Interreg Mediterranean

EUROPEAN UNION

MPA-Engage

Project co-financed by the European Regional Development Fund

Monitoring Climate-related responses in Mediterranean Marine Protected Areas and beyond:

ELEVEN STANDARD PROTOCOLS

CSIC · portofino · Parc national des Calanques · BRIJUNI · Generalitat de Catalunya Departament de Territori i Sostenibilitat · Area Marina Protetta Tavolara Punta Coda Cavallo · MIO-ECSDE · Future Oceans Lab · Regione Liguria · DAN Divers Alert Network Europe

How to cite these protocols:

Garrabou J., Bensoussan N., Di Franco A., Boada J., Cebrian E., Santamaria J., Guala I., Grech D., Cerrano C., Pulido T., Jou M., Marambio M. & Azzurro E., 2022.
Monitoring Climate-related responses in Mediterranean Marine Protected Areas and beyond: ELEVEN STANDARD PROTOCOLS.
74 pp. Edited by: Institute of Marine Sciences, Spanish Research Council ICM-CSIC, Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain.

DOI: https://doi.org/10.20350/digitalCSIC/14672

MPA-ENGAGE, Interreg Med Programme
(Grant number 5216 5MED18_3.2_M23_007)
85% co-funded by the European Regional Development Fund

Foreword

Global warming is having observable effects on the abundance, distribution and survival of living organisms worldwide, with serious consequences on the functioning of coastal ecosystems and the services that they provide to our societies. These effects are particularly alarming in the Mediterranean Sea, which is warming faster than the global oceans.

Documenting these changes is a key task to support mainly the Marine Strategy Framework Directive and the Ecosystem Approach process which and the associated Integrated Monitoring and Assessment Programme (IMAP). Nevertheless, the complexity of ecological transformation along with inadequate human and financial resources typically hampers our observation capabilities.

This series of standard protocols provides practical guidance to track climate-related impacts in Mediterranean Marine Protected Areas (MPAs) and beyond. The guiding principles and architecture of these tools respond to the requirements of the Ecosystem Approach undertaken under the auspices of UNEP/MAP Barcelona Convention and the EU MSFD, with the ultimate objective of achieving the Good Environmental Status (GES) of the Mediterranean Sea and Coasts. Technically these tools are inspired by the concept of Essential Climate and Ecosystem Variables and focus on a restricted set of simple measurements to capture greater dimensions of environmental change. Indicators have been chosen on the basis of their scientific relevance, feasibility and cost effectiveness. The engagement of local stakeholders is another key ingredient in some of these methodologies. Adopting these protocols, allow participants to join to a common and consolidated strategy to track climate change effects. This will improve, complement or extend the ongoing monitoring initiatives in the different Mediterranean countries.

Mediterranean MPAs as long-term designations can play a primary role in providing a systematic and harmonized observation system, translating principles already consolidated in the political framework, to the real world. The resulting outputs are key information to support mitigation strategies and effective adaptation plans.

PROTOCOL 1

LEK-1: Exploring Local Ecological Knowledge to reconstruct historical changes

PROTOCOL 1: LEK-1: Exploring Local Ecological Knowledge to reconstruct historical changes

RATIONALE AND OBJECTIVES

Local Ecological Knowledge is the information that people have about the ecosystems where they spend most of their time. This knowledge is gained by individuals during their daily activities, like fishing. The following protocol explains how to interview experienced fishers or other sea users, to reconstruct historical changes in species abundances and distribution. This approach, adopted by several Mediterranean countries, can be particularly effective for tracing back the arrival and subsequent explosion of an invasive species, to document the increasing success of thermophilic taxa but also to provide evidence of drastic population declines linked to any natural or anthropogenic factor. It can also be used for the early detection of new species. For periodical monitoring, please see **protocol LEK-2**, for the assessment of mass mortalities please see **protocol LEK-3**.

TARGET SPECIES

There are no target species. All the taxa perceived as increasing by the respondents should be considered. To help the elicitation process you might recall species that could have increased in that specific fishing area (for example warm adapted species or recent invaders).
Note: You can use the same protocol to collect data about decreasing species. In this case, it is suggested to only register the most drastic decreases.

MATERIALS

- ☑ Printed copies of the **Questionnaire for LEK-1** (**Fig. 1.1 and 1.2**) to carry out interviews
- ☑ A **field guide** or **pictures** of fish and other marine species, to assist species identification
- ☑ An **excel file** where to input data taken through the **Questionnaire for LEK-1** (see **DATA_LEK_1.xls**)

Note: all the materials and training tools are freely downloadable from the MPA-Engage web platform.

PROTOCOL 1: LEK-1: Exploring Local Ecological Knowledge to reconstruct historical changes

SAMPLING SITES

Interviews can be performed at any location, any time of the year. Interviewers may ask small scale fishers, recreational anglers and spear fishers, as well as scuba divers.

PERIODICITY OF MONITORING

This protocol can be used only once, to reconstruct past changes. After implementing **LEK-1** you can start with protocol **LEK-2** (for periodical monitoring). It is recommended to interview at least **20 respondents at each location**.

FIELD WORKERS EXPERIENCE

Interviewers should be practitioners skilled in species identification and with a good knowledge of local fisheries. Pre-existing contacts and good relationships between researchers and fishers are certainly helpful.

PROTOCOL 1: LEK-1: Exploring Local Ecological Knowledge to reconstruct historical changes

METHODS

INTERVIEWS

Respondents should be selected among artisanal fishers with more than 10 years of activity in the area where the interview is performed. You can also interview other sea users such as divers, spearfishers or recreational anglers.

APPROACHING RESPONDENTS

Fishers can be interviewed during their land activities, while they are cleaning the nets or when they are fixing their boats. Considering that fishers could distrust researchers and practitioners, special attention should be paid to the approach used during the interview. Remember: i) to 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.

A selection of respondents can be made on the basis of their trustworthy, experience and availability. Before starting the interview, explain the aims of the survey and ask the respondents his/her oral consensus to use these data for scientific purposes.

AN EXAMPLE

You are interviewing Pino, a professional fisherman, who began fishing in 1973.

☑ Draw a vertical line at 1973

YOU: *Do you know any species which increased or appeared in your fishing area?*

PINO: *Oh, everything decreased, but actually a few species increased in their abundance... the bluefish is one of them!*

☑ Verify the correct identification of the species (check, with the help of pictures, that the mentioned species is actually the bluefish, *P. saltatrix*)

☑ Take note of the perceived trend: Pino says the species is increasing and you write "(I)"

YOU: *Can you help me reconstructing how the abundance of bluefish changed in time?*

PINO: *Yes, I did not notice it until early 1990s, let's say 1992 when I got married. Then, the species remained occasional for a few years and in the last 10 years it became very, very, common.*

PROTOCOL 1: LEK-1: Exploring Local Ecological Knowledge to reconstruct historical changes

☑ Take note of the historical trends, according to 6 ranks of abundance: 0=ABSENT, 1=RARE (once in a year), 2=OCCASIONAL (sometimes in a year), 3=COMMON (regularly in a year), 4=ABUNDANT (regular in captures and abundant), 5=DOMINANT (always in captures and with great abundances)

YOU: *Which was your maximum catch, in a single day of fishing?*

PINO: *Oh, well, in a single day during Summer 2011, I caught about 20 fish, for a total of more or less 25 kilograms, with hooks.*

☑ Write down these numbers, in the **Max day capture field** (**Fig. 1.3**)

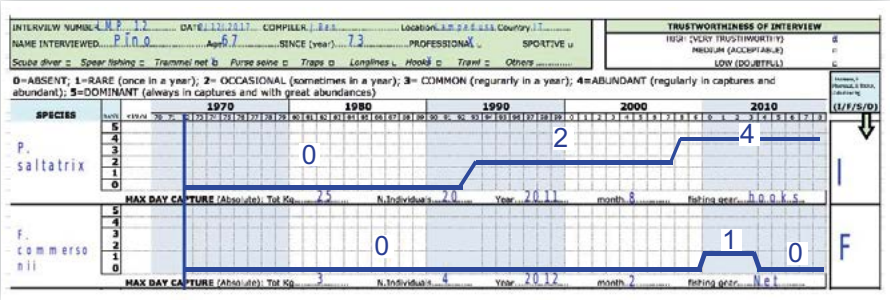

Figure 1.3: Screenshot of the LEK-1 questionnaire, with an example of the historical reconstruction of the abundance of two species (*Pomatomus saltati* and *Fistularia commersonii*).
The blue lines and the numbers illustrate how to write down the data during the interview.

YOU: *Do you know any other species that recently appeared in the area or increased in its abundance?*

PINO: *Oh, yes, I kow a new fish... the trumpetfish! I saw it for the first time in 2010, but then it disappeared for a few years.*

YOU: *Do you think that this species is increasing?*

PINO: *No, it is a new species but I don't think it is increasing right now.*

☑ You identify the bluespotted cornetfish (*Fistularia commersonii*). Rank it as "Fluctuating" (F) and keep interviewing Pino to reconstruct its historical timeline, as for the bluefish

☑ When Pino has listed ALL the new or increasing species, you might keep interviewing him about the disappearing ones. Rank them as "Declining" (D) and proceed as before, reconstructing their change in time

YOU: *Thanks Pino. Are you sure there is no other species which is increasing in the area? In this case, could you please tell me if any species disappeared from here? (...)*

PROTOCOL 1: LEK-1: Exploring Local Ecological Knowledge to reconstruct historical changes

DATA REPORTING AND DATA POLICY

Input data to **Data_LEK1.xlsx**. First, insert the interview number, which can be recalled from the drop-down menu. Then, compile the spreadsheet, inserting information on historical trends (Fig. 1.1) and records (Fig. 1.2). Collected data can be used for your needs, but they can also be shared with our the LEK Team, by sending them to: ernesto.azzurro@cnr.it.

PROTOCOL 1: LEK-1: Exploring Local Ecological Knowledge to reconstruct historical changes

1_HISTORICAL TRENDS

OUR QUESTION: *'What species have been increasing in the last decades? Are you fishing or observing species, which were not present before?'*

INTERVIEW NUMBER................................ DATE....................... COMPILER....................................... Location......................... Country.................

NAME INTERVIEWED...Age........................SINCE (year)...............................PROFESSIONAL □ SPORTIVE □

Scuba diver □ *Spear fishing* □ *Net* □ *Purse seine* □ *Traps* □ *Longlines* □ *Hooks* □ *Trawl* □ *Others* ....

TRUSTWORTHINESS OF INTERVIEW

HIGH (VERY TRUSTHWORTHY) □

MEDIUM (ACCEPTABLE) □

LOW (DOUBTFUL) □

0=ABSENT; **1**=RARE (once in a year); **2**= OCCASIONAL (sometimes in a year); **3**= COMMON (regurarly in a year); **4**=ABUNDANT (regularly in captures and abundant); **5**=DOMINANT (always in captures and with great abundances)

SPECIES	RANK	<1970	1970										1980										1990										2000										2010									I Increase, F Fluctuant, S Stable, D decreasing (I/F/S/D)
			70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	
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Credits: adapted from a first version of the CIESM *Tropical Signals* Pr.

Figure 1.1: Questionnaire for LEK-1: Historical trends spreadsheet.

PROTOCOL 1: LEK-1: Exploring Local Ecological Knowledge to reconstruct historical changes

2_RECORDS

Isolated/occasional observations or captures of 'new' species, never seen before should be collected here

INTERVIEW NUMBER..... COMPILER..... Location..... Country.....

NAME INTERVIEWED..... Age..... SINCE (year)..... PROFESSIONAL □ SPORTIVE □

Scuba diver □ Spear fishing □ Trammel net □ Purse seine □ Traps □ Longlines □ Hooks □ Trawl □ Others .....

SPECIES *	Year	Month	N. Ind.	Depth	**Exact Location** (and coordinates if available)	Fishing gear	Picture?**	**Notes**

If the interviewed has observed/captured something we can not identify write:* ***No ID* *and report general description (lenght, weight, color, shape..)*

***Ask if he/she took pictures of the species (in this case try to get it)*

Trustworthy (quality of the interview)

LOW □ MED. □ HIGH □

Figure 1.2: Questionnaire for LEK-1: Records spreadsheet.

PROTOCOL 2

LEK-2: Exploring Local Ecological Knowledge for periodical monitoring

PROTOCOL 2: LEK-2: Exploring Local Ecological Knowledge for periodical monitoring

RATIONALE AND OBJECTIVES

Local Ecological Knowledge is the information that people have about the ecosystems where they spend most of their time. This knowledge is gained by individuals during their daily activities, like fishing. In the following protocol, we explain how to interview experienced fishers or other sea users, to regularly (every year) monitor climate-sensitive species of both native and exotic origin. **Note:** Before implementing **protocol LEK-2** is highly recommended to have completed **protocol LEK-1**.

TARGET SPECIES

Based on previous experience gained at the Mediterranean level, target species are divided in three groups:

- **Med targets**
  7 target species selected a priori to be used in all the Mediterranean MPAs: *Pomatomus saltatrix, Sparisoma cretense, Lagocephalus sceleratus, Pterois miles, Siganus luridus, Siganus rivulatus, Sarpa salpa*

- **Local targets**
  Up to 5 additional species selected according to local monitoring needs and to the following criteria: i) easy to recognize, ii) interacting with fisheries, iii) emerging the most in the area, iv) impacting on environment, fisheries or human health

PROTOCOL 2: LEK-2: Exploring Local Ecological Knowledge for periodical monitoring

- **Fishermen's targets**
  Any species perceived by the respondent as increasing or drastically decreasing can be added. Note that target species may change at each interview, since they are spontaneously mentioned by each respondent

MATERIALS

- Printed copies of the **Questionnaire for LEK-2** (**Fig. 2.1**) to carry out interviews
- A **field guide** or **pictures** of fish and other marine species, to assist species identification
- An **excel file** where to input data (see **DATA_LEK_2.xls**)

Note: all the materials and training tools are freely downloadable from the MPA-Engage web platform.

SAMPLING SITES

Interviews can be performed at any location and once a year.

PERIODICITY OF MONITORING

Interviews should be performed once a year, every year.

FIELD WORKERS EXPERIENCE

Interviewers should be practitioners skilled in species identification and with a good knowledge of local fisheries. Considering the periodical use of the protocol, it will be important to maintain trustful and long-lasting relationships with the expert respondents.

METHODS

INTERVIEWS

Respondents should be selected among professional and/or recreational fishers with more than 10 years of activity in the area where the interview is performed. It is recommended to interview at least 5 respondents per gear, achieving a sample of **20 interviewed people**, who ideally should remain the same across time.

APPROACHING RESPONDENTS

Fishers can be interviewed during their land activities, while they are cleaning the nets or when they are fixing their boats. Considering that fishers might distrust researchers and practitioners, special attention should be paid to the approach used during the interview. Remember: i) to be humble, ii) to behave like a facilitator and not an expert, iii) to show a genuine interest towards what respondents say

PROTOCOL 2: LEK-2: Exploring Local Ecological Knowledge for periodical monitoring

but to follow the protocol but iv) to keep the interview on track and v) to critically review information. A selection of respondents can be made on the basis of their trustworthy, experience and availability.

DATA RECORDING

To carry out interviews, remember the following steps:

- Print the **Questionnaire for LEK-2** (**Fig. 2.1**) and bring with you a field guide or pictures of species to assist their identification
- Collect baseline information about respondents, which is reported on top of the questionnaire. The questionnaire can be used for scuba divers as well, but taking note of observations rather than captures
- Ask information about each target species, if clearly recognized by respondents
- Current trend of the species: "Do you think the species is currently Increasing(I)/Decreasing(D)/Fluctuating(F)/ Stable(S)/Don't know(Nk)?"
- Current abundance: 0=ABSENT; 1=RARE (once in a year); 2=OCCASIONAL (sometimes in a year); 3=COMMON (regularly in a year); 4=ABUNDANT (regularly in captures and abundant); 5=DOMINANT (always in captures and with great abundances)
- Best day-catch over the past 12 month, the fishing gear and the fishing period
- If the species is perceived as something "Bad/Good/ Neutral/Don't know" for fisheries
- If the species is perceived as something "Bad/Good/ Neutral/Don't know" for the environment

ISOLATED RECORDS

Isolated captures/observations of new, unusual or exotic species can be registered on the Records page of **DATA_LEK_2.xlsx**.

