areas play an important role in the recovery of these forests. Regulating some human activities, especially the fishing effort and habitat destruction, MPAs restore the equilibrium of trophic chains, affecting grazers abundance and the ratio preys/predators. There is a large body of scientific literature indicating that overfishing through trophic cascades is a major driver of degradation of macroalgal habitats. The lack of predators of sea-urchins species due overfishing result in the increase of the abundance of sea-urchins that graze on macroalgal species and ultimately resulted in the expansion of the urchin barrens in many unprotected areas of the Mediterranean.

Moreover MPAs are usually located in areas where main sources of contamination are limited or null. However, the effects of many chemical compounds even in low concentrations can have significant effects on macroalgal species persistence.

Regarding climate change, the most common sea-urchin species in the shallow rocky reefs display a differential thermal affinity and foraging behaviour, *Paracentrotus lividus* "cold-water" and *Arbacia lixula* "warm-water" species. Therefore, we can expect dramatic changes in the macroalgal beds due to changes in the abundance of sea urchins. Besides warming is favouring the colonization of alien herbivore species such as the invasive rabbit fish (*Siganus* spp.) which is also causing the transition to impoverished barrens. Monitoring the sea-urchin populations jointly with the Fish Visual Census and Benthic Alien Species Rapid Detection (BARD) monitoring protocols may contribute to define management actions towards conservation of the productive and high-diverse macroalgal beds in the Mediterranean MPAs.

3.2. OBJECTIVES

Determine the structure and the dynamics of sea-urchins populations in the shallow rocky habitats within the MPAs.

3.3. TARGET HABITATS AND SPECIES

Infralittoral rocky habitats with different cover of macroalgal species.

The target species to monitor are the sea urchins *Paracentrotus lividus* and *Arbacia lixula*.

3.4. MATERIALS

-Caliper

-Slate with pencil

-Measuring tape

-Gloves (thickness 3 mm at least)

3.5. METHODS

The abundance and population structure of *Paracentrotus lividus* and *Arabacia lixula* is determined by SCUBA diving along three parallel horizontal transects (50 m×1 m each) between 3 and 10 m depth at each selected study site.

Transects are divided into five 10 m² subtransects with intervals of 5 m. Within each transect, *P. lividus* and *A. lixula* >1 cm in diameter are counted and their diameters (test without spines) were measured with a calliper.

For the size structure, in each transect once we reached 100 sea-urchins individuals for each species we only count the abundance of sea-urchins. This facilitates the implementation while providing enough data for the size-structure analysis.

A standard underwater template board will be provided.

3.6. DETERMINATION OF FIELD WORKERS AND SPECIALIZATION

Fieldwork can be carried out by trained professional scuba divers, working in couple.

3.7. PERIOD OF MONITORING

Sampling should be performed once per year in the late summer since in this period facilitates the sampling because at this point in the year, the erect seasonal algae have disappeared. Besides in this period allow the detection of 1-year-old individuals of sea-urchin.

3.8. SAMPLING SITES. STUDY SITES LOCATION AND DEPTH

Select a minimum of 3 sites within the MPA separated by a minimal distance of about 0,2-0,5 km when possible. The sampling must be performed over the same type of substrates and slope e.g. rocky boulders, continuous rocky substrate or rocky walls. For each selected substrate 3 sampling sites should be considered.

3.9. DATABASE AND REPORTING

A specific Excel file will be provided for data storage, analysis and data reporting for each MPA.

