

Acronym: AMAre

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Priority Axis 3: Protecting and promoting Mediterranean natural and cultural resources

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To maintain biodiversity and natural ecosystems through strengthening the management and networking of protected areas

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List of abbreviations and terms

ACI	After-Control Impact
ANOVA	Analysis Of Variance
BACI	Before-After Control Impact
CAI	Coralligenous Assemblages Index
COARSE	Coralligenous Assessment by ReefScope Estimate
DW	Dry Weight
EEC	Environmental European Commission
EQBI	Ecological Quality Biological Index
ESCA	Ecological Status Coralligenous Assemblages
GES	Good Environmental Status
HD	Habitat Directive
ISEA	Interventi Standardizzata di Gestione Efficace in Aree Marine Protette
LIT	Line Intercept Transect
MPA	Marine Protected Area
MFD	Marine Framework Directive
MSFD	Marine Strategy Framework Directive
NIS	Non-indigenous species
OCI	Overall Complexity Index
PERMANOVA	Permutational Analsis of Variance
RAC/SPA	Regional Activity Center for Special Protected Areas
RVA	Rapid Visual Assessment
SST	Sea Surface Temperature
UNEPMAP	United Nations Environment Programme Mediterranean Action Plan
UWC	Underwater Census

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Executive summary

The monitoring protocol will include the sampling designs and the methodologies to address the effects of different combinations of human uses on vulnerable habitats. They will be tailored on the 3 MPAs, but extendable to other ecological contexts

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Elaboration of Common Monitoring Protocols, with a special focus on the descriptors of the Good Environmental Status (GES) of the Marine Strategy Framework Directive (MSFD).

INTRODUCTION

Marine protected areas are currently abundant in the Mediterranean Sea. From 2012 they increased up to 1231 MPAs in 2016, and already represented 7.14% of the total sea surface area (*"The 2016 Status of Marine Protected Areas in the Mediterranean"* MedPan, in prep.). It appears that protection is on track to reach the 10% recommended by the Aichi Biodiversity Targets of the Convention on Biological Conservation, by 2020. But Mediterranean MPAs have serious shortcomings:

- 1) Limited Protection: only 0.04% of the Mediterranean surface enjoys real protection in the form of no-take or no-take no-go zones, and MPAs in Categories IV (Management areas for habitats and species) and II (National Parks) are the most common management IUCN types (Day et al, 2012; Gabrié. et al., 2012).
- 2) Limited Management: the best indicator of the level of management of the Mediterranean MPAs is that only 80 out of 180 questionnaires were duly completed by the management entities (*"The 2016 Status of Marine Protected Areas in the Mediterranean"* MedPan, in prep.). Out of the 73 MPAs, only 20 report having a management plan which takes the marine part into consideration and which is being implemented, while 14 other managers only partially implement such a plan due to funding constraints or other problems. Of these, 21 management plans have been reviewed and adapted, or are less than 10 years old. 31 MPAs either don't have a management plan or don't implement it.
- 3) Limited monitoring: twenty-five per cent of the research projects undertaken in Mediterranean MPAs in the last decade were related to management and monitoring. Most of them were developed in Italy, France and Spain (Gabrié et al., 2012), in the few well-managed Mediterranean MPAs. Moreover, there are few

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published evidences of true long-term monitoring (> 10yrs) (but see Coll et al., 2012; Garcia-Rubies et al., 2013). Most long-term monitoring have been restricted to fish and specific populations. Habitats have been rarely monitored systematically.

The MPAs participating in the AMAre showed two distinct management models according their own main objectives. The marine reserve “Dels Freus d'Eivissa and Formentera” is mainly devoted to protect fishing resources and make sustainable local artisanal fisheries. The Marine Reserve depends on the autonomous Balearic Government, specifically the General Directorate of Fishing and Marine Environment (Direcció General de Pesca I Medi Marí; www.caib.es/sites/reservesmarines/ca/les_reserves_marines_a_les_illes_balears-850/). The management focuses almost entirely on fishing regulations and the control of underwater activities. The monitoring is based on UWC of commercially interesting fish. The census are repeated biannually. Catches and fishing effort are also controlled in the area (both, professional and recreational), according different modalities. The UWC are currently carried out by specialized scientists hired by the reserve, while fishing is controlled by reserve's own guards. In spite of not being a conservation MPA, it responds to the minimum zoning requirements, including a “no take no-go” zone and two buffer zones. The enforcement is good enough to guarantee an effective protection level of populations and habitats.