DATA REPORTING AND DATA POLICY

Collected data can be used for your local monitoring needs, but they can also be shared with the LEK Team, by sending them to: ernesto.azzurro@cnr.it.

PROTOCOL 2: LEK-2: Exploring Local Ecological Knowledge for periodical monitoring

INTERVIEW N..... Compiled by..... DATE..... LOCATION.....

NAME or CODE FISHERMAN.....Sex..... Age..... SINCE (year).....

Used GEARS: PROFESSIONAL — Set nets □ Trawl □ Others specify..... | RECREATIONAL — Angling □ Spear □ Other specify..... | DIVER □

FREQUENCY OF FISHING ACTIVITIES: Once a month □ Once a week □ More days per week □ Almost every day □

FISHERMAN'S EXPERIENCE: Expert and trustworthy □ Very Expert and very trustworthy □

HOW TO PERFORM THE INTERVIEWS:

For each geographical area **1)** identify a minimum of **5** EXPERT fishermen *per* **gear (N tot 5x4 = 20)** and EVERY 12 months: **2)** Ask relevant information related to **Med** and **local targets** - Ask for **additional species** they might perceive as new or increasing in their fishingh area (Fisherman's target); **3)** input the data to the excel file.

	SPECIES	What is the current **TREND** ? **I** = Increasing **D** = Decreasing **F** = Fluctuating **S** = Stable **Nk** = Not known — I/D/F/S/Nk	How **ABUNDANT** is the species in the fishing period? **0** = ABSENT **1** = RARE (once in a year) **2** = OCCASIONAL (sometimes) **3** = COMMON (regurarly captured) **4** = ABUNDANT (regular and abundant) **5** = DOMINANT (always and very abundant) — 0/1/2/3/4/5	What is the **BEST day-catch** in the past 12 months? — Kg	N.	With **which Gear**?	What is the **fishing period**? — From	To	Is this species: Bad/Good /I dont know - for **FISHERY** — B/G/Nk	Is this species: Bad/Good/I dont know - for the **ENVIRONMENT** — B/G/Nk	NOTES
Med Targets	*P. saltatrix*										
	S. cretense										
	S.salpa										
	L. sceleratus										
	S. luridus										
	S. rivulatus										
	P. miles										
Local Targets	.....										
	.....										
	.....										
	.....										
	.....										
Fisherman's	.....										
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Figure 2.1: Questionnaire for the LEK-2.

PROTOCOL 3

LEK-3: Exploring Local Ecological Knowledge for mass mortalities

PROTOCOL 3: LEK-3: Exploring Local Ecological Knowledge for mass mortalities

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.

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

MATERIALS

- Print copies of the **Questionnaire for LEK-3** (**Fig. 3.1**) to carry out the interview
- Excel file **DATALEK3.xls** where to input the recorded data

SAMPLING SITES

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

FIELD WORKERS EXPERIENCE

Interviewers should be practitioners skilled in species identification and with a good knowledge of fishery resources and/or underwater environments. Respondents can be either professional fishers, recreational fishers or scuba divers.

METHODS

FIND THE EXPERTS

Respondents should be selected among fishers or divers with more than 10 years of activity in the area where the interview is performed. It is recommended to interview **20 interviewed people**.

PROTOCOL 3: LEK-3: Exploring Local Ecological Knowledge for mass mortalities

APPROACHING RESPONDENTS

People can be interviewed during their land activities. Remember: i) to be humble, ii) to behave like a facilitator and not an expert, iii) to show a genuine interest towards what respondents say but to follow the protocol but iv) to keep the interview on track and v) to critically review information. A selection of respondents can be made on the basis of their trustworthy, experience and availability.

OPENING THE INTERVIEW

Before starting the interview you should introduce yourself and explain the aims of the interview. You should also mention that the elicited information can be used for scientific purposes. The interviewer must receive the oral consensus before proceeding with the questions.

TAKING THE DATA

If mortality events are mentioned by the respondents, you need first to correctly identify the species. It is recommended to ask for pictures or accurate descriptions. Once the species has been correctly identified, the interview will proceed according to the questions reported in the the **Questionnaire for LEK-3** (**Fig. 3.1**). If more than one mortality event is reported by the same respondent, you should use a new page to fill in the info. This is also the same when more than one mortality event is reported by the same respondent for the same species.

DATA REPORTING AND DATA POLICY

Collected data should be imputed to the excel file **DataLEK3.xls** following the example given within the same excel file. Collected data can be used for your needs, but they can also be shared with our LEK Team by sending them to ernesto.azzurro@cnr.it.

PROTOCOL 3: LEK-3: Exploring Local Ecological Knowledge for mass mortalities

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* ☐ *Spearfising* ☐ *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:

Figure 3.1: Questionnaire for the LEK-3.

PROTOCOL 4

Monitoring temperature conditions

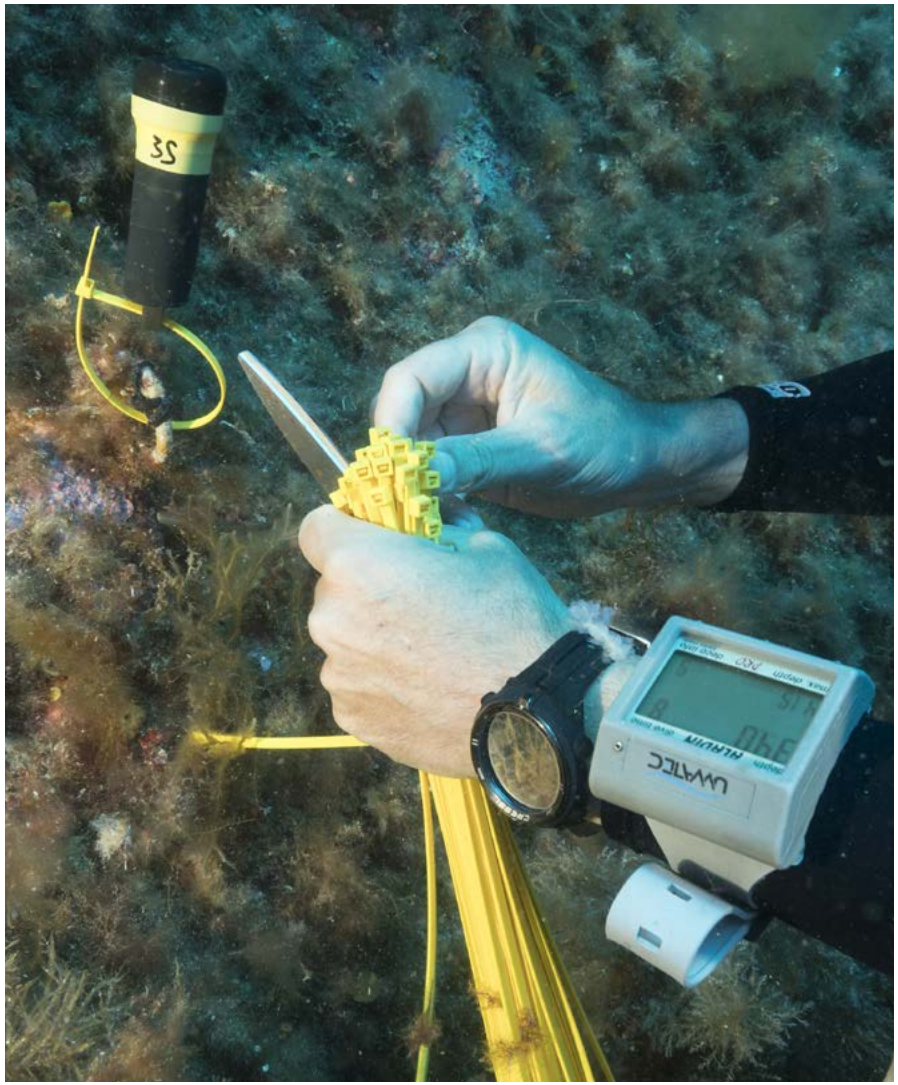
PROTOCOL 4: Monitoring temperature conditions

RATIONALE AND OBJECTIVES

The proposed method is issued from scientific and field experience gathered in the T-MEDNet network over the past 18 years. It is designed to acquire long-term and high resolution information on temperature conditions along the depth gradient in coastal waters. In this protocol we propose characterizing sea water temperature conditions recording temperature every hour using data loggers deployed and recovered over an annual or a semi-annual basis. Data loggers are placed every 5 m from surface down to 40 m or deeper in order to acquire information about seasonal stratification dynamics and temperature conditions at depth. Run over the long-term these data series build robust baselines and track hydrological changes (e.g. warming, heat waves, shifts in seasonality, stratification) to better understand the impacts of climate warming on marine coastal biodiversity.

The objectives are:

- ☑ Setup temperature data loggers to obtain hourly data series on local temperatures along the depth gradient
- ☑ How to operate with temperature data loggers
- ☑ Upload temperature records on the T-MEDNet web platform



PROTOCOL 4: Monitoring temperature conditions

MATERIALS

Materials for data collection include:

- **Temperature data loggers**
  HOBOTidbit v2 or HOBO-U22 (**Fig. 4.1a**)
- **Fixation kit**
  Colson rings, ankles Colson (**Fig. 4.1c**), putty for underwater sealing (**Fig. 4.1b**), plastic gloves and bag
- Tool to scratch the rock prior fixation (e.g. **diving knife**) and a cutting **pliers or scissors**

Figure 4.1: Illustrations of some of the material needed for protocol 4.

SAMPLING SITES

- The study site should be characterized by rocky bottoms with steep slope, facing open sea, to be exposed to dominant currents
- Standard depth levels to set up data loggers are every 5 m, from 5 to 40 m depth or more. However, different depth levels can be included if standard depths are not found at the study site
- Avoid known diving sites and inform diving centers about your data collection site

FIELD WORKERS EXPERIENCE

Temperature monitoring can be conducted by certified scuba divers, working in pairs.

METHODS

PERIOD OF DATA LOGGER SETUP AND RETRIEVAL

Setup/retrieve data loggers every 6 months, generally before and after the warm season (e.g. May, November). Yearly periodicity can be adopted for remote sites. Avoid interruption in data acquisition, particularly during the warm season. Ideally, using 2 sets of data loggers allows simultaneous recovery/setup in a single dive.

PROTOCOL 4: Monitoring temperature conditions

HOW TO OPERATE DATA LOGGERS:

DATA LOGGERS LAUNCHING

- ☑ Use Hoboware software, base station and the appropriate coupler to launch the temperature data loggers (**Fig. 4.2**)
- ☑ Set recording interval to 1 hour. Save logger's name as (site_yearmonth_depth)
- ☑ Label data loggers with depth of destination

DATA LOGGERS OFFLOADING

- ☑ Use Hoboware software, base station and the appropriate coupler to offload data from the loggers
- ☑ Make a backup copy of the data on external device (USB, hard drive)
- ☑ Warning, the loggers will need to be launched with correct parameters prior being placed again in the field

Figure 4.2: Optic base and coupler used for HOBO-U22 data logger launching and offloading.

PROTOCOL 4: Monitoring temperature conditions

HOW TO SETUP A NEW STUDY SITE:

STUDY SITE LOCATION AND DEPTHS

- Prepare the putty on boat, right before diving, using wet gloves
- Mix equal amount of the blue and yellow putties till it gets homogeneously green
- Keep putty in a wet plastic bag (e.g. ziploc). It is preferable to use it within 20 minutes

SEALING OF DATA LOGGER'S FIXATIONS

- Fixations should be set at standard depths (every 5 m from 5 to 40 m depth)
- To secure the temperature data loggers set 2 fixations for each logger
- Find a natural hole or small crevice on the rocky substrate where the 2 fixations can fit
- Scratch the substrate to bare rock and insert the putty to fill the hole/crevice. Press firmly the putty to increase adherence (**Fig. 4.3**)
- Insert the 2 fixations firmly. Press the putty around the fixations for robust sealing
- Let the putty harden 12 h before attaching the loggers
- Draw a plan to facilitate future finding of the different fixation points

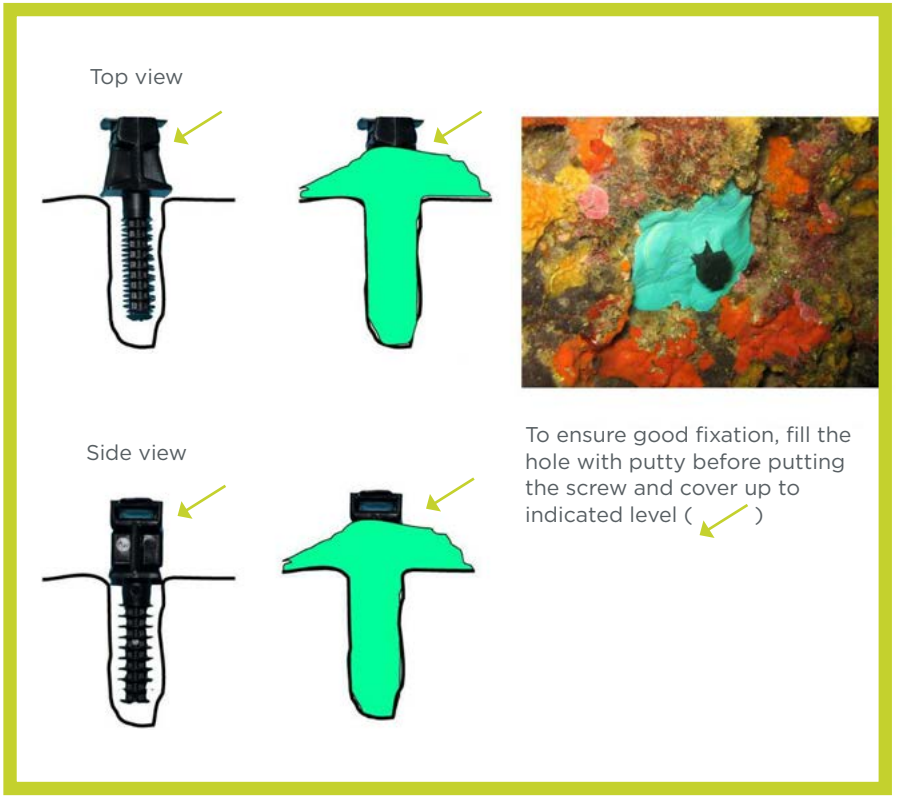


Figure 4.3: Illustration of the data loggers views and fixation.

PROTOCOL 4: Monitoring temperature conditions

DEPLOY/RECOVER THE DATA LOGGERS AT DEPTH

- ☑ Attach loggers to the fixation using Colson rings
- ☑ Retrieve data loggers using cutting pliers or scissors
- ☑ Warning: HOBO-U22 data loggers float!
- ☑ Keep note of date and time at which loggers were deployed/recovered at depth
- ☑ Check the video tutorial for illustration on the procedure (**Fig. 4.4**)

DATA REPORTING AND DATA POLICY

Register on the T-MEDNet web platform. Declare a new monitoring site or modify site/editor settings using online form. Upload raw data files using the dedicated interface. Receive notifications on data qualification and reporting. Access report on temperature conditions dedicated to each monitoring site. Video tutorials and presentations are available from the T-MEDNet and the MPA-Engage web platform describing the different actions to implement the protocol.