4. POFA: POSIDONIA OCEANICA FAST ASSESSMENT ON MEADOWS CONSERVATION STATUS FACE TO CLIMATE CHANGE (WEBINAR 4)

4.1. RATIONALE AND OBJECTIVES

The proposed protocol stems from the scientific evidence that seagrass shoot mortality rates increase with increased warming, thus leading to net shoot losses and consequent depletion of the meadows. The protocol aims to define the conservation status of *Posidonia oceanica* meadows by assessing the density of the leaf shoots, which is one of the main structural descriptors of the meadows health condition. Additional information on variables (i.e. flowering events, presence of seeds) potentially linked to warming will also be collected. Flowers appear during autumn while seeds are fully developed in spring. Flowering intensity and frequency have been recently correlated with the occurrence of marine heat waves conditions and they can be also correlated with the health status of the plant. Once, baselines have been established, annual surveys will provide indications on the evolution of the conservation status of the meadows while highlighting the impacts.

4.2. TARGET SPECIES

Posidonia oceanica

4.3. MATERIALS

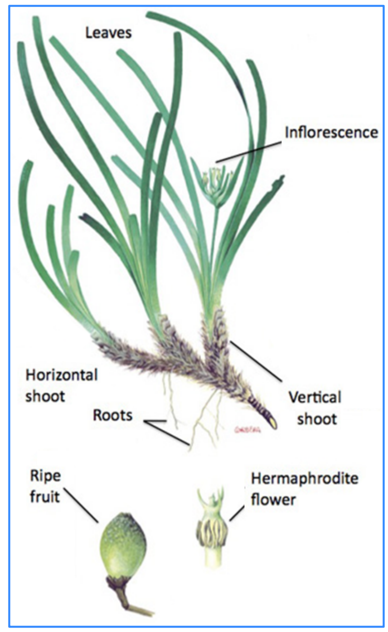
- Georeferenced maps of the meadows within the MPA (in preference divided into 100×100 m cells)
- A 50×50 cm quadrat
- A plastic board with pencil to collect data underwater, possibly pre-organised in data fields (see provided example)
- A deep gauge or diving computer to keep the depth of the survey
- A GPS (on board)
- A diving buoy with ballast or small anchor

4.4. METHODS

This methodology can integrate already active protocols the MPAs are using for the monitoring of Posidonia meadows or can be adopted where an institutional monitoring programme is not present. Depending on the extension of the meadow it is possible to define a grid of subareas (100x100m cells). This grid can be developed by the MPA-engage trainers if not available. Once on the selected monitoring site, locate the 100×100 m cell. Position the boat (and the diving buoy) in the middle of the monitoring cell and record the GPS position to confirm the precise positioning. A total of 9 quadrats (50×50 cm) will be sampled in each cell. The quadrats will be randomly placed within the meadow in a 50 m radius from the diving point, The minimum number of cells that have to be sampled will be defined in accordance with the MPA' staff.

As mentioned, each quadrat is randomly dropped onto the bottom (few meters each other) and then pushed to the base of the rhizomes. In case there are no shoots in the randomly positioned quadrat, the procedure should be repeated (move a few meters further) until there is at least one shot within the quadrat.

All the shoots inside the quadrat must be counted; each shoot consists of one rhizome and the leaves (see drawing); shoots in division are counted twice. Counting should start from one corner of the quadrat, taking care to separate with the arms the shoots already counted from those to be counted. Two divers, facing each other, can start counting on the opposite side and stop when they meet each other. For each quadrat, the total number of shoots and depth, carefully measured at the basis, is recorded to the slate.



Drawing author: Jordi Corbera. Picture taken from the book «Praderas y Bosques Marinos de Andalucía».

Photograph courtesy of Tavolara – Punta Coda Cavallo MPA (Italy) and Reef Check Italia onlus.

In autumn it is possible to find flowers (i.e. inflorescences), count the number of flowers found in the quadrats and record the presence outside them.

In spring it is possible to find fruits still on the plant, count the number of fruits found in the quadrats and record the presence outside them.

Record the type of substrate (sand, matte, rock) and whether the meadow is continuous or discontinuous.

The following additional observations can be recorded in each cell including ranges of abundance and depth.