In Italy, 29 marine protected areas have been setup with a single legal and administrative status based on the same model: a no take area surrounded by one or two ‘buffer’ areas where the prohibitions concern mainly recreational or industrial fishing (Meinesz and Biondini, 2015). That's the most coherent policy about marine protected areas in the Mediterranean Sea. That is why Torre Guaceto MPA shares the same standard management scheme of all the Italian MPAs. It is called ISEA and is represented by a conceptual map (<http://www.progettoisea.minambiente.it/mappeconcettuali/>) in which the target biodiversity is defined the first, following by the direct and indirect threats. After that the intervention strategies were determined in order to achieve the planned conservation objectives. If fully applied, ISEA is, in fact, a true adaptive management model. It can be

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adopted by the other Mediterranean MPAs participating in AMAre. The monitoring protocol is an important part of this scheme, and it's not only limited to environmental indicators. In any case, the number of indicators is impressive (www.riservaditorreguaceto.it/ISEA/esterno/7-indicators.aspx). Not all the biophysical indicators (the most expensive) are covered by current budget, but most of them (especially those on an annual basis) are covered by project. Surveys on fishery, benthonic habitats (the most important) are actually covered by EU and national programs. The socio-economic and Governance indicators are normally registered by the staff with current budget.

The large number of indicators, many of which require specialized personnel, and the lack of own funds to finance the monitoring of biophysical indicators, is the only weak point of this type of management, in order to ensure regular monitoring in the long term.

Malta is still in the process of preliminary studies, which focus on the location and intensity of threats of human origin, such as fishing and aquaculture, or the presence of NIS. The meadow of *P. oceanica* of the MPA of the NE of Malta, as well as the population of *Pinna nobilis* are also being evaluated.

1. Purpose of the Guidelines

Some local stressors and global threats related to global change are well beyond the scope of MPAs in spite how well protected they may be. In any case, MPAs can be used as sentinels in which, both the threats and their effects can be documented, taking advantage, if possible, of the infrastructure that MPAs should already have. It is also possible to verify the impact of these threats on the unexploited areas in no-take zones, which are, in fact, the most “pristine” areas that can be currently found in the Mediterranean. The use of these non-take zones as a laboratory is undoubtedly one of the most remarkable values that MPAs can bring to science.

The guidelines presented here aim to provide managers of MPAs with simple monitoring

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tools that can produce reliable information, both on the effects of protection, and also on the effects of global change in MPAs (Figure 1) that are geographically far from each other. Obviously, the monitoring protocol proposed here is not exhaustive. Different MPAs have diverse intended origins and types of management. It is not a question of abandoning the monitoring that is being presently carried out on a regular basis in each MPA (Coll et al., 2012), but rather to establish a minimum monitoring protocol in such a way that the different Mediterranean MPAs can begin to act as a true network sharing a common monitoring protocol as a seed of a future common minimum management.

The objectives of the monitoring methods presented here have a dual purpose: on the one hand, they seek to know what is the effect of the protection on biological assemblages, which may be obvious (i. e. fish populations) or much more subtle (i. e. *Posidonia* meadows, or coralligenous habitats); on the other hand, they also seek to verify what the effects of global change are, with a special emphasis on the progressive warming water temperature (SST). There is a strong likelihood of increasing frequency of mass mortalities events in the Mediterranean Sea (Garrahou et al., 2013). The possibility of comparing the seasonal evolution of SST (using *in situ* data loggers at different depths) and their immediate effects on benthic habitats at a local scale (each MPA) in a geographically wide context (all MPAs), can yield an interesting information about the spatial range of thermal anomalies and their effects.

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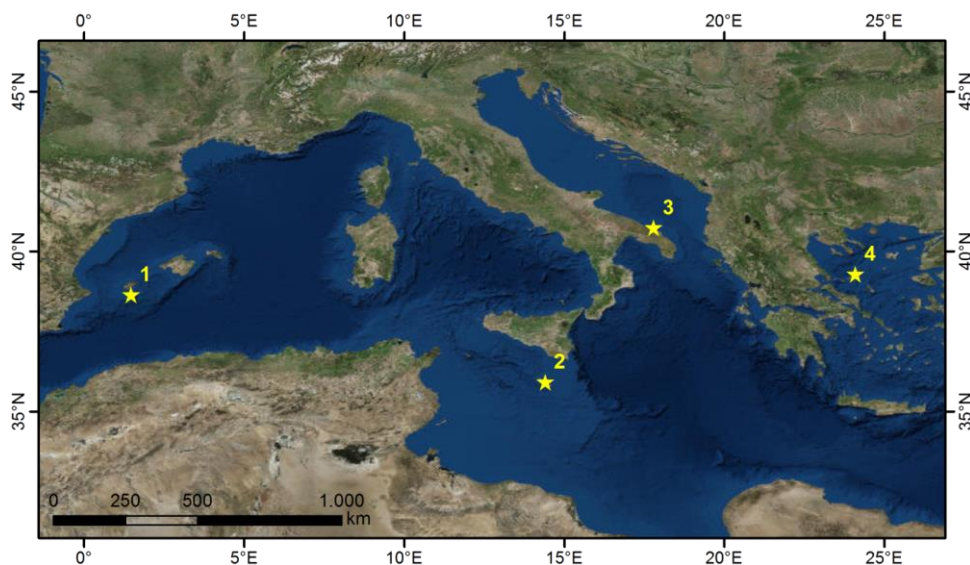


Figure 1. Location of the MPAs included in AMAre; from East to West: Freus d' Eivissa i Formentorera; Malta, Torre Guaceto and Sporades (Deliverable 3.1).