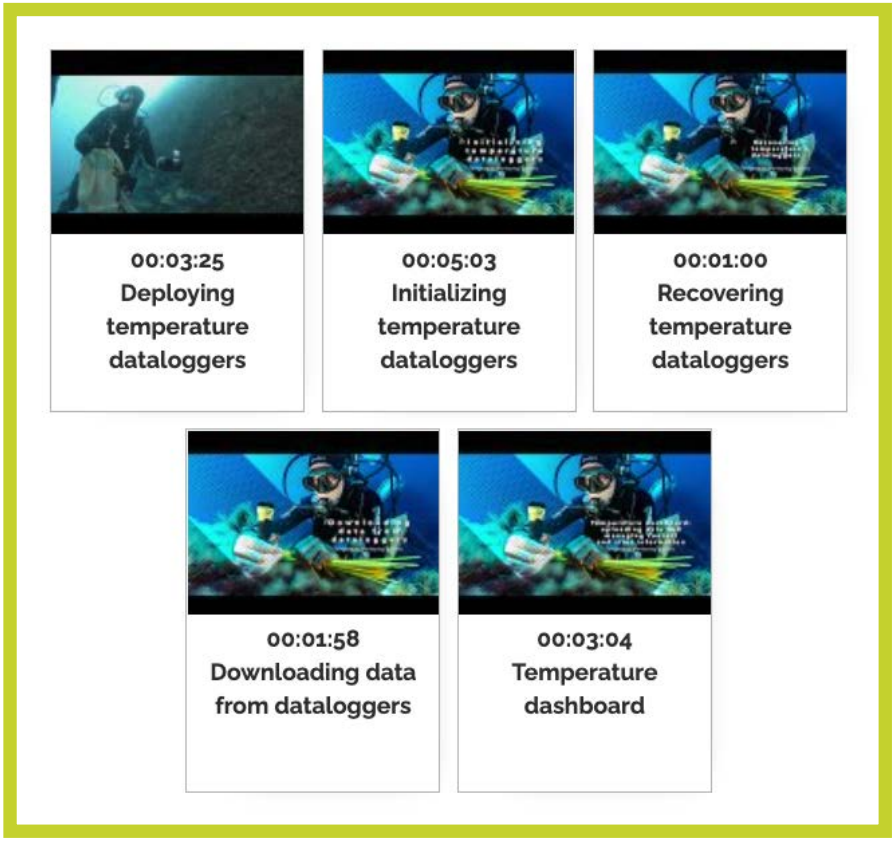


Figure 4.4: Screenshots of the different videotutorials available at the T-MEDNet web platform.

PROTOCOL 5

Assessment and Monitoring of Mass Mortality

PROTOCOL 5: Assessment and Monitoring of Mass Mortality

RATIONALE AND OBJECTIVES

The proposed protocol stems from the scientific and field experience gathered during the impact assessment of mass mortality events in different areas of North-Western Mediterranean. The protocol has been succesfuly applied to monitor the effects of mass mortality events in macrobenthic species such as gorgonians, sponges and bryozoans. The protocol aims to quantify the degree of affected specimens by mass mortality events within the surveyed populations. The mass mortality events are mainly associated with the onset of marine heatwaves, but there are other factors such as severe storms, blooms of mucilagenous algal species and sedimentation that can result in similar effects.

TARGET SPECIES

The protocol aims to monitor mass mortality events on some target species, notably:

- **Gorgonian species**
  Paramuricea clavata, Eunicella singularis, E. cavolini, Corallium rubrum, Leptogorgia sarmentosa
- **Corals**
  Cladocora caespitosa, Oculina patagónica, Astroides calycularis
- **Sponges**
  Ircinia fasciculata, Sarcotragus spinosulus, Spongia spp., Ircinia variabilis
- **Bryozoans**
  Myriapora truncata, Pentapora fascialis
- Criteria to select other target species: i) they have to be abundant in the study area and ii) easy to identify underwater

MATERIALS

Data collection will require the following materials:

- A **plastic board** to collect data underwater
- Diving **computer** to keep the **depth** of the survey
- A **reference** (e.g. a quadrat 50 × 50 cm or 25 × 25 cm, a bar 50 cm)

PROTOCOL 5: Assessment and Monitoring of Mass Mortality

SAMPLING SITES

Select 3 sites within the MPA separated by a minimal distance of about 0.2-0.5 Km. At each site select the upper distribution limit of the selected species to conduct the mortality monitoring surveys. However, it is recommended (when possible) to include a second mortality survey for each selected site below the seasonal thermocline depth (20-25 m). The selection of depth levels should correspond whenever possible to the depths where the temperature data loggers are set up (i.e. every 5 m from 5 to 40 m depth).

PERIOD OF MONITORING

Every 12 month after summer, from mid-September to mid-October. If this period is not possible you can also perform mortality assessments at any other time of the year, or in case of observed mass mortality.

FIELD WORKERS EXPERIENCE

Fieldwork can be carried out by trained professional scuba divers, working in pairs, as well as by recreational divers who received adequate training.

METHODS

The goal of the surveys is to observe a minimum of 100 specimens per mortality survey (if the species is not abundant in the monitoring site the total number of observed specimens per survey can be reduced to 20-30). To avoid bias in data collection use a reference (e.g. quadrat, bar, line) and define a criteria for the observation. For instance, using a bar, one could only count the colonies in contact with the bar or all inside an imaginary rectangle formed as a basis the length of the bar and the height of 10-15 cm. Surveys should be carried out around the selected depths (± 1 m).

In the surveys, do not take into account small colonies or specimens (e.g. < 15 cm in height for gorgonians), since looking for small colonies is not straightforward during the sampling.

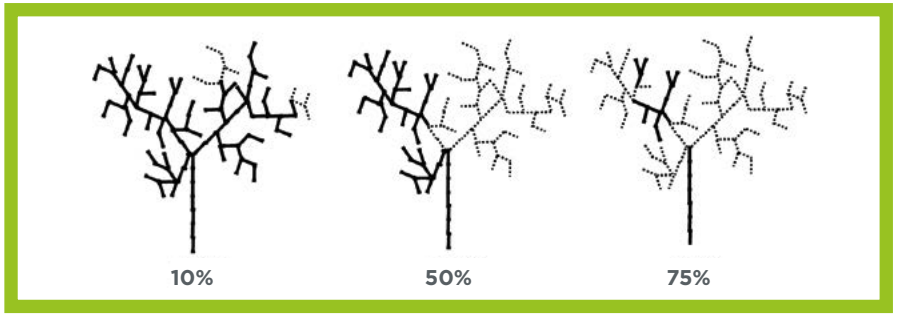


Figure 5.1: Estimation of the colony's extent of injury (adapted from Perez et al. 2000). According to the proposed protocol, colonies with >10% injured surface are considered as affected.

PROTOCOL 5: Assessment and Monitoring of Mass Mortality

During the surveys, for each observed colony (specimen), determine whether or not is affected by mortality. For gorgonian species we consider an affected colony when the colony displays more than 10 % of tissue/ skeleton necrosis/epibiosis (**Fig. 5.1**). For other macroinvertebrate species, in general, if they display necrosis they should be considered as affected (e.g. denuded skeletons of horny sponges). Field observations can be collected through photo quadrat sampling for species displaying small-sized colonies (e.g. *Astroides calycularis*).

Besides, for gorgonian surveys, for each affected colonies it should be also noted whether the mortality is: i) recent i.e. colonies showing presence of recent necrosis and/ or denuded skeletons and/or skeletons colonized by pioneering species such as hydrozoan species; ii) old i.e colonies displaying skeleton covered by epibionts species with thick calcareous skeletons such as bryozoans, calcareous algae); iii) or both with the features of recent and old mortality signs (see above, indicating that the colony suffered recent and past impacts of mortality). Examples of the three considered types of affected colonies are shown in the underwater board (**Fig. 5.2**). More examples on healthy and affected colonies are displayed at the end of the protocol description (**Fig. 5.3**).

Check the mortality assessment video-tutorials for operational details.

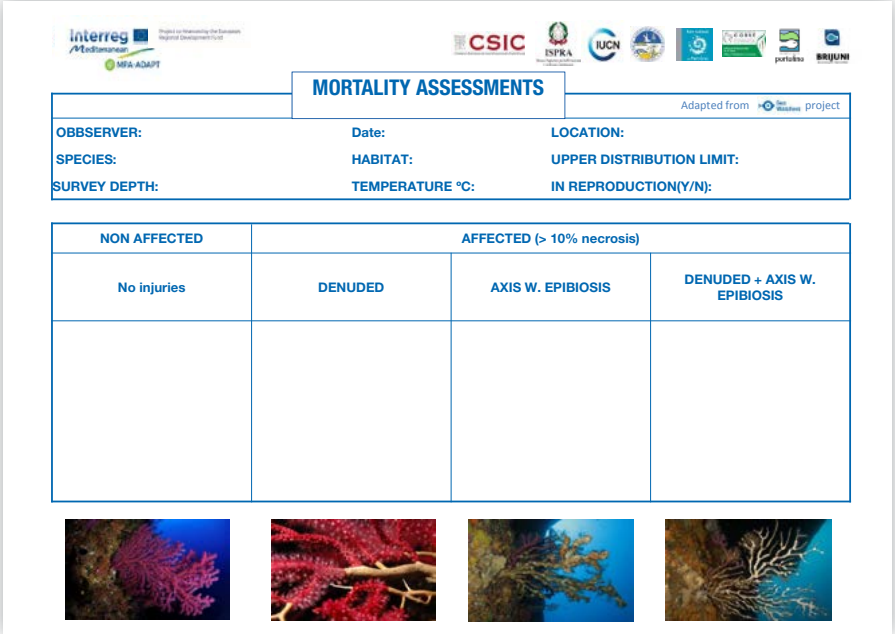


Figure 5.2: Underwater board template for mortality assement monitoring protocol.

DATA REPORTING AND DATA POLICY

Upload the data collected for each site and depth in the T-MEDNet web platform. Video-tutorials and presentations will be available at the T-MEDNet and the MPA-Engage web platform describing the different actions to conduct mass mortality assessments monitoring protocols from data collection to data reporting.

PROTOCOL 5: Assessment and Monitoring of Mass Mortality

Red gorgonian *(Paramuricea clavata)*

White gorgonian *(Eunicella singularis)*

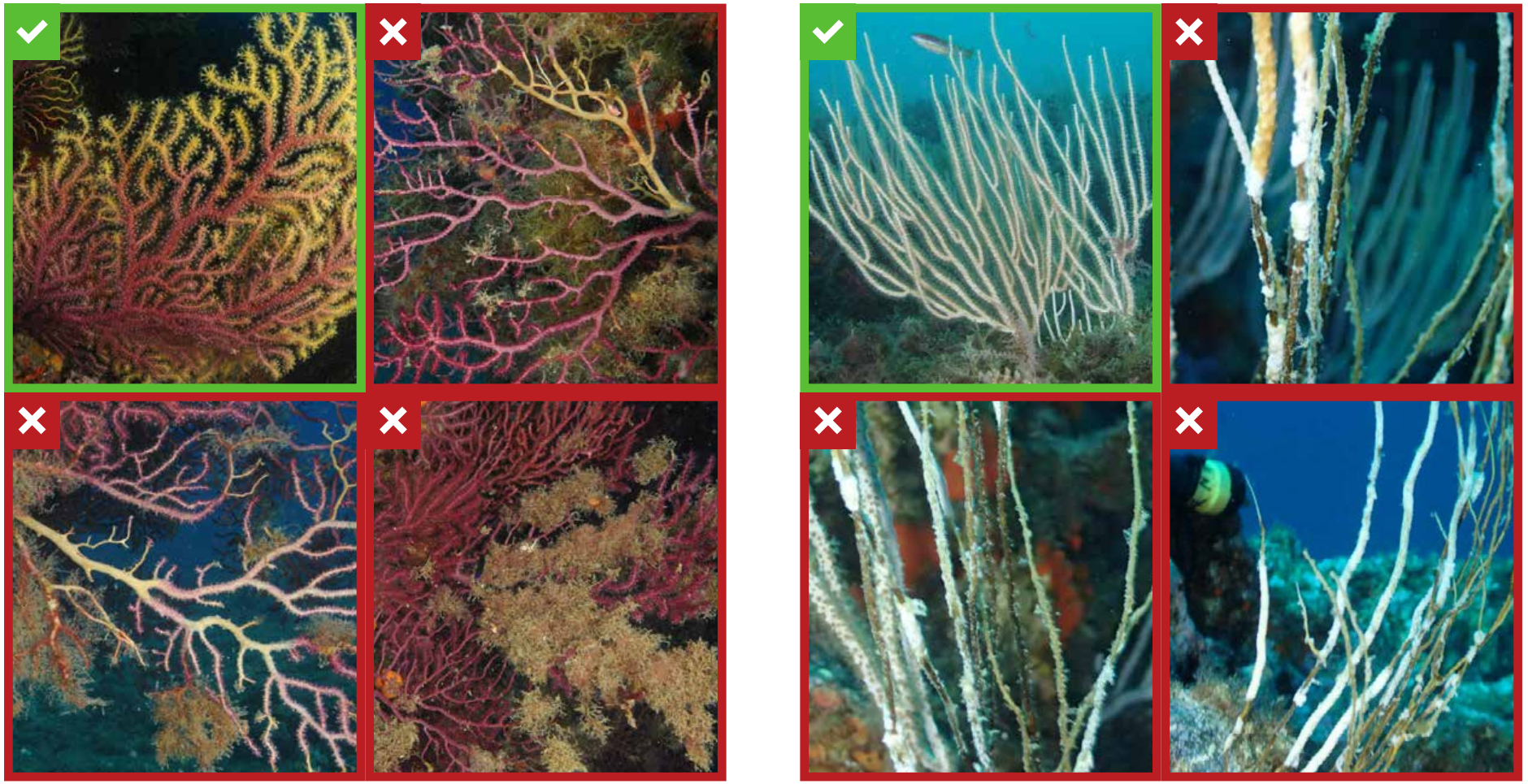


Figure 5.3: Illustration of healthy (✓) and affected (✗) gorgonian and coral colonies and sponge specimens.

PROTOCOL 5: Assessment and Monitoring of Mass Mortality

Yellow gorgonian *(Eunicella cavolini)*

Cushion coral *(Cladocora caespitosa)*

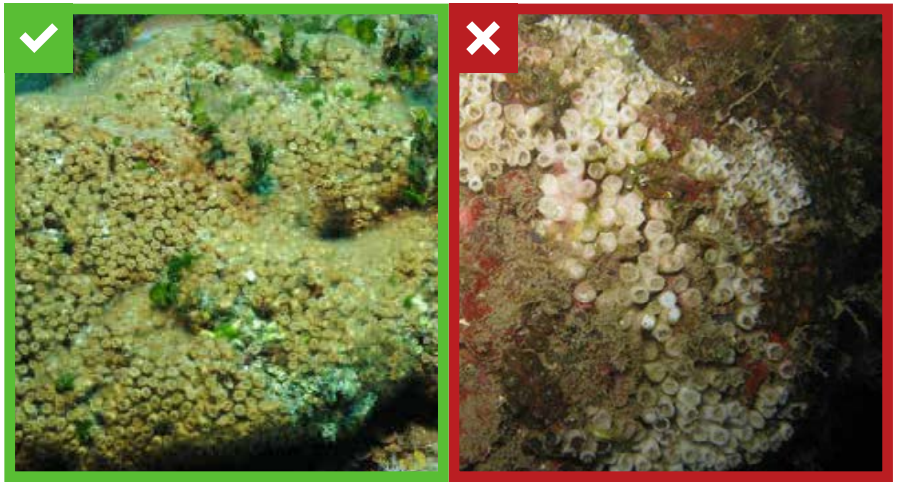


Figure 5.3: Illustration of healthy (✓) and affected (✗) gorgonian and coral colonies and sponge specimens.