- presence of noble pen shells (*Pinna nobilis*) and status (dead or alive). This information can be used to identify sampling sites for the *P. nobilis* monitoring protocol;
- presence of other seagrasses (e.g. *Cymodocea nodosa*);
- presence of alien species (e.g. *Caulerpa cylindracea*, *Asparagopsis taxiformis, A. armata*);
- presence of mucilaginous aggregates;
- presence of potential pressures (e.g. mooring systems, concrete blocks, pier, anchors, chains, ropes, trash);
- presence of clear signs of damage of the meadow (e.g. detached shoots, detached plates of matte, damages due to trawling or anchoring).

4.5. PROCESSING

Data will be reported in a specific excel file associated to the monitoring protocol.

It is recommended that two persons, in pairs, enter data and check the accuracy. There should be a clear link between GPS coordinates and monitoring stations in the data, and it should be checked if GPS data is accurately recorded.

The average number of leaf shoots detected in 50×50 cm quadrats is reported to the m^2 (i.e. multiplying the counting by 4 in order to assess the meadow density at each station/site and depth. Temporal trends may be used for assessing the conservation status of the meadows for each specific depth according to the interpretation scale proposed by UNEP/MAP-RAC/SPA (2015), with the identification of five quality classes as defined under the Directive, 2000/60/EC.

4.6. DETERMINATION OF FIELD WORKERS AND SPECIALIZATION

Fieldwork can be carried out by trained scientific scuba divers, working in couples as well as by volunteer divers after receiving adequate training.

4.7. PERIOD OF MONITORING

Summer to autumn on annual basis. Please note, from the end of September-October, flowering events might be more likely, this period is recommended for surveys. Anyway, also the presence of fruits on the plant is an indicator and allows monitoring during spring (April-May).

4.8. SAMPLING SITES

At each MPA, different study sites must be established depending on the variety of habitats present and the possible threats occurring on the protected area, applying (if possible) the same criteria in terms of number of sampling sites or depth range selection. Large meadows can be divided into 100×100 m cells.

4.9. DATABASE, REPORTING AND DATA POLICY

An Excel file will be provided for data storage, analysis and data reporting for each MPA.

4.10. REFERENCES

UNEP/MAP-RAC/SPA (2015).

https://www.rac-spa.org/sites/default/files/doc_mkh/vegetation/algue_eng_bd_cover.pdf

EXAMPLE OF FIELD FORM

Date ___________________ **Cell/site n.** ____________________

\ **Coordinates** _______________________ **N**

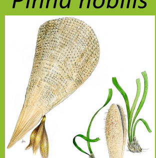
(WGS84) ____________________**E/W**

QUADRATS	1	2	3	4	5	6	7	8	9
DEPTH BOTTOM									
SHOOTS IN 50X50 CM									
FLOWERS									
FRUITS									
SUBSTRATE S= SAND R= ROCKS M= MATTE									

MEADOWS CONTINUITY	CONTINUOUS	SMALL CLEARINGS	LARGE CLEARINGS
FLOWERS OUTSIDE QUADRATS	YES	YES	NO
FRUITS OUTSIDE QUADRATS	YES	YES	NO
OTHER SEAGRASSES			
MUCILAGINOUS AGGREGATES			
POTENTIAL PRESSURES			
SIGNS OF DAMAGE			
ALIEN SPECIES			

OBSERVATIONS IN THE WHOLE EXPLORED AREA (WITHIN THE MONITORING CELL)

Pinna nobilis



Alive

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3-5
- ☐ 6-10
- ☐ 11-50
- ☐ >50

Dead

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3-5
- ☐ 6-10
- ☐ 11-50
- ☐ >50

Caulerpa cylindracea

- ☐ Absent
- ☐ An isolated specimen
- ☐ Some scattered specimens
- ☐ Several scattered specimens
- ☐ A crowded area
- ☐ Some crowded areas
- ☐ Several crowded areas

Caulerpa taxifolia

- ☐ Absent
- ☐ An isolated specimen
- ☐ Some scattered specimens
- ☐ Several scattered specimens
- ☐ A crowded area
- ☐ Some crowded areas
- ☐ Several crowded areas