But sharing a common monitoring protocol is not only very interesting from a scientific point of view. It is also the beginning to solve some of the shortcomings of the Mediterranean MPAs that, according to Rodriguez-Rodríguez (2015):

- Sound and regular monitoring is essential to effective MPA management.
- Monitoring should focus on protected features, pressures and socioeconomic effects of conservation measures.
- Most existing MPA monitoring programmes are still scarce, short-term, poorly funded and inconsistent.
- Partnering with research institutions, securing monitoring funds and citizen science can help to streamline MPA monitoring efforts.

2. Monitoring the MPAs

A common monitoring protocol focusing on the most ecologically important Mediterranean benthic habitats and sensitive organisms to protection can be a start to unify a minimum common management among all the MPAs involved in the AMAre project (Figure 1) in a frame of a collaborative, transnational network (Braun, 2017).

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After (Lindenmayer and Likens 2010) the operational definition of long-term monitoring consists in the repeated field-based empirical measurements that are collected continuously and then analysed for at least 10 years. Desirable conditions of any long-term monitoring protocol should be characterized by:

1. Good temporal replicability
2. Simple sampling methods
3. To be long-term designed
4. Rigorous experimental design considering the aims

These basic conditions were not always fulfilled in most of the simple monitoring methods that have been carried out so far in some Mediterranean MPAs (Fraschetti et al., 2002) that criticized above all the poor statistical design of the monitoring and, especially, the lack of correct replication when comparing protected and unprotected areas.

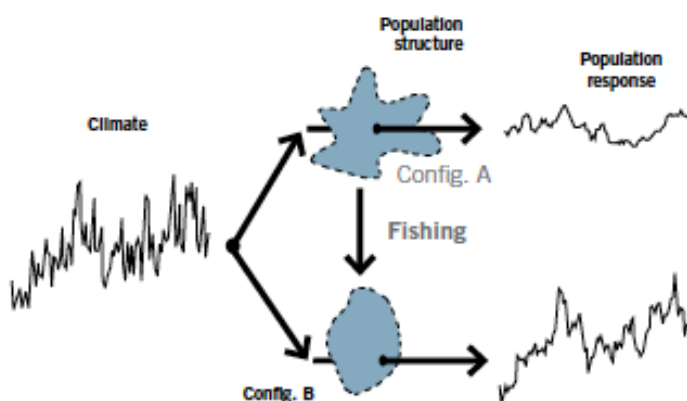


Figure 2. Schematic representation of the effects of climate change on a population with (A) and without (B) other stressors (Brock et al 2012).

Here, we propose experimental designs taking into account the possible effect of protection. The “reserve effect” seems clear in the case of fish, but is much subtle when dealing with complex benthic habitats, that may be largely affected by other factors not directly linked to fish exploitation (i. e. *P. oceanica* meadows). There are some evidence that MPAS enhance temporal stability in some benthic habitats (Fraschetti et al., 2013),

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suggesting that in absence of local stressors the habitats are more estable and thus, more resilient (Figure 2).

Besides the study of the effects of protection, it has to be stressed that, in general, we recommend to start including high resolution data on the distribution of habitats such as *P. oceanica* and environmental stressors at the scale of individual MPAs. The collection of *in-situ* data into MPAs will be of crucial importance to obtain specific local information into those coastal zones, not available now, and to validate the high resolution numerical models in order to provide more realistic results in a near future.

2.1. *Posidonia oceanica* meadows

The *Posidonia oceanica* meadow is one the most quantitatively and ecologically important benthic sublittoral habitat characteristic of the Mediterranean Sea. The meadows can range from near surface down to 40-45m (Gobert et al., 2014). Telesca, et al., (2015) reveals that 1224707 ha, along 11907 km of coastline (12% of the Mediterranean coastline) are covered by *P. oceanica* meadows although there is a substantial lack of reliable data from 2147km of the Mediterranean coastline (43%).

P. oceanica meadows carry out crucial ecological functions in coastal waters, some of which have regional or even global repercussions: *P. oceanica* is an important habitat engineer, because both leaves and stems, modified in the form of rhizomes, form a solid support that gives protection to a huge variety of plant and animal species, acting as biodiversity reservoir. Up to 660 epiphyte species have been recorded (430 spp on leaves and 437spp on rhizomes, according the review of (Piazzi et al., 2016).

It also is an important primary producer (51-142 g DW m⁻² yr⁻¹ for sheaths; 167-530g DW m⁻² yr⁻¹ for leaves, and 20 – 42g DW m⁻² yr⁻¹ for rhizomes, after (Pergent et al. 1994), playing an important role in the trophic networks.

Moreover, *P. oceanica* meadows provide also a wide array of ecological services, such as oxygen production (Borum et al., 2004). Since remineralisation in the sediment only

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returned $15.6 \text{ g C m}^{-2} \text{ yr}^{-1}$, yielding a net carbon accumulation of $182 \text{ g C m}^{-2} \text{ yr}^{-1}$, therefore *P. oceanica* meadows are important sites of carbon sequestration. *P. oceanica* meadows also plays an important role in stabilizing the substrate and represent an effective protection against the action of the waves and the erosion of the coast (Gacia and Duarte, 2001).