PROTOCOL 5: Assessment and Monitoring of Mass Mortality

***Spongia* spp.**

Ircinia variabilis

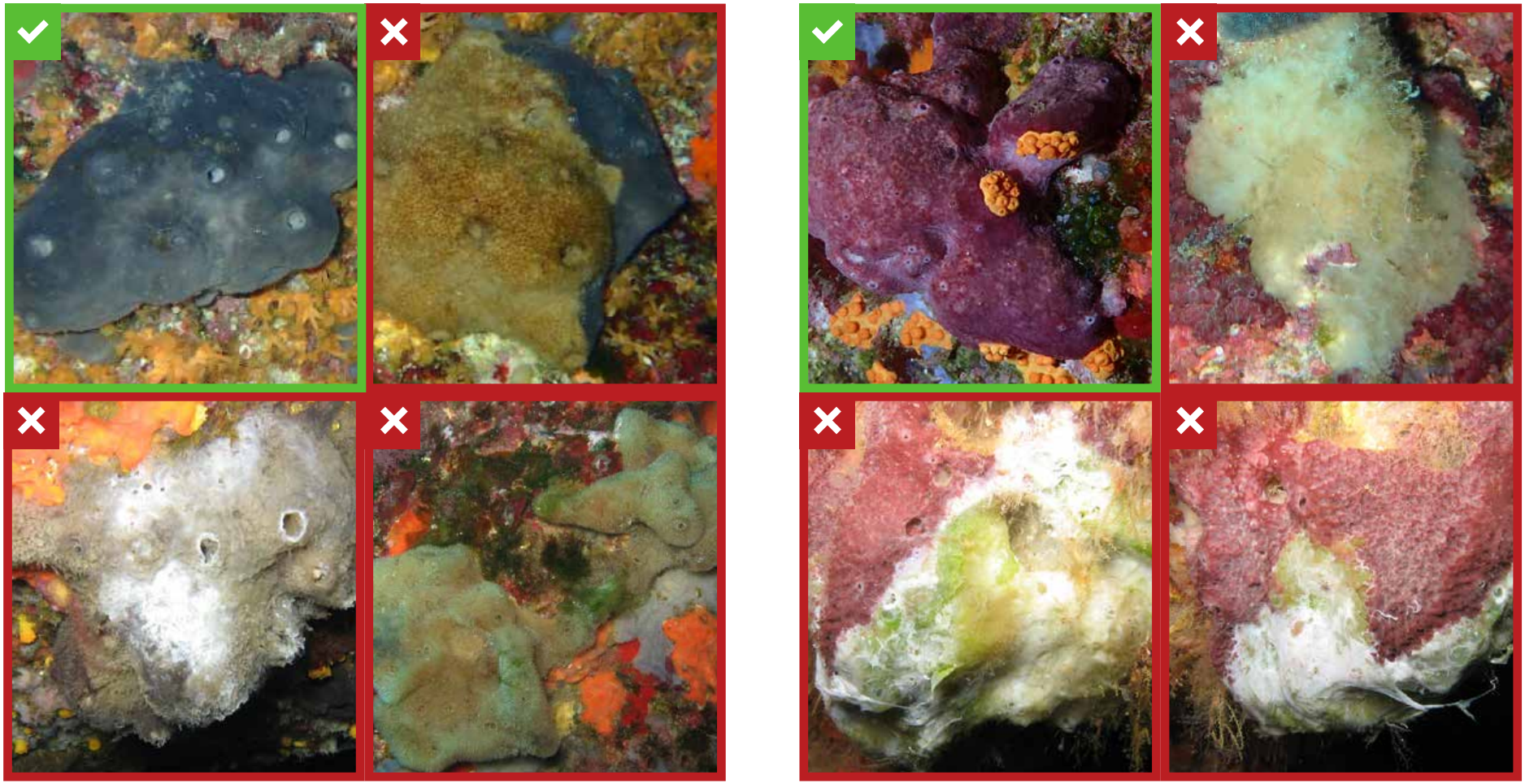


Figure 5.3: Illustration of healthy (✓) and affected (✗) gorgonian and coral colonies and sponge specimens.

PROTOCOL 6

Fish Visual Census of Climate Change Indicators

PROTOCOL 6: Fish Visual Census of Climate Change Indicators

RATIONALE AND OBJECTIVES

Changes in distribution and abundance of fish species is one of the most apparent signals of climate warming worldwide. This is particularly clear in a number of coastal Mediterranean fishes, which are becoming more or less distributed, according to their thermal affinity and origin. Here is described a simplified visual census methodology to monitor this phenomenon on a set of coastal fish species. The method, adopted by several Mediterranean countries, was specifically intended to be used by a wide group of observers over large geographical scales and on a periodical basis.

TARGET SPECIES

Based on previous scientific experiences, the following fish species (MED Targets) are used as reliable indicators of climate change in all the Mediterranean MPAs:

- Eight **native fishes**: *Sparisoma cretense, Epinephelus marginatus, Thalassoma pavo, Sarpa salpa, Serranus scriba, Coris julis, Serranus cabrilla* (**Fig. 6.1**)
- Plus ALL the observed **exotic fishes** (*Siganus luridus, Siganus rivulatus, Fistularia commersonii, Parupeneus forskali, Torquigner flavimaculosus, Pterois miles* and others) (**Fig. 6.2**)
- Additional species (local targets) can be eventually added by each MPA, according to local monitoring needs (e.g. vulnerable species), easiness of recognition, interaction with fisheries, increase/decrease in the area, potential impacts on the environment/fisheries/human activities

PROTOCOL 6: Fish Visual Census of Climate Change Indicators

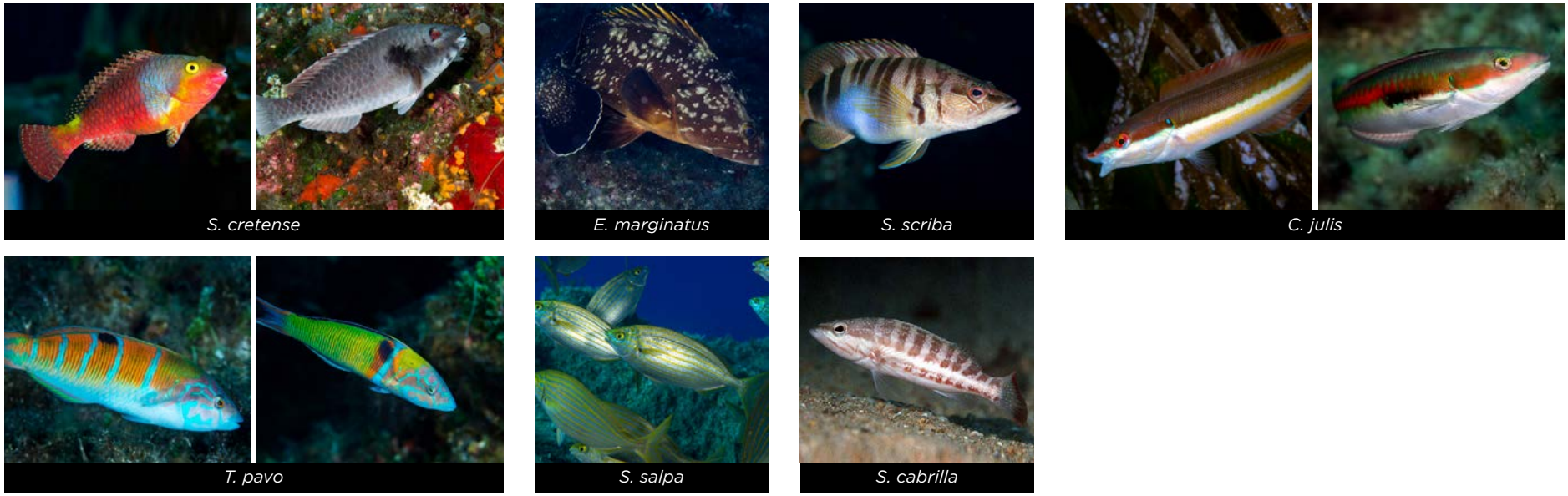


Figure 6.1: Native species.

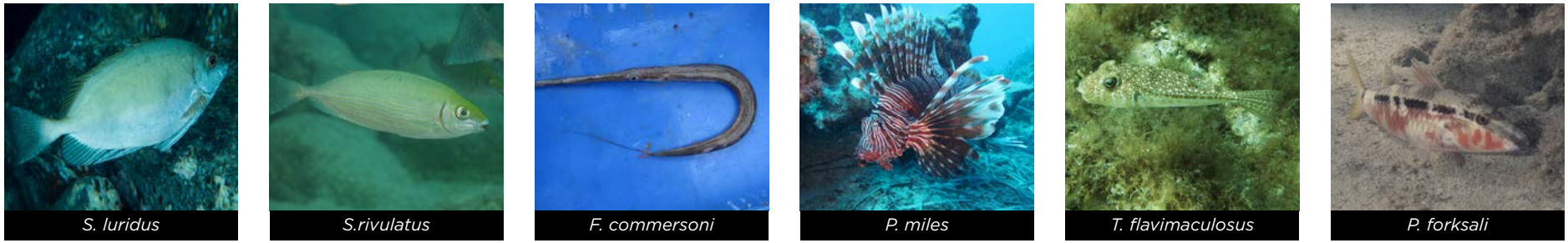


Figure 6.2: Exotic species.

PROTOCOL 6: Fish Visual Census of Climate Change Indicators

MATERIALS

- Pre-printed **Board for collecting underwater data** (**Fig.6.3**) to collect data underwater
- **Underwater watch** to measure 5 minutes
- **Paper sheet** or logbook where to copy the data from the plastic board
- **Computer/thermometer** to measure water temperature

Note: all the materials and training tools are freely downloadable from the MPA-Engage and the T-MEDNet web platform.

SAMPLING SITES

Fish counts can be only performed over **Rocky bottoms with moderate slope**. Sandy bottoms or rocky bottoms covered by *Posidonia oceanica* must be avoided. Depth ranges are 1-3 m, 5-10 m, 11-20 m, 21-30 m. At 1-3 m, censuses can be performed by either snorkeling on the surface or SCUBA.

PERIODICITY OF MONITORING

Between August and October, every 12 months.
For recreational divers, the censuses can be performed any time of the year.

FIELD WORKERS EXPERIENCE

Scientific divers are skilled to recognize and count species underwater. **Note:** the protocol can also be adopted by trained recreational divers (to this aim, see the project *ClimateFish* at Observadores del Mar web platform).

METHODS

SAMPLING DESIGN

Within your study area select at least 3 permanent locations separated by a minimal distance of about 0.5 Km. At each location and for each depth layer, each diver will perform 4 consecutive transects. It is suggested to work in pairs and to perform 4 transects each (8 transects in total).

The depth of 1-3 m is the most important one: you might choose to monitor only this layer by snorkeling. In this case, the minimum number of transects that is requested each year will be equal to: (4 transects + 4 transects) × 3 permanent locations × 1 depth layer = 24 transects.

PROTOCOL 6: Fish Visual Census of Climate Change Indicators

HOW TO COUNT FISHES

- Swim **VERY slowly** underwater (speed about 10 m/minute) for 5 minutes, covering a distance of about 50 m (**Fig. 6.4**)
- While swimming forward, count all the species and individuals you observe within a radius of 2.5 m, because the transect is 5 m-wide. Hence, do not count fishes if they are very far from you
- Do not count fishes if they are smaller than 2 cm
- Once you have finished the first transect (after 5 minutes) you can proceed in the same direction starting a new transect

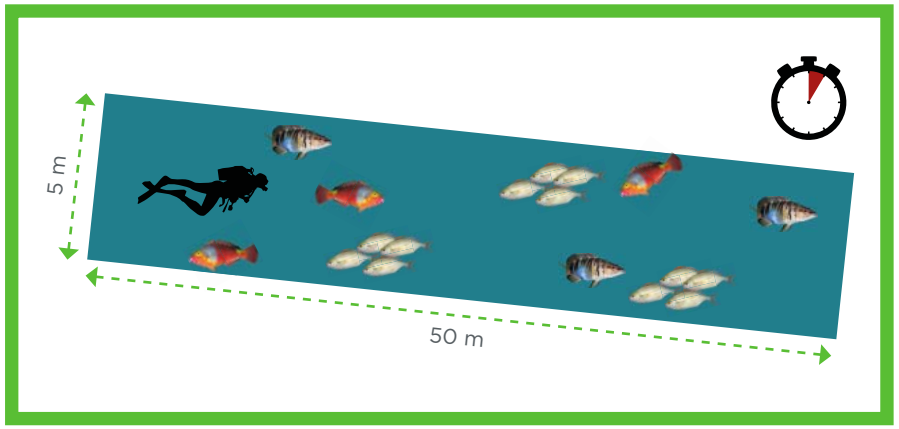


Figure 6.4: Illustration of the transect for counting fishes in protocol 6.

DATA REPORTING AND DATA POLICY

Upload the data collected for each site and depth in the T-MEDNet web platform. Input your data to **DATA_Visual.xlsx**. Data can be used by each MPA, to build time series and track changes in the relative abundance of indicator species. Your data can also be shared with a large Mediterranean network of participating MPAs and other relevant stakeholders. For further information on data collection, sharing and reuse, please contact ernesto.azzurro@cnr.it or garrabou@icm.csic.es.

Recreational divers can upload their data through the project *ClimateFish*.

PROTOCOL 6: Fish Visual Census of Climate Change Indicators

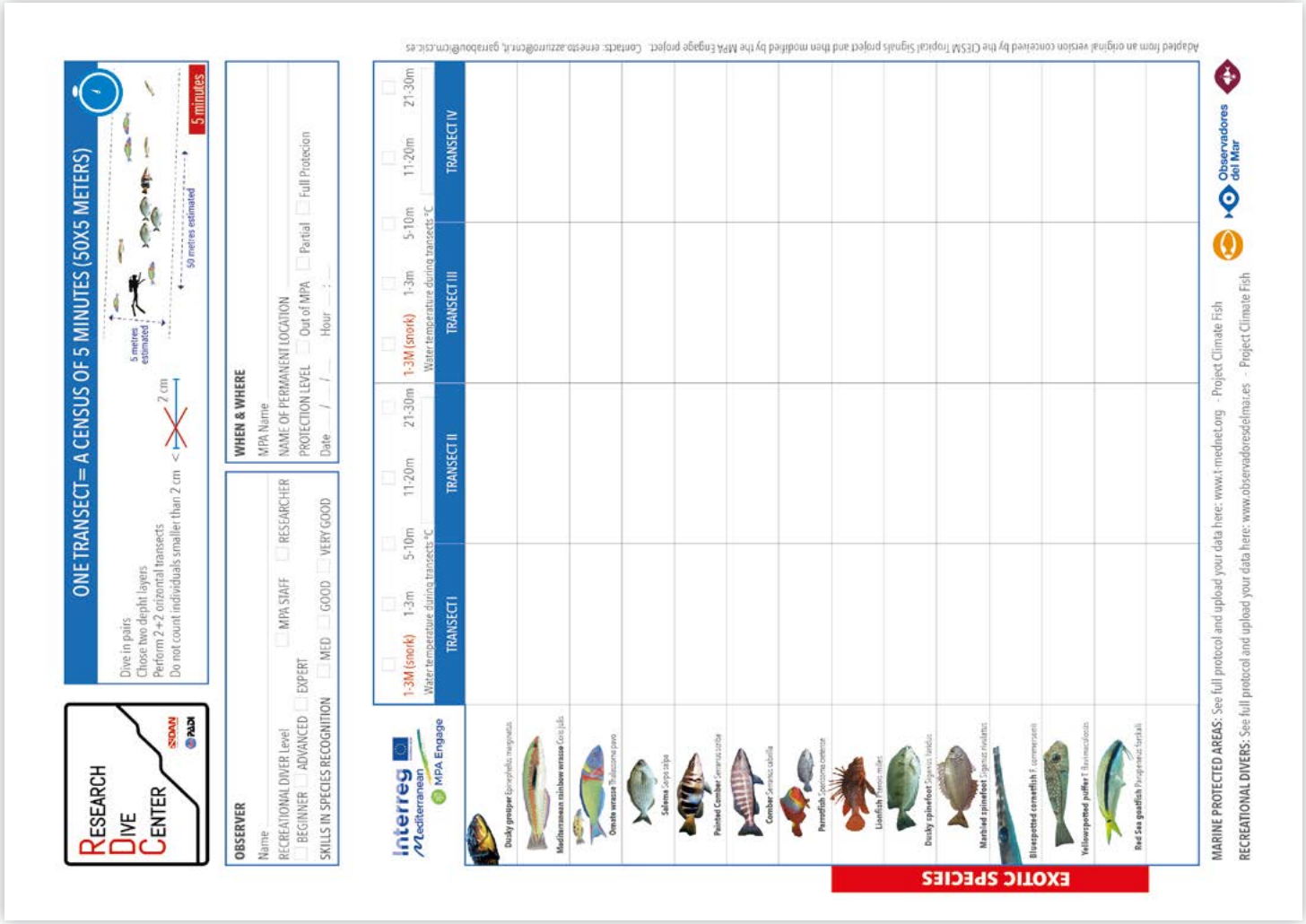


Figure 6.3: Board for collecting underwater data.