5. FAP: FAST ASSESSMENT *PINNA NOBILIS* (WEBINAR 4)

5.1. RATIONALE AND OBJECTIVES

The noble pen shell *Pinna nobilis* Linné, 1758 is the largest endemic Mediterranean bivalve, reaching a size of up to 120 cm living mainly in soft-sediment areas and beds of seagrass *Posidonia oceanica* (Linnaeus), Delile and *Cymodocea nodosa* (Ucria) Aschers, but occasionally found also on irregular rocky substrates (scattered boulders), at depths ranging from 0.5 to 60 m. *P. nobilis* populations have greatly reduced in the last 40 years as a result of the impact of human impacts such as artisanal and recreational fishing, trawling, anchoring and seagrasses regression. A gradual recovery especially inside MPAs, has been documented but since 2016, different pathogens never reported before (an haplosporidian and a mycobacterium) have caused dramatical mortalities of *P. nobilis* along the whole Western Mediterranean basin. The present protocol offers a rapid tool to assess the health status of *P. nobilis* populations in Mediterranean MPAs.

5.2. OBJECTIVES

Determine the abundance, the size structure and the health status of *Pinna nobilis* populations within the MPAs.

5.3. TARGET SPECIES

The noble pen shell *Pinna nobilis*

5.4. MATERIALS

- Underwater compass
- GPS
- Buoy
- Deco buoy
- Metric-tape
- Plastic sheet

- Pencil
- Rule or frame with subquadrats
- Underwater camera with housing and electronic strobes or focus providing continuous lighting

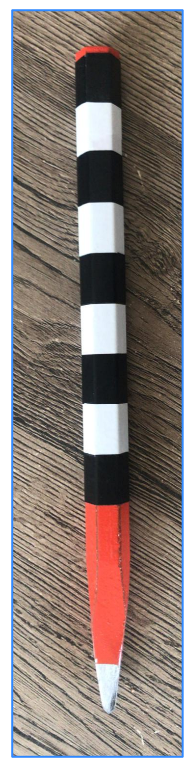
5.5. METHODS

In order to determine the density and the individual vitality for each specimen accurately, a **metric-tape** must be used aiming to count within each transect all individuals.

At each selected sampling site, 3 transects (100 m long and 6 m wide) are performed. All individuals observed inside the transects are counted and alive individuals measured. Data will be expressed as N individuals/600m^2

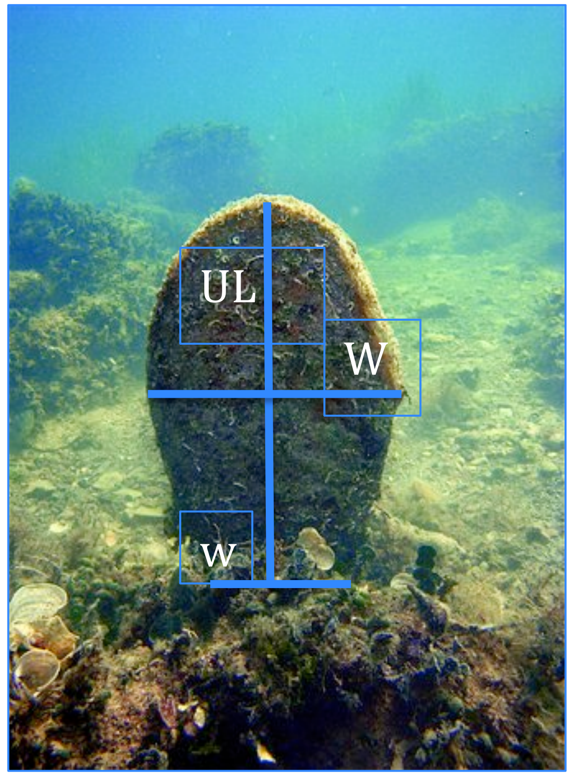
For each transect, at least the geographic coordinates of the starting point should be recorded with a GPS and once in the bottom a constant direction (marked by a submersible compass) is followed. When possible a marking buoy is deployed at the starting point of the transect as well at the end of the transects (deco buoy) to record the geographic coordinates of both buoys are taken with a GPS.