2.1.1. Main threats

P. oceanica meadows, as well as other marine angiosperms, are very sensitive to anthropic drivers, so there is a global concern for its future. After (Waycott et al., 2009) around 1.5% of sea grass beds are lost every year, and up to 29% have been estimated to be lost from 1879. Marbà et al., (2014) estimated that between 13% and 50% of seagrass areal extent of *P. oceanica* in the Mediterranean basin appear to be lost, and the remaining meadows may have thinned shoot density by 50% during the last 20 years. Telesca et al., (2015) estimated that the lost area of *P. oceanica* was 124091 ha over the past 50 years, which corresponds to an average regression of 10.1% of the total known area. Stressors may be local or global.

1) Local stressors:

- Coastal development has been accelerated during the second half of the 20th century, with massive littoral urbanization and the extension of existing ports (Meinesz et al., 1991; Montefalcone et al., 2007).
- Pollution, excess of sediment input and turbidity caused severe regression of *P. oceanica* meadows near large urban centres (Pérès, 1984, Duarte 2002, Boudouresque, 2003).
- Trawling can be considered one of the main responsible of seagrass losses. A single standard trawling can uproot 99000 to 362000 shoots h^{-1} (Martín et al., 1997). It accounts for a reduction of 80% in the Gulf of Gabés in Tunisia (Zaouali, 1993), 50% in the coast of Alicante (Esplá 1995), or 12% in Corsica (Pasqualini et al., 2000).
- Mooring in sheltered bays has been a cause of seagrass destruction along the history (Ruíz et al., 2017). Big cruise ships are currently involved in significant destruction

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of *P. oceanica* meadows, while small pleasure boats can produce a serious uprooting shoots in heavy mooring sites (around 68000 shoots ha⁻¹ are ripped annually in a 1.4ha of Elbu Cove, Corsica (Boudouresque et al., 1995). Mooring can be also highly deleterious for *Pinna nobilis* inhabiting *P. oceanica* meadows (Centoducati et al., 2007).

- Dredging, sand extraction and dumping, are major causes of worldwide seagrass loss (Erftemeijer and Lewis, 2006). *P. oceanica* meadows can be directly destroyed by sediment pumping or indirectly by turbidity and sand re-deposition (Gambi et al., 2005; González-Correa et al., 2008, González-Correa et al., 2009). The laying of underwater cables and pipes, brine discharges, introduced, and explosives have also undergone important roles in the regression of *P. oceanica* meadows.
- Fish farming facilities increase the nutrients and organic matter that lead to a reduction in light intensity (Pergent-Martini et al., 2005, Pergent-Martini et al., 2006). The negative impact can reach as far as 1km from large fish cages (Marbà et al., 2006).

2) Global change

- Non-indigenous algae species (especially invasive NIS) have deleterious effects when *Caulerpa taxifolia* (Devillele and Verlaque, 1995) and *Lophocladia lallemandi* (Ballesteros et al., 2007); (Marba and Duarte, 2010) infested and cover *Posidonia oceanica*, producing a decrease in size and weight of the seagrass shoots, leaf chlorosis, leaf necrosis and, finally, shoot death as the main symptoms. Bare sand or low densities of *P. oceanica* shoots facilitate the invasion of *Calulterpa racemosa* (= *cilindracea*), according (Ceccherelli et al., 2000).
- Climate change is mainly characterized by an increase in SST, but also by a progressive sea level rise, which may be not so evident (between 2.75 and 5.50cm from 1940 to 2000), according to Calafat and Gomis, (2009). Mayot et al. (2006) hypothesize that the sea level rise may be responsible for the regression of the lower limit of the *P. oceanica* meadows along 556 km of the French coast, regardless of the generalized increase in coastal water quality and the recovery of the state of *P.*

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oceanica in shallower areas. The regression has also been verified, in pristine areas, free of other anthropogenic threats. It has been estimated that sea level will rise between 9.8 and 25.6cm by the decade 2040-2050, so it can be expected that the lower limit of the meadows continue to recede in the coming years. On the other hand, Marba and Duarte, (2010) blamed the rising SST as a main responsible of the increasing mortality of *P. oceanica*. Jordà, et al. (2013) predict that the *P. oceanica* meadows can reach functional extinction (10% of the current density values) depending on the increase of SST and the effect of other anthropic threats from 2049 to 2093 (Jordà, et al. 2012)(table 1). Although this study has been criticized (Altaba, 2013), it seems clear that the mortality of *P. oceanica* shoots is exacerbated during severe warming events (Marba and Duarte, 2010).