PROTOCOL 7

URCH - Sea Urchins population

PROTOCOL 7: URCH -Sea Urchins population

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 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.

The human activities caused the degradation of macroalgal forests mainly overfishing, contamination and habitats 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.

Focusing on climate change effects, the most common sea-urchin species in the shallow rocky reefs display a differential thermal affinity, *Paracentrotus lividus* "cold-water" and *Arbacia lixula* "warm-water" species. The ongoing warming in the Mediterranean is affecting the abundance and distribution of sea-urchins in different areas, including the local extinction of *P. lividus* populations in the eastern Mediterranean. Therefore, we can expect dramatic changes in the macroalgal beds due to changes in the abundance of sea-urchins. Besides warming is favoring 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 HAbitat Rapid Assessment (BHARA) monitoring protocols may contribute to define management actions towards conservation of the productive and high-diverse macroalgal beds in the Mediterranean MPAs.

The main objective of the URCH protocol is to determine the structure and the dynamics of sea-urchins populations in the shallow rocky habitats within the MPAs.

PROTOCOL 7: URCH -Sea Urchins population

TARGET SPECIES

The target species to monitor are the sea urchins *Paracentrotus lividus* and *Arbacia lixula* (**Fig. 7.1**) dwelling in the infralittoral rocky habitats with different cover of macroalgal species.

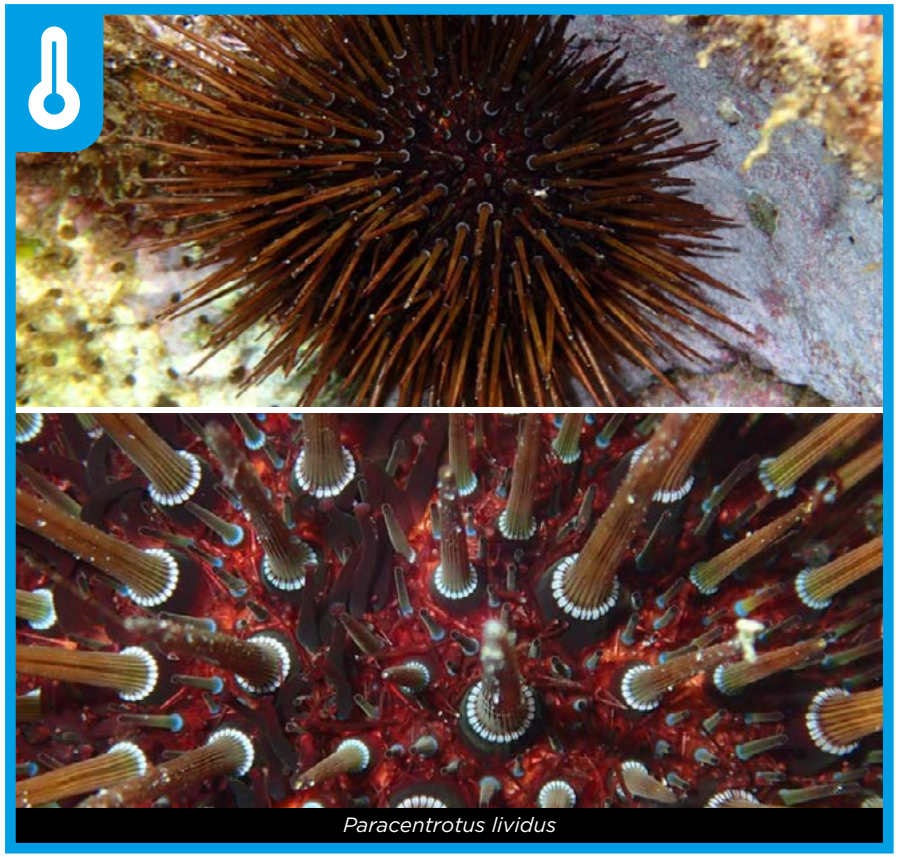


Figure 7.1: Images of the species *P. lividus* and *A. lixula*.

PROTOCOL 7: URCH -Sea Urchins population

MATERIALS

- ☑ Caliper
- ☑ Slate with pencil
- ☑ 1 m bar
- ☑ 50m measuring tape
- ☑ Gloves
- ☑ Underwater board (**Fig. 7.2**)

SAMPLING SITES

The study sites should be characterized by rocky bottoms (preferably with moderate slopes) between 3 and 10 m depths.

PERIODICITY OF MONITORING

Sampling should be performed every 12 months in the late summer since in this period the erect seasonal algae shows a lower development facilitating the sampling.

FIELD WORKERS EXPERIENCE

MPA managers and scientific divers skilled to recognize and count species underwater, working in couple. The protocol can be as well adapted for trained recreational divers.

METHODS

SAMPLING DESIGN

Within your study area select at least 3 permanent locations with similar habitat conditions separated by a minimal distance of about 0,2-0.5 Km. At each location set a depth between 3 and 10 m where 3 (50m × 1 m) transects will be deployed (**Fig. 7.3**).

CHARACTERIZING SEA-URCHINS POPULATIONS

The abundance and population structure of *Paracentrotus lividus* and *Arbacia lixula* is determined by SCUBA diving along three consecutive transects (50 × 1 m each).

Transects are divided into five 10 m^2 subtransects with intervals of 5 m and they shoulb be paralel to the coast line and keeping similar depth. Within each transect, *P. lividus* and *A. lixula* >1 cm in diameter are counted and their diameters (test without spines) were measured with a caliper.

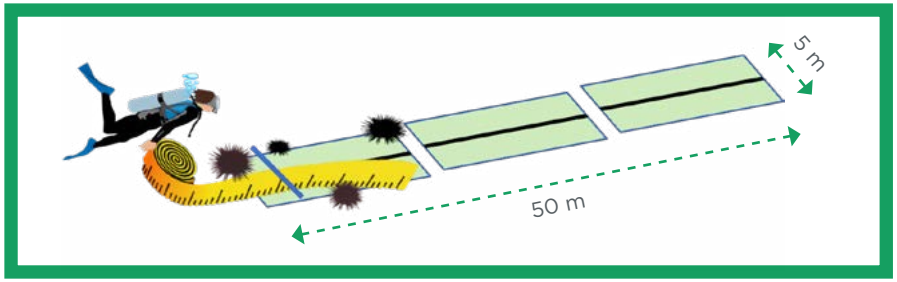


Figure 7.3: Illustration of the transects for the protocol 7.

PROTOCOL 7: URCH -Sea Urchins population

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.

DATA REPORTING AND DATA POLICY

Input your data on the provided specific Excel file which was designed for data storage, analysis and data reporting is available.

Note: all the materials and training tools are freely downloadable from the MPA-Engage web platform.

PROTOCOL 7: URCH -Sea Urchins population

Site:			Depth:		Name:
Date:			Habitat:		
SIZE	SUBTRANSECT 1	SUBTRANSECT 2	SUBTRANSECT 3	SUBTRANSECT 4	SUBTRANSECT 5
1					
2					
3					
4					
5					
6					
7					
8					

Figure 7.2: Underwater board to conduct the sea-urchins monitoring.

PROTOCOL 8

BHARA - Benthic Habitat Rapid Assessment

PROTOCOL 8: BHARA - Benthic Habitat Rapid Assessment

RATIONALE AND OBJECTIVES

Marine benthic ecosystems provide a wide variety of goods and services, including the provision of habitat, food and shelter for most organisms in coastal habitats. Monitoring changes on biological characteristics, species composition, the arrival and colonization of non native species as well as the impacts of major disturbances such as climate change, is required to assess the conservation status of rocky benthic habitats. When provided with cost-effective protocols, MPA managers can substantially contribute to this monitoring effort and thus, reduce management response-time for the identification and the implementation of measures to support the recovery of rocky benthic habitats.

TARGET SPECIES AND HABITATS

Infralitoral and circalitoral rocky habitats.

Specially important groups in terms of habitat structure (e.g. canopy forming algae, massive or erect invertebrates, barrens) and alien species that have been identified in other areas in the Mediterranean.

A list of target categories is provided in the methods section.

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
- ☑ Guides of native and non indegenous species and main benthic categories to be identified from photographs. Specifically, for the non indigenous species, we will provide a plastic board, bearing identification drawings of the main benthic exotic species identified in the Mediterranean Sea. However, during your sampling, you can discover new species introduced in the Mediterranean Sea. Therefore, close up pictures of any strange or new species will be very helpful for their identification.

SAMPLING SITES

Select a minimum of 3 sites within the MPA separated by a minimal distance of about 0,2-0,5 km when possible. Depending on the habitat diversity and features in the MPA, the number of sampling sites should be increased to ensure the detection of changes in the benthic condition and the alien benthic species.

PROTOCOL 8: BHARA - Benthic Habitat Rapid Assessment

PERIODICITY OF MONITORING

Sampling should be run twice a year in summer and winter to detect the presence of non indigenous 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 spring-summer, and at the same time each year. Perennial algae species display their greatest growth over spring-summer making them easier to detect at this time.

FIELD WORKERS EXPERIENCE

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

METHODS

SAMPLING DESIGN

In the selected sites, dive along a linear transect perpendicular to the shore. When you reach 30m depth (or the deepest point in the monitored site) take 10 photographs of 25 x 25 cm at random within an area of 100 m^2 (**Fig. 8.1**). Repeat the same procedure at 20 and 10 meters depth (**Fig. 8.2**). During the dive you can take additional photographs or notes on the presence of conspicuous alien benthic species in other depths ranges.

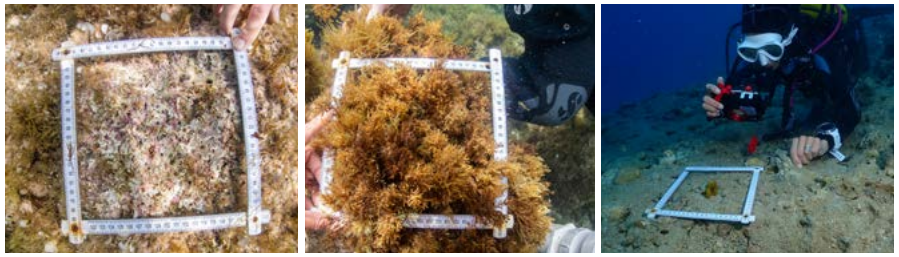


Figure 8.1: Examples of photographs obtained from the implementation of BHARA.

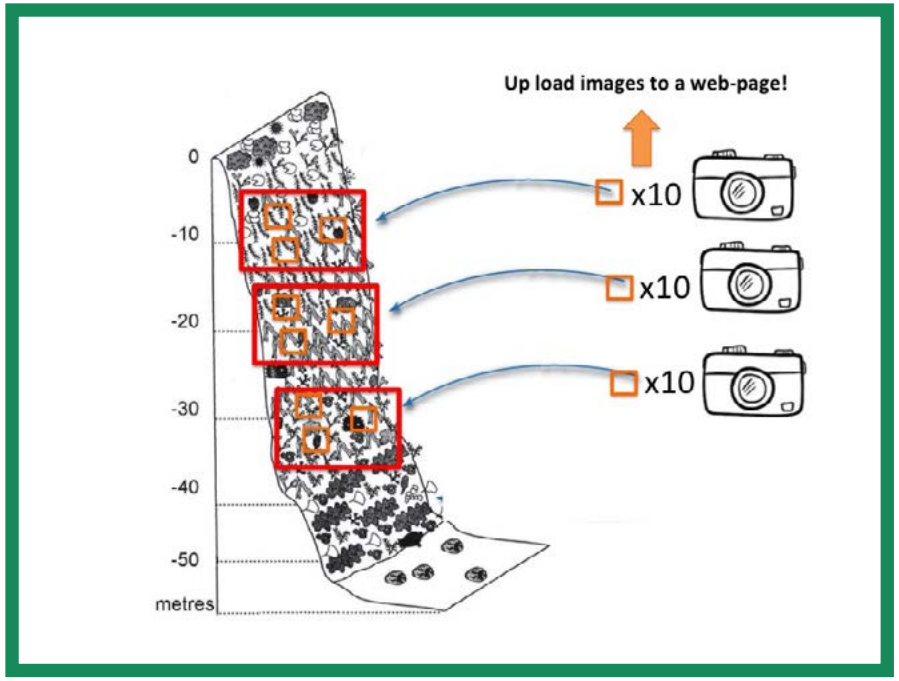


Figure 8.2: Perpendicular transect to the shore, with photograph sampling at -30, -20 and -10m.

PROTOCOL 8: BHARA - Benthic Habitat Rapid Assessment

IMAGE ANALYSIS

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.

For each photo the percentage cover of the species and categories is obtained from their presence in 5 x 5 cm subquadrats.

To analyze the pictures and determine the abundance of each invasive species and of the main benthic categories/ species in the community, see section benthic categories.

BENTHIC CATEGORIES

- ☑ Bare rock
- ☑ Holes
- ☑ Sediment
- ☑ Calcareous red algae
- ☑ Dead calcareous red algae
- ☑ Brown turf
- ☑ Red turf
- ☑ Green turf
- ☑ Red erect and canopy algae
- ☑ Brownish erect algae
- ☑ Brown canopy algae
- ☑ Encrusting invertebrates
- ☑ Dead encrusting invertebrates
- ☑ Erect invertebrates
- ☑ Death erect invertebrates
- ☑ Massive invertebrates
- ☑ Death massive invertebrates
- ☑ Solitary invertebrates
- ☑ Death solitary invertebrates

DATA REPORTING AND DATA POLICY

A specific Excel file is provided for data reporting and for the calculation of different indicators for the different depth ranges sampled.

Divers can also report their observations of non indigenous benthic species to Observadores del Mar web platform.

PROTOCOL 9

POFA - *Posidonia oceanica* fast assessment

PROTOCOL 9: POFA - *Posidonia oceanica* fast assessment

RATIONALE AND OBJECTIVES

The proposed protocol stems from the scientific evidence that seagrass shoot mortality rates increase with increased temperature, thus the ongoing warming in the Mediterranean is one of the drivers, along with various direct anthropogenic activities (e.g. pollution, fishing, anchoring, coastal works), which lead 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 meadow health condition. Also, flowering events (or the presence of fruits) will also be collected as their intensity and frequency have been recently correlated with the occurrence of marine heat waves. Once the baselines have been established, long-term monitoring based on annual surveys will provide indications on the evolution of the conservation status of the meadows. Coupled to the assessment of the main local pressures, the POFA protocol can contribute to unravel the effects of different impacts and define management actions for the conservation of this outstanding Mediterranean habitat.

TARGET SPECIES

The target species to monitor is *Posidonia oceanica* (Linnaeus) Delile, 1813 (**Fig. 9.1**).