Once an individual of *P. nobilis* is noted
-alive,
-affected by infection (still alive but with slow valves-closure reaction when disturbed),
-recently death (buried parts with no epibiosis or byssus still abundant),
-not recently death individuals (broken shells and evident epibiosis of buried parts) or
- death individuals found still orthogonal, into the substrate
pictures of all observed individuals together with a measurement reference scale should be taken (a frame with quadrats or a sort of rule with black and white band guarantees the most accurate measurement).



Back in the office biometric information is obtained by image analysis using open source software:

- unburied length (UL) for alive individuals or total length for death individuals;
- maximum width (W);
- minimum width (w)



The monitoring on *P. nobilis* is providing:

- Density of individuals
- % affected of individuals
- % mortality of individuals

Size structure of alive individuals and of former population

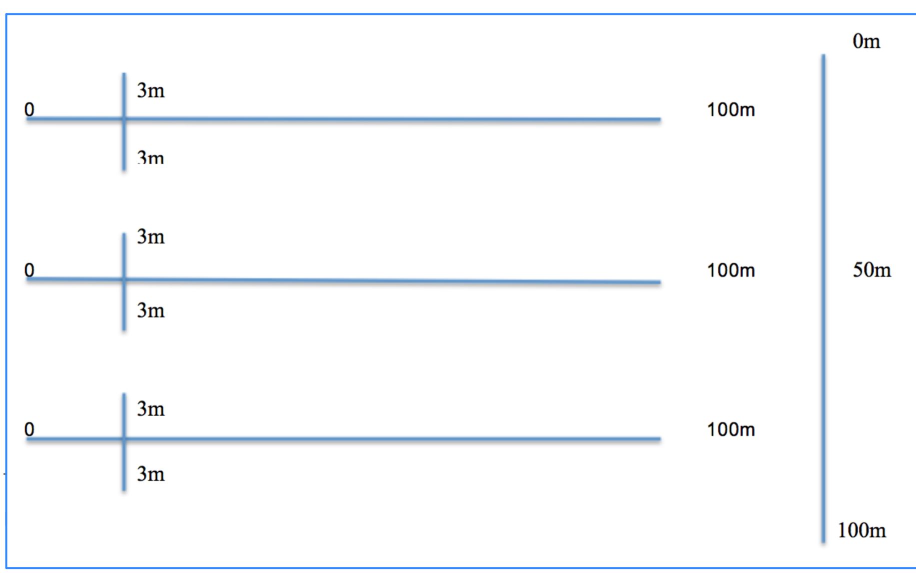


Fig. 1: Transects positioning (from MSFD protocol of ISPRA)

5.6. DETERMINATION OF FIELD WORKERS AND SPECIALIZATION

Fieldwork and processing steps should be conducted by the MPA staff after receiving a quick training. The recorded data will be uploaded to the file Pinna_monitoring.xls for further analysis in collaboration with a scientific team.

5.7. PERIOD OF MONITORING

Late summer/Early autumn

5.8. SAMPLING SITES

At each MPA, a minimum of 3 study sites should be established. The sites should be set in habitats suitable for *P. nobilis* e.g. seagrass beds, detritic bottoms, rocky boulders. The total number of sampling sites will depend on the variety of habitats present in each MPA.

5.9. DATABASE, REPORTING AND DATA POLICY

An Excel file will be provided for data storage, analysis and data reporting for each MPA. The % of affected and % mortality can be uploaded to the T-MEDNet database devoted to the mass mortality events http://t-mednet.org/mass-mortality/mass-mortality-events

6. SFM: PHOTOGRAMMETRY AS MONITORING TOOL FOR BENTHIC HABITATS STRUCTURE AND DYNAMICS (WEBINAR 5)

6.1. RATIONALE AND OBJECTIVES

Recent technologies are allowing to improve marine habitat mapping, moving from the traditional bidimensional maps to more detailed three-dimensional ones.