The ecological importance of the *P. oceanica* meadows and its regression has made the EU take measures aimed at its protection. *Posidonia oceanica* meadows are found in Annex I of the Habitats Directive (Directive 92/43 / EEC, of 21 May), which refers to the natural habitats of community interest for which it is necessary to designate special areas of conservation. The Marine Strategy Framework Directive (MSFD) (2008/56/EC) has

Simulation	Year functional of extinction (10% of the present density)
No warming but local anthropogenic pressures	2093 ± 12 yrs
Warming and local anthropogenic pressures	2049±10 yrs
Warming but complete mitigation of local anthropogenic pressures by year 2010	2061±13 yrs
Warming but complete mitigation of local anthropogenic pressures by year 2030	2053±14 yrs
Table 1. Estimated average (± s. e.) year when <i>P. oceanica</i> meadows would reach 10% of their density in 2010 under different combinations of forecast warming and with or without local anthropogenic pressures at the year 2000 (from (Jordà et al., 2012))	

established a framework according to which each Member States shall take the necessary measures to achieve or maintain “Good Environmental Status”(GES) in the marine environment. Angiosperms have been listed as a biological feature in Table 1 of Annex III “Indicative list of characteristics, pressures and impacts” and *P. oceanica* has been selected

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as representative species of the angiosperm quality elements for the Mediterranean marine environment, as a consequence, each EU Member State has defined its own method to evaluate the health status of *P. oceanica* meadows according to the WFD (2000/60/EC)13

2.1.2. Monitoring *P. oceanica* meadows

Under the current European regulations (MSFD) several ecosystem-based approach indices have been developed trying to evaluate the Good Environmental Status (GES). These indices are not only based on habitat descriptors (i.e. on *P. oceanica* variables) but on most of the functional components of the whole ecosystem (such as primary producers, herbivores, filter feeders, etc.) (Personnic et al., 2014). These indexes are, however, too complex to apply in a systematic long-term regular monitoring protocol. Non-destructive, simple, easy-to-apply sampling methods that do not necessarily require highly qualified personnel, seems far more advisable in this case (Table 2). A detailed map of *P. oceanica* meadows, already available for the three MPAs participating in AMAre, is critical and represent a baseline for the management of each MPA (Figure 3).

- The lower limit

Lower limits are mostly related to the light extinction coefficient (Dennison, 1987; Duarte 1991; Carter et al., 2013). It is normal for shoot density to decrease as light decreases in depth; as a consequence, any factor that reduces the amount of light will push back the lower limit of the meadow. Any increase in nutrients that favour algal blooms and/or the development of leaf epiphytes can compromise the viability of the plants in the lower limit of the meadow causing its regression.

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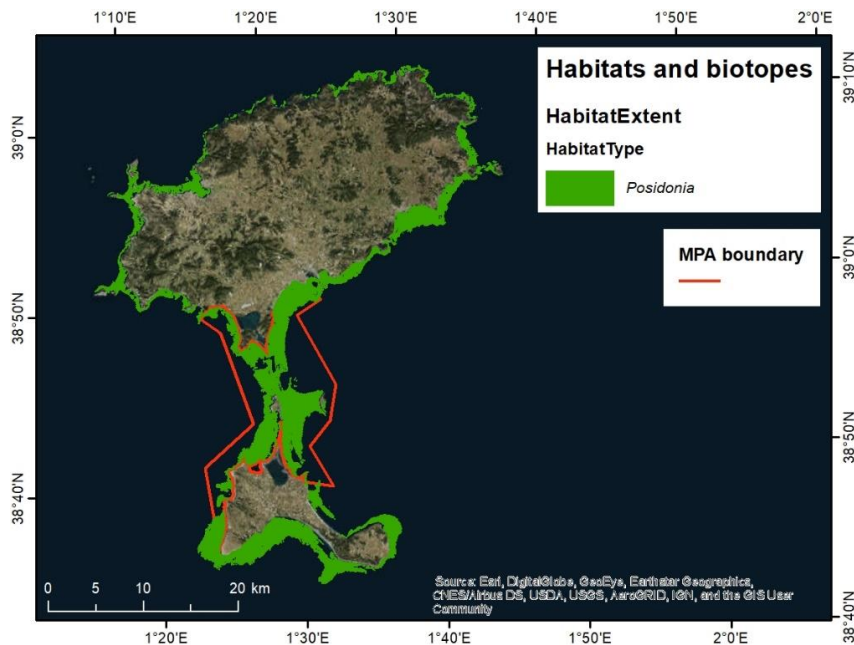


Figure 3. *Posidonia oceanica* meadow is the dominant benthic habitat in the *Freus d'Eivissa i Formentera* marine reserve.

The lower limit can also be affected by excess of sedimentation. If the sediment input exceeds between 5 and 7 cm yr⁻¹ (the annual growth of the orthotropic rhizomes), the vegetative apices are buried and die (Boudouresque and Jeudy de Grissac, 1983). Opposite and less frequent than the previous one, is the sedimentation deficit which leaves the rhizomes exposed and very vulnerable to water movement, trawling or anchorage (Boudouresque et al., 2006).

Montefalcone (2009) defined up to 9 morphologies of lower limit: 3 are natural and 6 are regressive (figure 4). The main characteristic that differentiates natural from regressive limits is that in the latter there are dead remains known as “dead mat”.