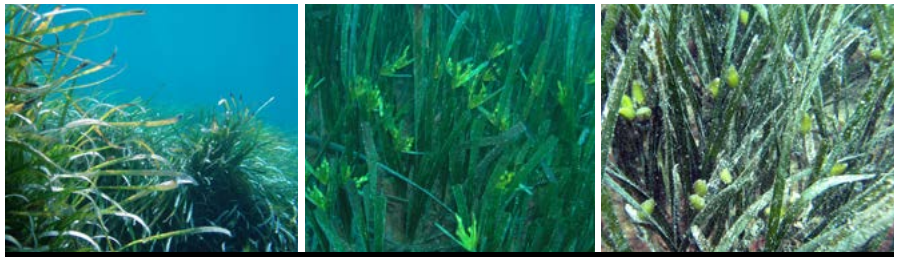


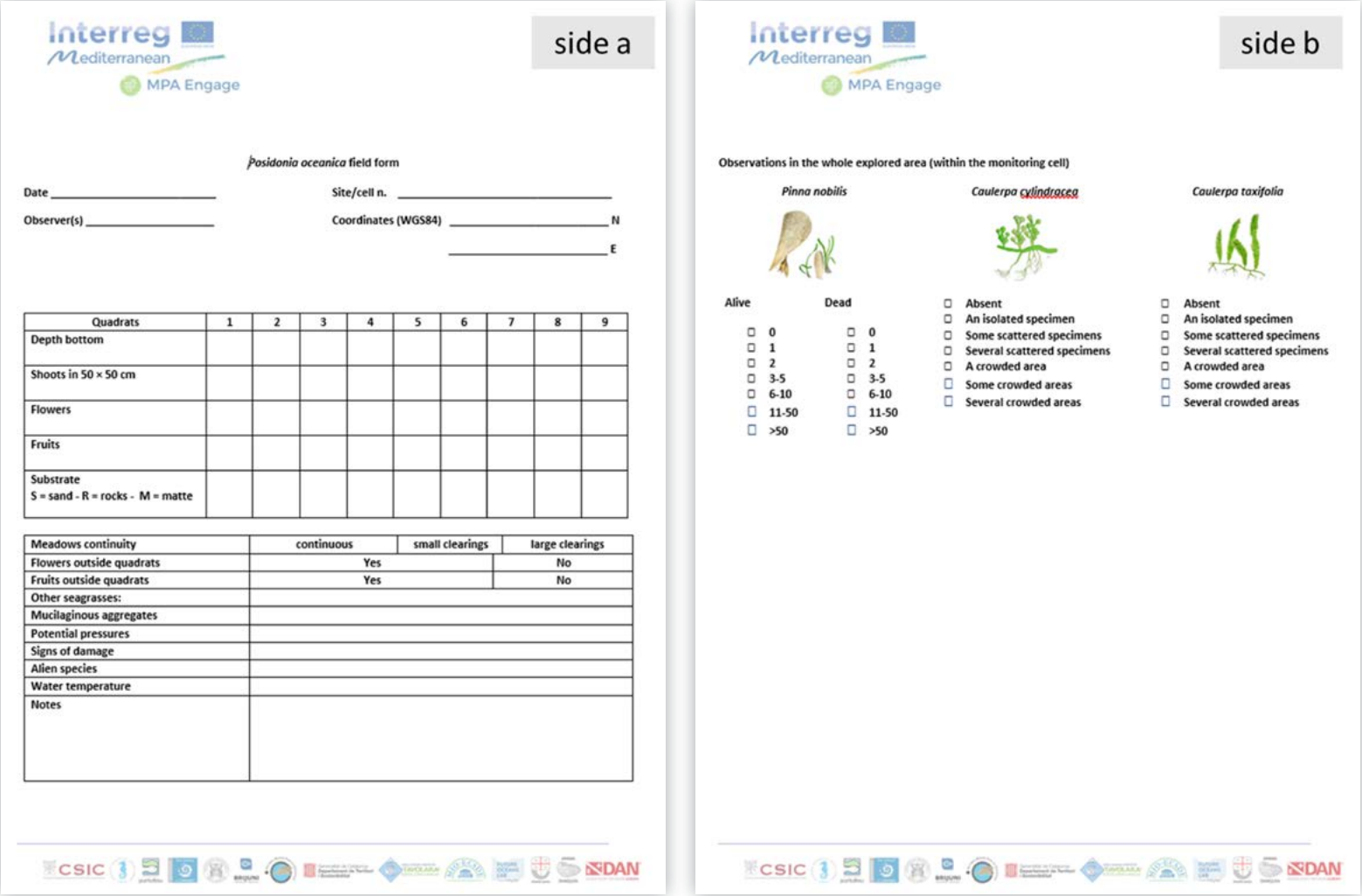
Figure 9.1: Images of the target species *Posidonia oceanica*.

MATERIALS

Data collection will require the following materials:

- ☑ Greferenced maps of the meadows within the MPA, in preference divided into 100 × 100 m cells (optional)
- ☑ A 50 × 50 cm quadrat
- ☑ A plastic slate, possibly pre-organised in data fields on both sides (see example below)
- ☑ A diving computer to record the depth of the survey
- ☑ A GPS (on board)
- ☑ A diving buoy with ballast
- ☑ Board for data collection (**Fig. 9.2**)

PROTOCOL 9: POFA - *Posidonia oceanica* fast assessment



side a

Posidonia oceanica field form

Date _____ Site/cell n. _____

Observer(s) _____ Coordinates (WGS84) _____ N

_____ E

Quadrats	1	2	3	4	5	6	7	8	9
Depth bottom									
Shoots in 50 × 50 cm									
Flowers									
Fruits									
Substrate S = sand - R = rocks - M = matte									

Meadows continuity	continuous	small clearings	large clearings
Flowers outside quadrats	Yes		No
Fruits outside quadrats	Yes		No
Other seagrasses:			
Mucilaginous aggregates			
Potential pressures			
Signs of damage			
Alien species			
Water temperature			
Notes			

side b

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

Figure 9.2: Board for data collection for protocol 9.

PROTOCOL 9: POFA - *Posidonia oceanica* fast assessment

SAMPLING SITES

The number of study sites depends on the variety and extent of the habitat and the possible pressures occurring in the MPA area. Extensive meadows with moderate slopes should be prioritized. Depending on local conditions, different depth levels between 1 and 40 m can be selected (if possible, choose some sampling stations at depths of 15 m for greater comparability between MPAs on a Mediterranean scale.

PERIODICITY OF MONITORING

Period for monitoring should be summer to autumn on an annual basis. From the end of September-October, flowering events might be more likely, thus 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).

FIELD WORKERS EXPERIENCE

Fieldwork can be carried out by skilled scientific divers, working in pairs, as well as by volunteer recreational divers who received adequate training.

METHODS

SAMPLING DESIGN

The minimum number of sites to be sampled depends on local conditions and will be defined by the MPA staff in agreement with the experts, if necessary. For large meadows it is possible to define a grid of sub-areas, for example by dividing them into cells of 100 × 100 m; a site can include multiple cells. Once in the middle of the cell (sampling station), anchor the boat (or the diving buoy) and mark the GPS position. A total of 9 quadrats (50 × 50 cm) will be sampled in each sampling station in a 50 m radius from the diving point (**Fig. 9.3**).

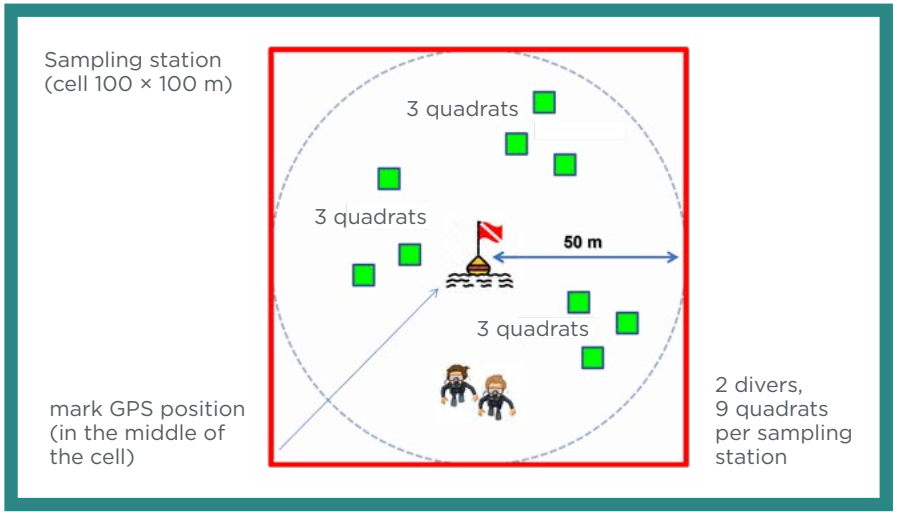


Figure 9.3: Illustration of the sampling design for porotocol 9.

PROTOCOL 9: POFA - *Posidonia oceanica* fast assessment

HOW TO COUNT LEAF SHOOTS

The quadrats will be randomly dropped onto the bottom (few meters each other) within the meadow. Each quadrat is pushed to the base of the rhizomes, depth is recorded and all shoots inside must be counted; each shoot consists of one rhizome and the leaves, shoots in division are counted twice (**Fig. 9.4**). Counting should start from one corner of the quadrat, taking care to separate with the hands the shoots already counted from those to be counted. Once number of shoots and depth have been recorded on the slate, move randomly a few meters to drop the following quadrat; repeat the procedure until you have counted 9 quadrats for each sampling station.

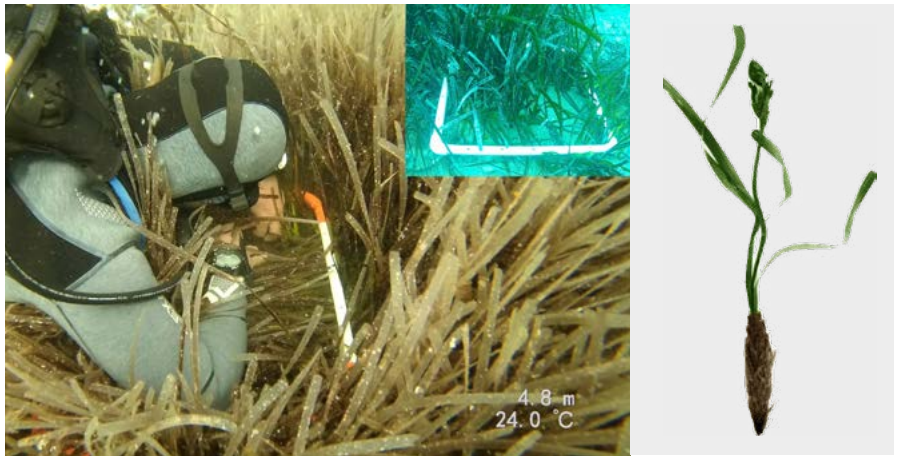


Figure 9.4: Picture of the position of the quadrats (left) and image of one shoot (right).

FLOWERING EVENTS

Count the number of flowers or fruits found within each quadrant; also record their presence outside the quadrats, if any.

ADDITIONAL OBSERVATIONS

Record the type of substrate (sand, matte, rock) and whether the meadow is continuous or discontinuous; for the latter, indicate whether there are small (i.e. 1-2 m) or large (<2 m) clearings.

Other additional observations can be recorded in each sampling station including ranges of abundance:

- ☑ Presence of flowers or fruits outside the quadrats
- ☑ Presence of noble pen shells (*Pinna nobilis*) and status (dead or alive). This information can be used to identify sampling sites for the *Pinna nobilis* monitoring protocol (FAP)
- ☑ Presence of other seagrasses (e.g. *Cymodocea nodosa*)
- ☑ Presence of alien species (e.g. *Caulerpa cylindracea*, *C. taxifolia*, *Asparagopsis* spp.)
- ☑ Presence of mucilaginous aggregates
- ☑ Presence of potential pressures (e.g. mooring systems, concrete blocks, pier, anchors, chains, ropes, trash, other)

PROTOCOL 9: POFA - *Posidonia oceanica* fast assessment

☑ Presence of clear signs of damage of the meadow (e.g. detached shoots, detached plates of matte, damages due to trawling or anchoring, other)

DATA REPORTING AND DATA POLICY

Input your data on the provided specific Excel file (**POFA_ datafile_Annex II**) which was designed for data storage, analysis and data reporting. Follow the guidelines and enter data and additional observations at both the scale of site and quadrat. Shoot density (n. shoots per m^2) and Flowering Intensity (FI = the ratio of the number of inflorescences and the number of shoots counted within sampling quadrats) are automatically generated as field data are entered. The Flowering Prevalence (FP = the ratio of the number of sites/ meadows flowering in a given year and the total number of sites monitored at each MPA) can also be calculated.

Note: all the materials and training tools are freely downloadable from the MPA-Engage web platform.

Collected data can be used by each MPA, to track changes of meadow status over time. Your data can also be shared with a large Mediterranean network of participating MPAs and other relevant stakeholders. For further information on data collection, sharing and reuse, please contact i.guala@fondazioneimc.it, garrabou@icm.csic.es, c.cerrano@staff.univpm.it.

PROTOCOL 10

FAP - Fast assessment of *Pinna nobilis* conservation

PROTOCOL 10: FAP - Fast assessment of *Pinna nobilis* conservation

RATIONALE AND OBJECTIVES

The noble pen shell *Pinna nobilis Linnaeus*, 1758 is the largest endemic Mediterranean bivalve, reaching a size of up to 120 cm. It lives mainly in soft-sediment areas and beds of seagrass *Posidonia oceanica* (Linnaeus), Delile and *Cymodocea nodosa* (Ucria) Ascherson, but occasionally found also on irregular rocky substrates (scattered boulders), at depths ranging from 0.5 to 60 m. It is an ecosystem engineer species, providing hard substrate for many other organisms and able to filter high volumes of water. *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 has been documented since the end of the last century following the ban of illegal practices and the adoption of conservation measures. However, since 2016, different pathogens (an haplosporidian, mycobacteria and vibrios), with some of them never reported before, have caused dramatic mortality events of *P. nobilis* along the Western Mediterranean basin and approaching to the Eastern side. The present protocol offers a rapid tool to assess the health status of *P. nobilis* populations in all the Mediterranean MPAs.

The main objective is to determine the abundance, the size structure and the health status of *Pinna nobilis* populations within the MPAs.

TARGET SPECIES

The target species to monitor is noble pen shell *Pinna nobilis* (Linnaeus 1758) ranging from 0.5 to 60 m in different habitats. Also the eventual co-occurrence of the co-generic *Pinna rudis* will be assessed (**Fig. 10.1**).

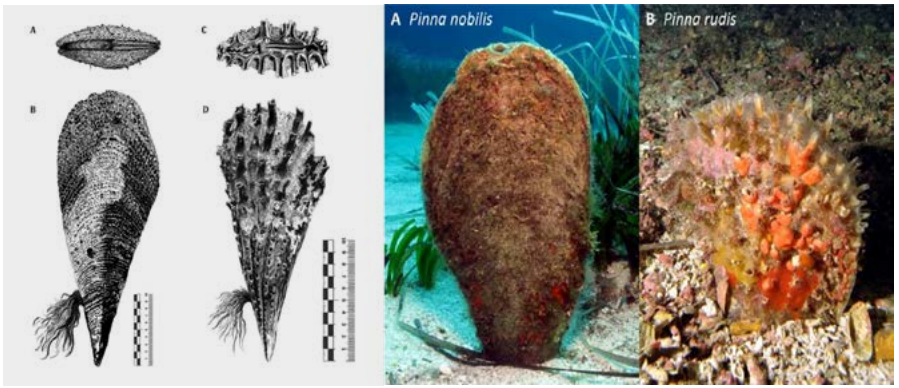


Figure 10.1: Images of the target species *P. nobilis* and *P. rudis*.

PROTOCOL 10: FAP - Fast assessment of *Pinna nobilis* conservation

MATERIALS

Data collection will require the following materials:

- ☑ Underwater compass
- ☑ GPS
- ☑ Buoy
- ☑ Deco buoy
- ☑ Metric-tape
- ☑ Underwater board (**Fig. 10.2**)
- ☑ Pencil
- ☑ Rule or frame with sub-quadrats
- ☑ Underwater camera with housing and electronic strobes or focus providing continuous lighting

SAMPLING SITES

The study sites should be characterized by depths ranging from 0.5 to 60 m (reported occurrence of the species in literature).

PERIODICITY OF MONITORING

Sampling should be performed every 12 months.
The period of monitoring should be late summer/ autumn.

FIELD WORKERS EXPERIENCE

MPA managers and scientific divers, skilled to recognize, count and measure specimens underwater, working in couple. The protocol can be as well adapted for trained recreational divers under the supervision of a trained diving center professional diver or a scientific diver.

METHODS

SAMPLING DESIGN

At each MPA, a minimum of 3 study sites should be established. The sites should be set in habitats suitable for *P. nobili*s, 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 and their size.

At each selected sampling site, 3 parallel transects (100 m long and 6 m wide) are placed about 50 m each other. In order to assess the density of population and determine the vitality of each specimen, a 100m-metric-tape must be used to identify all individuals within approximately 3 m of each side (left and right) of the tape.

PROTOCOL 10: FAP - Fast assessment of *Pinna nobilis* conservation

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. If applicable, a marking buoy is deployed at the starting point of the transect as well at the end of the transects (deco buoy); the geographic coordinates of both buoys are marked with a GPS (**Fig. 10.3**).