Over the past decades, habitat **complexity has decreased** along the global ocean causing a decline in abundances and diversity of the fish and invertebrates associated communities. Although it is known that climate change can affect ecosystems assemblage and complexity, little is known about their synergy and how ecosystem functions will be threatened within their decrease under future climate conditions. Further data is needed for a deeper understanding of habitat complexity dynamics and communities shifting.

Structure from Motion (SfM) photogrammetry is proposed as a suitable technology to monitor changes in habitat structure and complexity allowing as well to monitor population changes of key species. Structure from motion (SfM) steamed from scientific and field experience over the past 10 years, SfM monitoring allows to implement cost-effective high-resolution surveys over relatively large areas (>100s m^2).

Structure from Motion (SfM): name given to the technique used for the extraction of three-dimensional data and camera positions from a collection of photographs. The main advantage of this technique would be the automatic assignment and alignment of control points along the image dataset without any knowledge of the camera position.

6.2. OBJECTIVES

Generate 3D information on MPAs key habitats and species. More precisely the implementation of the photogrammetry protocols will allow to characterize habitat complexity and distribution patterns of populations of key species and track their changes over time.

6.3. TARGET HABITATS AND SPECIES

The protocol aims to monitor both habitat and species present on the benthic area of interest. Depending on the studied habitat, different species would be targeted. Main criteria to select habitats and organisms of interest by the MPA managers would be:

i) easy to identify visually
ii) of interest as indicator (e.g. for climate change)
iii) of interest for conservation (protected species and/or ecosystem engineer)

It is recommended to align the implementation of the photogrammetry protocol with the habitats and species targeted in other monitoring protocols. In this way we will obtain a more comprehensive view on the conservation status of the targeted habitats in each MPA.

6.4. MATERIALS

- **Underwater camera and housing,** e.g. sport cameras such as GoPro can be used; depending on the aimed resolution of the reconstruction maybe other models must be considered.
- **Ground control points (GCPs)** for geo-reference processing (Fig.1) .

Fig.1. *Example of a ground control point. The dimension of this GCP was 22 cm _ 22 cm _ 22 cm. The chessboard extension was 21 cm long by 8 cm wide, but size can be different, it is a reference point useful to correctly reconstruct the surveyed area (Palma et al., 2018).*

- **A metric reference** to scale the reconstruction.
- **Artificial light** could be necessary if natural light is not enough to take quality images caused by turbidity or light attenuation through the water column.
- **Colour chart** is also recommended, especially in cases of reduced visibility, where it could help in terms of colour correction of the images. It will be provided during the training

6.5. METHODS

In order to produce accurate 3D models of the targeted habitats and key species populations, it is necessary to use references to **Ground Control Points** (GCPs) and **metric-scales**. These references should be deployed along the studied area prior to photosampling in order to later scale and georeference the reconstruction. It is recommended to use bright contrast colours for reference **Ground Control Points** (GCPs), as white and black, to better recognise the scale subdivisions on the 3D models. **Metric-tapes** can also be used aiming to better define study sites dimensions and standardised the same area of study on the different habitat approaches on the different MPAs.