The characterization of the lower limit only requires a visual inspection to verify the depth to which it is found and the morphology it presents. In “natural” conditions the maximum

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depth of lower limit is only conditioned by the transparency of the water, that is, by the amount of light that reaches a given depth. It can naturally vary from one area to another, according the water transparency, from about 15m in relatively eutrophic waters, to about 50m in very oligotrophic waters. Four typologies were defined by (Meinesz and Laurent, 1978) and (Pergent and Pergent-Martini, 1995) were revised by (Montefalcone et al., 2007) that defined 9 different typologies: only 3 of them can be categorized as “healthy” or “natural” (Figure 4):

- 1) A natural shaded limit, characterized by a high percentage of plagiotropic rhizomes (horizontal rhizomes, arranged parallel to the angle of slope), and by the absence of mat below them. A limit of this type indicates that the progression in depth of the meadow is hindered only by the gradual decrease in light.
- 2) A clear-cut limit, characterized by a sudden interruption of the meadow and by the presence of both orthotropic (vertical) and plagiotropic (horizontal) rhizomes and by the absence of matte. The type of substrate or sediments seems to be the conditioning factor.
- 3) An erosive limit, characterized by the presence of a pronounced step of mattes, prevalently with orthotropic rhizomes. The hydrodynamics (e.g. the bottom currents) seem to be the main factor limiting the meadow’s progression

In all typologies the presence of dead mat beyond the current lower limit implies that a regression of the meadow has occurred or is taking place. The dead mat, with some live shoots, extends beyond the current limit to which the living *P. oceanica* homogeneous meadow is found in the case of the shaded boundary. In some cases, the regression is not homogeneous and can form prolongations (belts) orthogonal to the coastline, of living (in the first instance) or dead *P. oceanica* (in a stage of more advanced degradation). In other cases patches of living *P. oceanica* remain among the dead mat, beyond the current limit of the homogeneous meadow.

The regression of the meadow can be easily measured by determining the distance at which the dead bush extends from the limits of the current homogeneous meadow.

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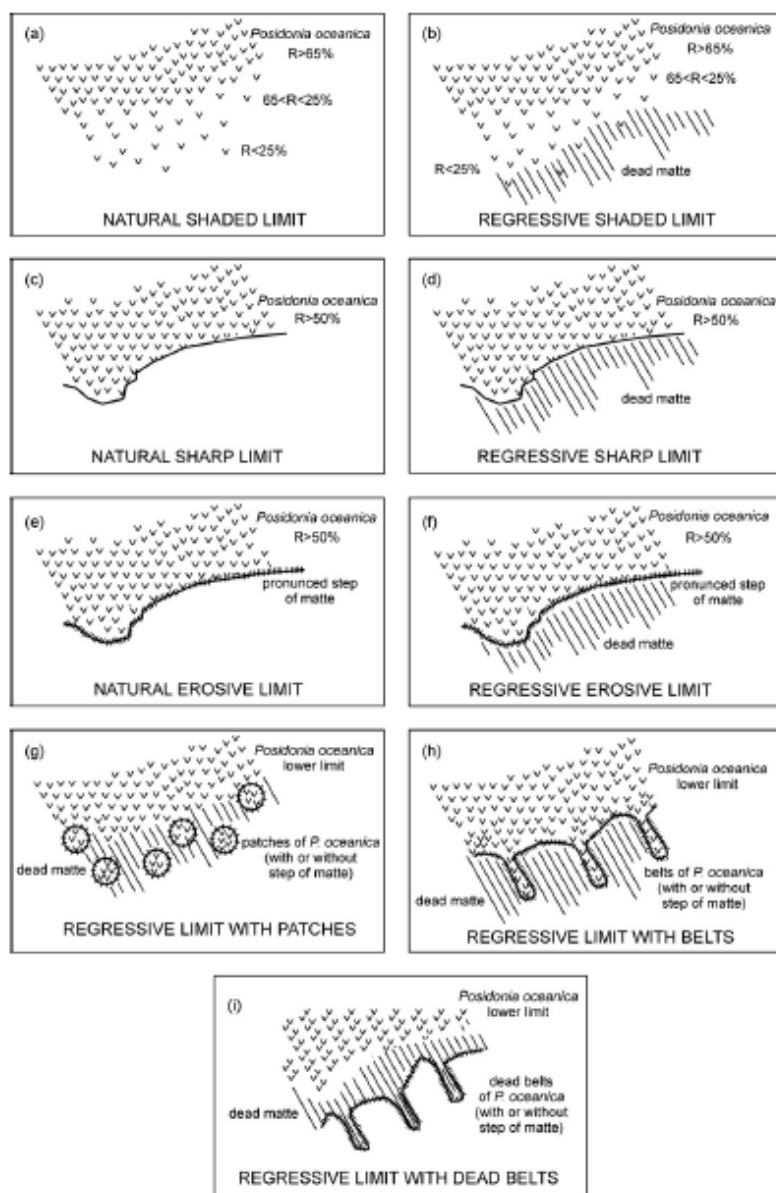


Figure 4. Natural (a, c, e) and regressive (b, d, f, g, h) lower limits of *P. oceanica* meadows (after Montefalcone, 2009)

To monitor the possible variation (either regression or progression) of the lower limit of the *P. oceanica* meadow, fixed, solid, non-degradable marks (i. e. cement blocks), can be

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positioned at a certain known distance from the existing limit, so as not to interfere with the dynamic of the meadow. The regular visit to these marks (annually, for example), will give us exact information of the displacement in time of the lower limit.