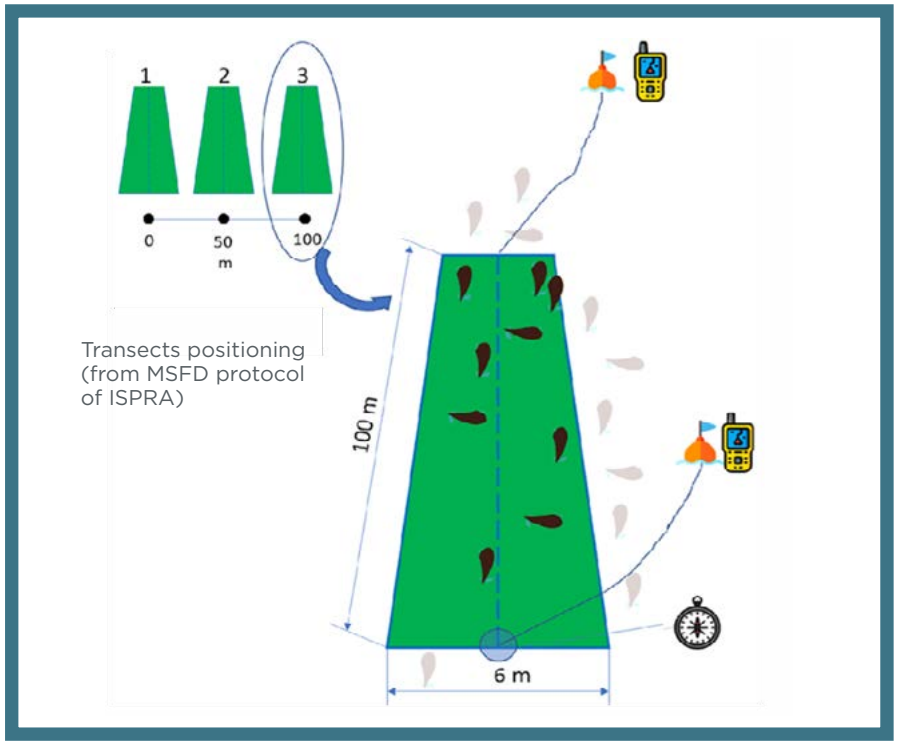


Figure 10.3: Sampling design for protocol 10.

CHARACTERIZING PINNA CONSERVATION STATUS

All individuals observed inside the transects are counted and abundance will be then expressed as the number of individuals per 100 m^2. Once an individual of P. nobilis is identified, approaching the shell, it should be noted if:

- ☑ Alive (fast valves closure)
- ☑ Affected by infection (still alive but with slow valves-closure reaction when disturbed)
- ☑ Dead individuals found still orthogonal, steady into the substrate (undamaged shells)
- ☑ Dead individuals lying on the bottom

All alive individuals detected along the transect are measured; biometric data are (**Fig. 10.4**):

- ☑ Unburied length **(UL)**
- ☑ Maximum width **(W)**
- ☑ Minimum width **(w)**

PROTOCOL 10: FAP - Fast assessment of *Pinna nobilis* conservation

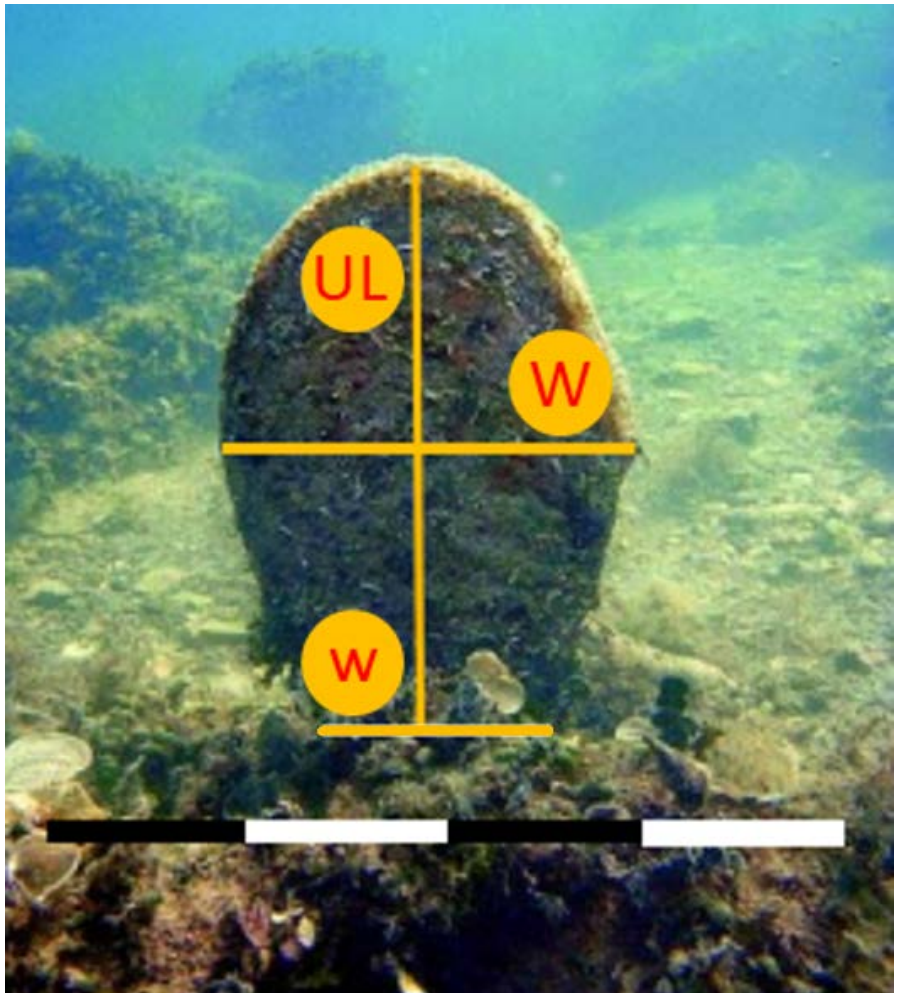


Figure 10.4: Biometric data of alive individuals.

In addition, the substrate in which each living individual is found must be recorded, distinguishing between:

- ☑ Sand
- ☑ Posidonia
- ☑ Matte
- ☑ Rock

The monitoring on *P. nobilis* is providing:

- ☑ Density of alive individuals
- ☑ Size structure of alive populations
- ☑ % of individuals affected by infection
- ☑ % mortality of individuals
- ☑ Ratio undamaged shells vs total dead individuals

Pictures of all observed individuals together with a measurement reference scale can be taken; a frame with quadrats or a rule with black and white bands guarantee the most accurate measurement. Back in the office biometric information (i.e. UL, W and w) is obtained by image analysis using open source software (e.g. ImageJ).

In some cases, *P. nobilis* shares the same habitats with the co-generic *P. rudis*, the latter is reported to not be

affected by the MME occurring in the Mediterranean Sea. *Pinna rudis* is distinguished by large and widely spaced spines on 5-10 radial ribs, well marked even when adults (**Fig. 10.1,** from Vázquez-Luis et al., 2021). Spines are also present in *P. nobilis*, especially in the juveniles, but they are small, distorted and very close to each other. Potential hybrids, recently reported (Vázquez-Luis et al., 2021) can be confused depending on the external characteristics expressed. The present protocol offers a rapid tool to also census the eventual co-occurring resistant *P. rudis*. Each specimen of *P. rudis* detected along the transect must be treated as *P. nobilis* (i.e. recording vitality and biometric data), just reporting in the notes that it is a different species. In case of doubtful specimens (potential hybrids) a photo should be provided and attached.

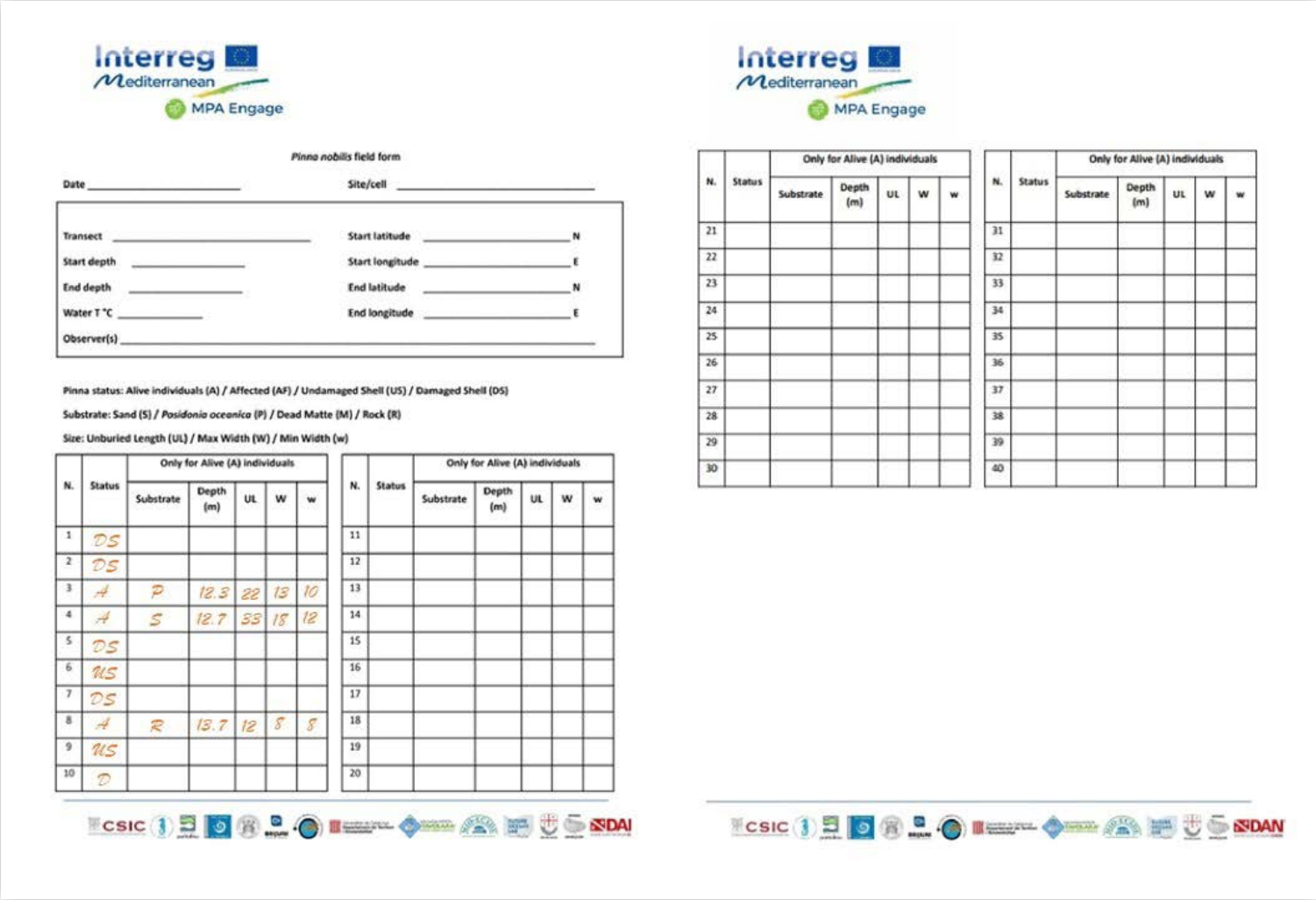
DATA REPORTING AND DATA POLICY

Input your data on the provided specific Excel files which were designed for data storage, analysis and data reporting.

For further information on data collection, sharing and reuse, please contact d.grech@fondazioneimc.it, garrabou@icm.csic.es, c.cerrano@staff.univpm.it.

Note: all the materials and training tools are freely downloadable from the MPA-Engage web platform.

PROTOCOL 10: FAP - Fast assessment of *Pinna nobilis* conservation



Interreg Mediterranean MPA Engage

Pinna nobilis field form

Date _____ Site/cell _____

Transect _____ Start latitude _____ N

Start depth _____ Start longitude _____ E

End depth _____ End latitude _____ N

Water T °C _____ End longitude _____ E

Observer(s) _____

Pinna status: Alive individuals (A) / Affected (AF) / Undamaged Shell (US) / Damaged Shell (DS)

Substrate: Sand (S) / *Posidonia oceanica* (P) / Dead Matte (M) / Rock (R)

Size: Unburied Length (UL) / Max Width (W) / Min Width (w)

N.	Status	Only for Alive (A) individuals				
		Substrate	Depth (m)	UL	W	w
1	DS					
2	DS					
3	A	P	12.3	22	13	10
4	A	S	12.7	33	18	12
5	DS					
6	US					
7	DS					
8	A	R	13.7	12	8	8
9	US					
10	D					

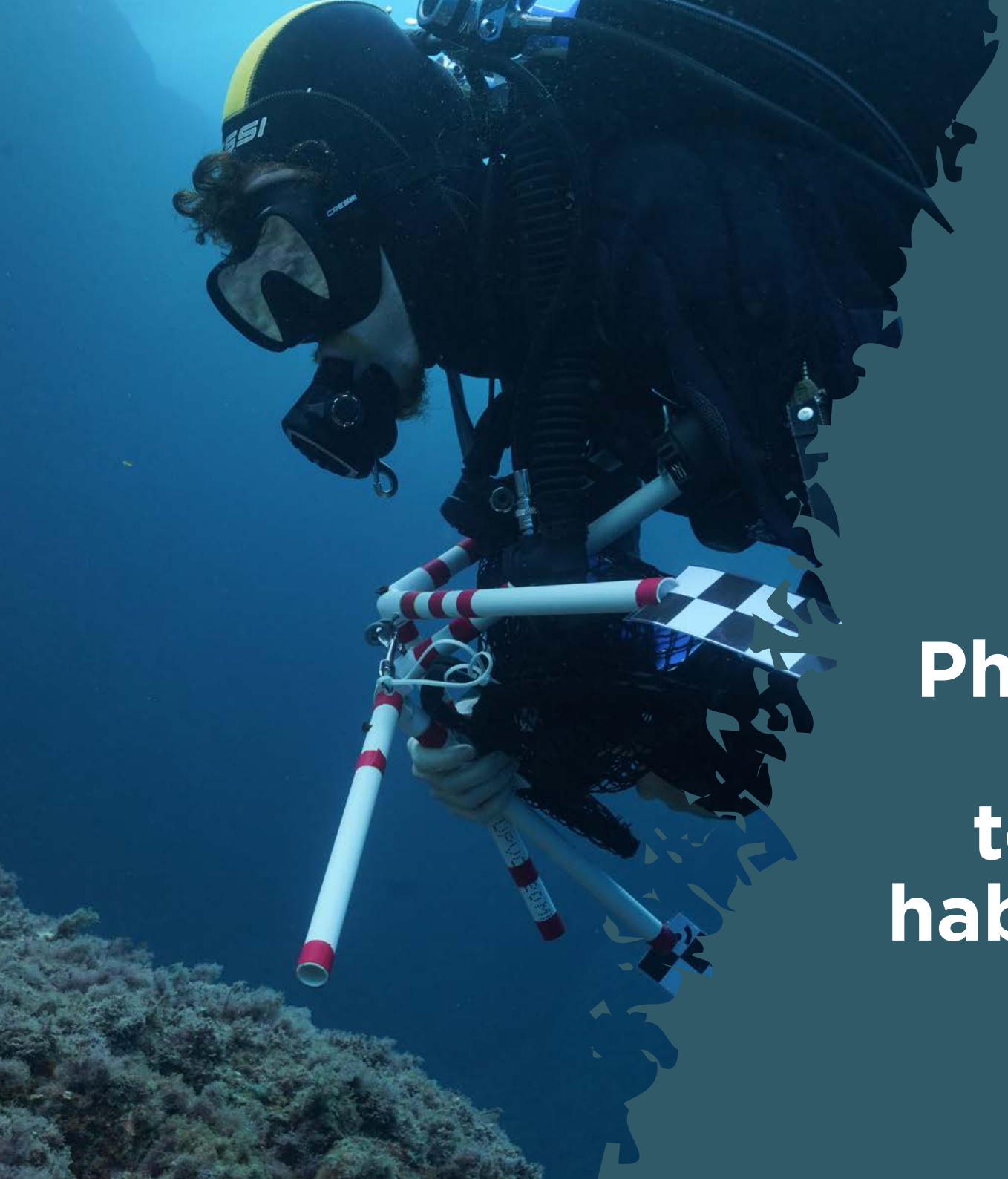
N.	Status	Only for Alive (A) individuals				
		Substrate	Depth (m)	UL	W	w
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						

Interreg Mediterranean MPA Engage

N.	Status	Only for Alive (A) individuals				
		Substrate	Depth (m)	UL	W	w
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						

N.	Status	Only for Alive (A) individuals				
		Substrate	Depth (m)	UL	W	w
31						
32						
33						
34						
35						
36						
37						
38						
39						
40						

Figure 10.2: Underwater board to conduct FAP- Fast assessment of Pinna nobilis conservation.