To start with a step by step process it is possible to test the methodology on a small area:

1) define the monitoring site, i.e. 10 x 10m 20 x 10m, 20 x 20 m;
2) setup the GCPs;
3) before taking the photographs, place the metric-scales and colour chart. (it is important to remind that the metric scale should not be moved during the sampling process);
4) Select the monitoring site being aware that you must be able to dive over the scene slowly in order to take the images at a predefined short time interval in order to obtain a sequence of photographs with an overlap of around 50-80%;
5) follow a zig-zag pattern to avoid holes in the 3D reconstruction;
6) download the images and save them in a folder with date_sitename_depth;
7) check the photographs quality and implement corrections if necessary;
8) photogrammetric processing will be carried out by the researchers collaborating with the MPA, consisting mainly on the 3D reconstruction of the scene and its escalation and georeferentiation;
9) data treatment

Once the methodology is adjusted to the study site and the final collection path adapts to sea floor complexity, the divers will take the images from approximately at 1 meter above the substrate in order to obtain the video? Or photographs records on the habitats. Following two perpendicular zigzag patterns (Fig. 1) the operators will try to **cover all the possible perspectives** and swim slowly in order to obtain the proper **overlap** between images. Any missing area on the image collection will be traduced as “holes” on the result models.

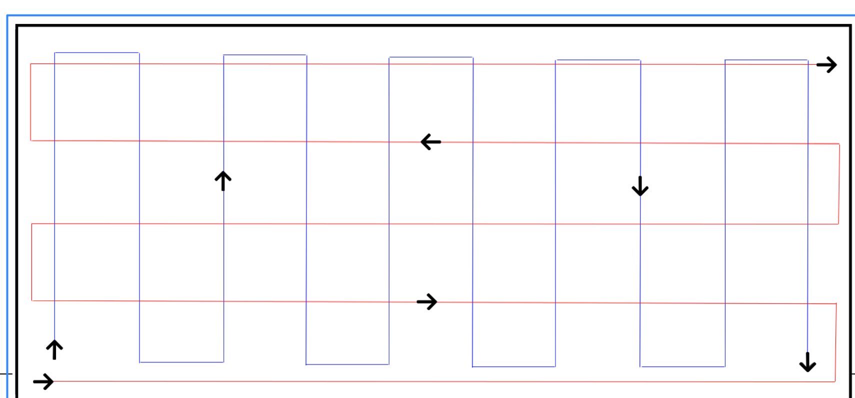


Fig. 1: Image collection zig-zag pattern.

6.6. DETERMINATION OF FIELD WORKERS AND SPECIALIZATION

Fieldwork steps should be conducted by the staff of MPA after receiving training. The sampling, image treatment and analysis will be carried out with the support of the MPA-Engage research institutions (referral Prof. Carlo Cerrano).

6.7. PERIOD OF MONITORING

Since initial image quality can affect the photogrammetric process causing anomalies on the analysis outcomes due to the extra difficulty extracting features from the images. Monitoring period must be adapted to each MPA depending on which period it hosts better water transparency conditions, usually during summer or early fall.

6.8. SAMPLING SITES

At each MPA, different study sites could be established depending on the variety of habitats present and the possible threats occurring on the protected area. However, it is suggested to focus the first efforts on the different diving sites present on the MPAs since the reconstructions could be also useful for the local industry, boosting collaboration and participation of volunteer divers.

The minimum sampling effort should be characterizing the habitat features covering 100s m^2 in 3 sites (with the same/different habitat) within the MPA. The final sampling design will be defined with the project research team.

6.9. DATABASE, REPORTING AND DATA POLICY

A specific procedure for the photo storage and processing will be provided. Eventually a web-platform will be designed to upload the photographs and facilitate the photo analysis. The photographs processing will be carried out with the support of the MPA-Engage research institutions

6.10. REFERENCES

Palma, M., Rivas Casado, M., Pantaleo, U., Pavoni, G., Pica, D., & Cerrano, C. (2018). SfM-based method to assess gorgonian forests (Paramuricea clavata (Cnidaria, Octocorallia)). *Remote Sensing*, *10*(7), 1154.

Palma, M., Magliozzi, C., Rivas Casado, M., Pantaleo, U., Fernandes, J., Coro, G., ... & Leinster, P. (2019). Quantifying Coral Reef Composition of Recreational Diving Sites: A Structure from Motion Approach at Seascape Scale. *Remote Sensing*, *11*(24), 3027.