Conservation status	Surveillance	Information and descriptors
Area	Mapping	- Detailed GIS map including the habitat extent and bathymetric range. - Local stressors
Structure (basic indicators)	Monitoring	- Shape and depth of lower limit (observational) - Cover (Large scale: LITransects) - Cover (small scale) - Shoot density (shoots m⁻²) - Canopy height (in cm) - Flowering events (flowers m⁻²; flowers per shoot) - Fish (50 x 5m transects) - <i>Pinna nobilis</i>; (50 x 1m transects)
Environmental data	Monitoring	- Main local threats (from GIS Map) - Water Transparency (satellite) - SST (satellite) - SST (data loggers)
Experimental design	Monitoring	- Evaluation (shoot density according depth) - Analysis (just an example): - o Protection: inside vs. outside each MPA - o Locations: replicated (within protected and not protected stretches of coast) - o Site: replicated in each location - o Replicated in time one/ twice every year
Structure (suppl. Indicators)	Monitoring	- Shoot demography (fixed 40 x 40 quadrats); tagged shoots - Permanent transect
Table 2 . Summary of the <i>P. oceanica</i> meadow monitoring protocol and experimental design		

- Cover of living *P. oceanica*

Cover of living *P. oceanica* is defined as % of living plant respect total extent of substrate. Cover is, in itself, a good indicator of the *P. oceanica* meadow health. It can be said that higher the cover of living *P. oceanica* the higher the ecological status of the meadow.

The fastest method to estimate cover is the Line Intercept Transect (LIT); (Montefalcone et al., 2007). It consists on a centimetre marked tape from 25m to 50 long, laid on the bottom. The diver swims over the tape and records the length of the sections with or without *P.*

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oceanica to the nearest centimetre. The percent cover (C%) along a transect of 10 m length, was calculated by the following formula $R\% = \Sigma(Lx/L \cdot 100)$, being Lx the distance between intersects, and L the transect length (Montefalcone, 2007). Several indexes can be derived from cover (Montefalcone, 2009) in function of the substrate that alternates with *P. oceanica*.

At small scale, cover can be also estimated by the number of shoots within a 40 x 40 cm square frame subdivided in 4 (20 x 20cm) square cells. The ratio of cells with or without *P. oceanica* shoots is then an estimate of cover (Pergent-Martini et al., 2005).

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Very disturbed beds		Disturbed beds		Beds in equilibrium			
Depth (m)	AD	Shoots m ⁻²	LSD	Shoots m ⁻²	ND	Shoots m ⁻²	HSD
1	←	822	↔	934	↔	1158	→
2	←	646	↔	758	↔	982	→
3	←	543	↔	655	↔	879	→
4	←	470	↔	582	↔	806	→
5	←	413	↔	525	↔	749	→
6	←	367	↔	479	↔	703	→
7	←	327	↔	439	↔	663	→
8	←	294	↔	406	↔	630	→
9	←	264	↔	376	↔	600	→
10	←	237	↔	349	↔	573	→
11	←	213	↔	325	↔	549	→
12	←	191	↔	303	↔	527	→
13	←	170	↔	282	↔	506	→
14	←	151	↔	263	↔	487	→
15	←	134	↔	246	↔	470	→
16	←	117	↔	229	↔	453	→
17	←	102	↔	214	↔	438	→
18	←	88	↔	200	↔	424	→
19	←	74	↔	186	↔	410	→
20	←	61	↔	173	↔	397	→
21	←	48	↔	160	↔	384	→
22	←	37	↔	149	↔	373	→
23	←	25	↔	137	↔	361	→
24	←	14	↔	126	↔	350	→
25	←	4	↔	116	↔	340	→
26			←	106	↔	330	→
27			←	96	↔	320	→
28			←	87	↔	311	→
29			←	78	↔	302	→
30			←	70	↔	294	→
31			←	61	↔	285	→
32			←	53	↔	277	→
33			←	46	↔	270	→
34			←	38	↔	262	→
35			←	31	↔	255	→
36			←	23	↔	247	→
37			←	16	↔	240	→
38			←	10	↔	234	→
39			←	3	↔	227	→
40			←		↔	221	→

Table 3. Shoot densities according depths in very disturbed, disturbed and non-disturbed *P. oceanica* meadows (after Pergent, 1996)

- Shoot density, flowering events and canopy height

Despite the fact that *P. oceanica* cover is a good indicator of GES, the density of shoots m⁻² is a more subtle indicator of the environmental status of the meadow. After Heidelbaugh and Nelson (1996), the degradation of a meadow can only be detected by cover when the number of shoots has reduced by half. One could say that the shoot density *per se* would

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define the actual environmental state of a meadow. Giraud (1977) created a scale of the state of conservation of the meadows only based on the shoot density, that was later corrected by (Pergent et al., 1995) depending on the depth (Table 3).