PROTOCOL 11

SFM - Photogrammetry as monitoring tool for benthic habitats structure and dynamics

PROTOCOL11: SFM - Photogrammetry as monitoring tool for benthic habitats structure and dynamics

RATIONALE AND OBJECTIVES

Recent technologies are allowing the improvement of marine habitat mapping, moving from the traditional bidimensional maps to a more detailed three-dimensional approach.

Structure from Motion (SfM) photogrammetry is proposed as a suitable cost-effective technology to monitor changes over relatively large areas (>100s m^2) in habitat complexity allowing as well to monitor population change. SfM is a technique used for the extraction of three-dimensional data and camera positions from a collection of photographs.

The main objective of its implementation in MPAs is to document and measure habitat complexity and distribution patterns of populations of key species, tracking their changes over time owing to several stressors including climate change.

TARGET SPECIES

Depending on the studied habitat, different species may be targeted. Main criteria to select habitats and organisms of interest by the MPA managers should be:

- ☑ Easy to identify visually
- ☑ Of interest as indicator (e.g. for climate change, pollution, tropicalization)
- ☑ Of interest for conservation (protected species and/or ecosystem engineer)

MATERIALS

- ☑ **Underwater camera**, e.g. sport cameras such as GoPro can be used; depending on the aimed resolution of the reconstruction more performance models must be considered.
- ☑ **A metric reference** to scale the reconstruction (**Fig. 11.1**).

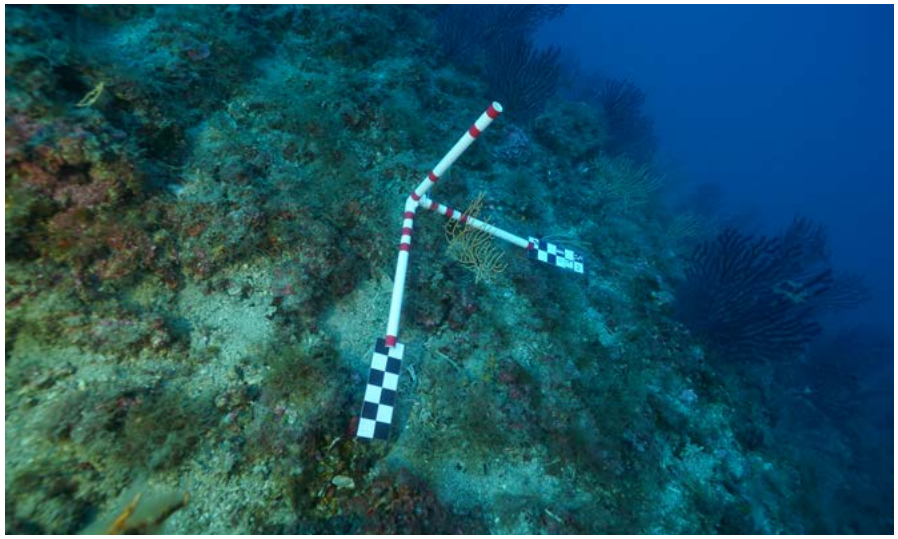


Figure 11.1: Example of a metric reference. The dimensions were 22 cm × 22 cm × 22 cm. The chessboard extension was 21 cm long by 8 cm wide, but size can be adapted (Palma et al., 2018).

PROTOCOL11: SFM - Photogrammetry as monitoring tool for benthic habitats structure and dynamics

- ☑ **Artificial light** may be necessary if natural light is not enough to take quality images.

SAMPLING SITES

The study sites should be characterized by rocky bottoms from 0 to 40 m depth. Depending on the characteristics of the site, the protocol can be applied within 3 different bathymetric ranges:

- ☑ Rocky shores (0-17.5 m)
- ☑ Coralligenous (17.5-27.5 m)
- ☑ Coralligenous (27.5-40 m)

A minimum sampling effort of 100 m^2 to characterize a site depth. Within a bathymetric range at least 3 sampling areas per site should be acquired.

PERIODICITY OF MONITORING

Since initial image quality can affect the photogrammetric process, the monitoring period must be adapted to each MPA depending on the season hosting the best water conditions. The protocol is expected to be performed every 12 months.

FIELD WORKERS EXPERIENCE

MPA managers, scientific divers, and recreational trained divers skilled in underwater photography.

METHODS

SUB-SECTIONS

The different steps to implement this protocol are the following:

1. Select a suitable monitoring site being aware that you must be able to slowly dive following the seabed in order to take the images at a predefined short time interval, obtaining a sequence of photographs with an overlap of around 60-80%
2. Define the area to be surveyed, i.e., 10 × 10 m 20 × 10 m, 20 × 20 m
3. Place the metric-scales and record the depth at each reference location. (It is important to remind that the metric scale should not be moved during the sampling process)

PROTOCOL11: SFM - Photogrammetry as monitoring tool for benthic habitats structure and dynamics

4. The collection path should be adapted to the substrate complexity, taking the images from approximately 1 meter above the substrate. Following two perpendicular zigzag patterns (**Fig 11.2**) the operators will try to cover all the possible perspectives of the seafloor, obtaining the proper image overlap
5. Upload the images to the cloud platform and save the images in a folder with a name as follow "date_sitename_depth"
6. Photogrammetric processing and data treatment will be carried out by the researchers collaborating with the MPA

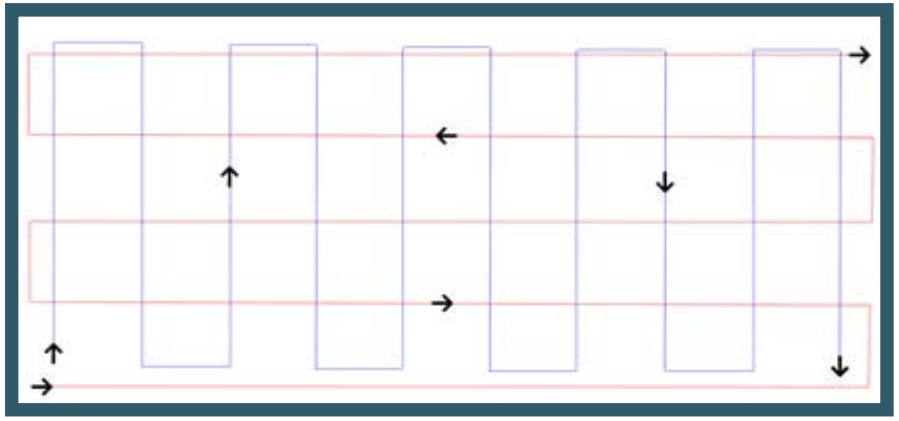


Figure 11.2: Image collection path, a double zig-zag pattern.

DATA REPORTING AND DATA POLICY

Share the captured images on the cloud platform built specifically for the protocol. To access the cloud platform and request instructions, please contact c.cerrano@staff.univpm.it and/or m.coppari@staff.univpm.it.

Monitoring tool: PowerBI

Monitoring tool: Power BI

RATIONALE AND OBJECTIVES

After conducting the different protocols the MPAs will have collected a vast amount of data corresponding to each of them. The purpose of the Monitoring Tool is to easily display all of the data of the different protocols under a single layout in order to ease the process of revision and quality control as well as to offer a pleasant design to showcase the results for the MPA managers and to a broader audience.

MATERIALS

In order to properly run and understand the Monitoring tool, you will need the following:

- ☑ Hardware: Computer with Microsoft Windows
- ☑ Software: Power BI Desktop Version
- ☑ The Monitoring Tool .pbix file
- ☑ The protocols datasets in Microsoft Excel templates
- ☑ The Monitoring Tool User Manual

PERIODICITY OF MONITORING

The Monitoring tool is meant to be used each time all of the protocols of an MPA are fulfilled for a given period of time. It has been designed to support annual datasets as well as built time series data.

LAYOUT

The layout of the monitoring tool is designed to be simple and pleasant as well as easy to use and understand. On the left part of the screen there is a menu to all of the different protocols and on the top there are different buttons to navigate inside the protocol, modifiers such as depth, etc.

The layout is consistent for most of the protocols consisting of a map pointing to the sites where the data was gathered, the main graph with the data of a given indicator of the protocol and an assessment of it if applicable (**Fig. 12.1**).

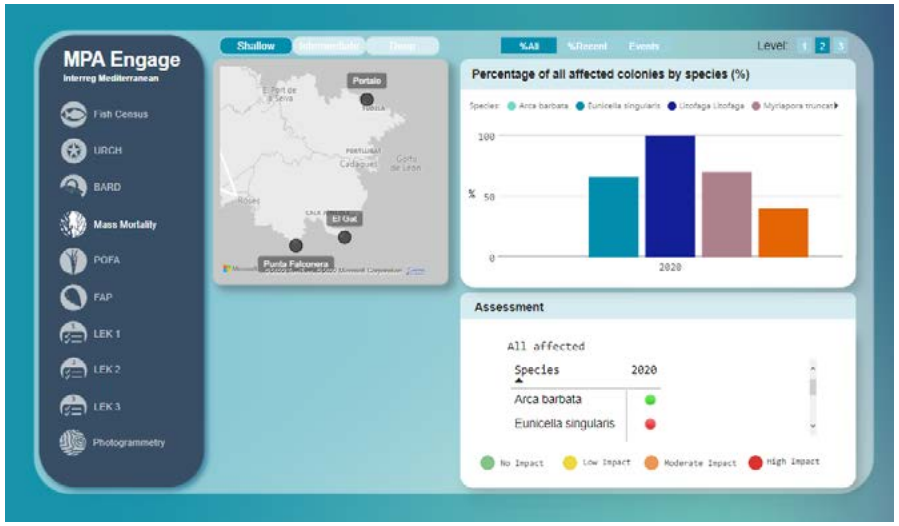


Figure 12.1: Layout of the monitoring tool developed within MPA-Engage project.

Monitoring tool: Power BI

METHODS

INSTALLATION

In order to use the Monitoring Tool it would be necessary to have installed on your PC the Power BI Desktop version (this software is only compatible with Microsoft Windows).

CREATING THE DAHSBOARD

All the visualizations are already in place, it would only be necessary to load the protocol data (on the Excel templates) to the Monitoring Tool. Once it has been loaded, the corresponding visualizations will be updated automatically.

EXPORTING THE DATA

Once all the files have been loaded the visualizations can be exported into a PDF or can be extracted individually.

Note: all the materials and training tools are freely downloadable from the MPA-Engage web platform.

Credits

LEK-1: EXPLORING LOCAL ECOLOGICAL KNOWLEDGE TO RECONSTRUCT HISTORICAL CHANGES

A first version of this protocol has been originally developed by the CIESM project Tropical Signals and adapted for the purposes of the Interreg project MPA_Adapt, with the contribution of FAO projects AdriaMed and MedSudMed.

References: https://doi.org/10.1371/journal.pone.0024885

Pictures: Gianpaolo Rampini

Concepts: Ernesto Azzurro

LEK-2: EXPLORING LOCAL ECOLOGICAL KNOWLEDGE FOR PERIODICAL MONITORING

The protocol has been conceived for the purposes of the MPA-ADAPT project and capitalizes the experience and scientific discussions made through the FAO projects AdriaMed MedSudMed projects.

Pictures: Jamila Ben Souissi

Concepts: Ernesto Azzurro

LEK-3: EXPLORING LOCAL ECOLOGICAL KNOWLEDGE FOR MASS MORTALITIES

Picture: Manuela Petrocchi

Concepts: Ernesto Azzurro

MONITORING TEMPERATURE CONDITIONS

The protocol has been originally developed by the T-MEDNet initiative and adapted for the purpose of the project MPA-Adapt.

Pictures: Joaquim Garrabou

Concepts: Joaquim Garrabou and Nathaniel Bensoussan

ASSESSMENT AND MONITORING OF MASS MORTALITY

The protocol has been originally developed for the project and adapted for the purposes of the project MPA-Adapt.

Pictures: Joaquim Garrabou, Alexis Rosenfeld and Observadores del Mar

Concepts: Joaquim Garrabou

FISH VISUAL CENSUS OF CLIMATE CHANGE INDICATORS

This protocol is adapted from a previous version developed by the CIESM project Tropical Signals.

Pictures: Giovanni Ombrello

Cover image: Matteo Varenna

Graphical inputs: PADI and DAN

Concepts: Ernesto Azzurro

URCH -SEA URCHINS POPULATION

The protocol has been adapted from the Parc Natural del Montgrí, Illes Medes i Baix Ter monitoring program started in 1990.

Pictures: Joaquim Garrabou

Concept: Joaquim Garrabou and Jordi Boada

BHARA: BENTHIC HABITAT RAPID ASSESSMENT

The protocol has been originally developed for the project MPA-Engage.

Cover image: Joaquim Garrabou

Pictures: X. Calsina & J. Garrrabou

Graphical input: adapted transect form Jordi Corbera

Concepts: E. Cebrian & J. Santamaria

POFA - *POSIDONIA OCEANICA* FAST ASSESSMENT

The protocol was inspired by the Tavolara Lab experience and is in line with ISPRA methods for monitoring *Posidonia oceanica* meadows in Italy.

Cover image: Ivan Guala

Pictures: Ivan Guala, Dusan Varda and Daniele Grech

Concepts: Ivan Guala and Carlo Cerrano

FAP - FAST ASSESSMENT OF *PINNA NOBILIS CONSERVATION*

The protocol was inspired by the ISPRA Handbooks and Guidelines for the monitoring of Pinna nobilis in Italy and the citizen science project *Pinna nobilis Ricerca per la sopravvivenza*

Cover image: Daniele Grech

Pictures: Maite Vázquez-Luis, Ivan Guala and Daniele Grech

Concepts: Daniele Grech and Carlo Cerrano

SFM - PHOTOGRAMMETRY AS MONITORING TOOL FOR BENTHIC HABITATS STRUCTURE AND DYNAMICS

Cover image: Ubaldo Pantaleo

Pictures: Ubaldo Pantaleo

Concepts: Carlo Cerrano, Ubaldo Pantaleo and Torcuato Pulido Mantas

MONITORING TOOL: POWERBI

The monitoring tool has been conceived for the purposes of the MPA-Engage project to provide a dashboard to join and visualiza all the data gathered for the eleven protocols presented.

Pictures: Marc Jou

Concepts: Joaquim Garrabou and Marc Jou

Cover image: Alexis Rosenfield

Graphic design & layout: Clara Comín

Graphical concepts of all the protocols: Jacopo Cerri

MPA-ENGAGE: Engaging Mediterranean key actors in Ecosystem Approach to manage Marine Protected Areas to face Climate Change, Interreg Med Programme

(Grant number 5216/ 5MED18_3.2_M23_007)
85% co-funded by the European Regional Development Fund

Interreg Mediterranean
MPA-Engage

Project co-financed by the European Regional Development Fund

Garrabou J., Bensoussan N., Di Franco A., Boada J., Cebrian E., Santamaria J., Guala I., Grech D., Cerrano C., Pulido T., Jou M., Marambio M. & Azzurro E., 2022.

Monitoring Climate-related responses in Mediterranean Marine Protected Areas and beyond: ELEVEN STANDARD PROTOCOLS

74 pp. Edited by: Institute of Marine Sciences, Spanish Research Council ICM-CSIC, Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain.
DOI: https://doi.org/10.20350/digitalCSIC/14672

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