The most common method to measure the shoot density is based on a simple square grid frame of known surface, subdivided into smaller sub-quadrats (40 x 40 squares divided in four 20 x 20 cm). The procedure is based on posing randomly the frames on the meadow and counting the number of shoots of each sub-frame, adding them below. The result is then extrapolated to the density of shoots m^{-2} . The same method can allow to quantify the flowering events (flowers m^{-2} ; flowers shoot $^{-1}$).

Canopy height and total leaf height are a good estimate of the herbivory rate of the meadow (Tomas et al., 2005). The height of the canopy is measured with a ruler located vertically in the centre of the same square. Canopy height is normally measured as the sum of the heights of all leaves in a shoot (which obviously requires the extracting of some shoots).

-Shoot demography (Optional)

In order to accurately quantify the variations of shoot density over time a permanent transect (a rope marked each meter) should be laid following the maximum slope of the meadow. Shoot density is measured, using the 40 x 40 cm frame, in a quadrat each two metres over the transect (see below experimental design). Permanent transects are useful to observe on a small scale, density changes throughout the range of depths covered by the meadow, from year to year.

Another method to assess changes in shoot density involves marking all the shoots within permanent plots within the meadow (Díaz-Almela et al., 2009). Each plot measures 250 cm^2 (a quadrat of 50 x 50 cm) and a rope running around four metal poles inserted in the sediment delimits its perimeter. All the shoots within the plot must be tagged with a plastic cable tie around the rhizome, 2cm below the leaf meristem. In each new census, total shoot density within the plot was counted again. The new untagged shoots are the new recruits

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and must be tagged with a plastic cable tie of a different colour. The shoot deaths that took place between two censuses was the difference between the shoots initially tagged with a specific colour and the number of survivors tagged with the same colour in the following census.



Figure 5. Tagged *P. oceanica* within a 20 x 20cm plot (photo courtesy of E. Díaz-Almela)

This is a quite time consuming method, but is the best way to accurately quantify both death and the recruitment of shoots in a meadow since it reveals exact changes in shoot density (Figure 5). Plots can be grouped in clusters of three in order to facilitate the localization and spare diving time. Four clusters of three plots (a total of 12 plots) will be enough to characterize a meadow (Díaz-Almela et al., 2009).

- Fish assemblage on *P. oceanica* meadows

P. oceanica meadow is an important habitat for fish. The qualitative composition of fish fauna is similar to that found on hard substrates at similar depths (Guidetti, 2000).

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In studies conducted on *P. oceanica* meadows, the effect of protection on fish is less noticeable (in terms of abundance and biomass) than in rocky reefs. The dominance of Labridae in *P. oceanica* meadows, which are less vulnerable to fishing than Sparidae, that are dominant on rocky reef habitats, could be the main cause of lack of significant differences between protected and unprotected *Posidonia* meadows (Francour, 1996).

Fish also play a significant role on *P. oceanica* meadows, particularly in some MPAs. The herbivorous *Sarpa sarpa* is also favoured by protection, rising its abundance and size, and making the rate of herbivory to increase substantially. In rather small meadows in well-protected marine reserves (such as Medes Islands or Banyuls marine reserves) the increasing herbivory rate can result in overgrazing, although *P. oceanica* seems to overcome it and stay alive (Prado et al., 2008). Most *P. oceanica* consumption (approximately 75%) has been attributed to *S. salpa* activity (Cebrian et al., 1996), although the relative importance of fish grazing can vary both spatially and temporally (Alcoverro et al., 1997; Peirano et al., 2001). Grazing on *Posidonia oceanica* by *S. salpa* is especially intense in shallow waters during summer months (Verlaque, 1990; Tomas et al. 2005), which seems to be associated with the reproductive behaviour of this fish. As a result, *S. salpa* grazing greatly exceeded sea grass leaf growth during summer, causing a clear decrease in shoot biomass and sexual reproduction inside protected areas due to overgrazing (Ferrari et al., 2008).

Sampling fish by means of visual censuses in *P. oceanica* meadows clearly underestimates the specific richness and abundance of the actual fish assemblage. In fact, an appreciable part of the fish is hidden inside the canopy and cannot be observed during daytime (Harmelin-Vivien and Francour, 1992). This bias has to be always expected when performing visual censuses, assuming it as constant in all the counts. Moreover, the number of species per count increases remarkably in ecotones in which *P. oceanica* alternates with other types of substrate, such as sandy or rocky patches. The mere proximity of a single rock can significantly increase the number of fish observed in a census, and the observed number of fish species tends to decrease as the cover and density of *P. oceanica* increases (Figure 6; Garcia-Rubies unpublished data). The cover of *P. oceanica* and other types of

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substrate should be considered as covariates, when visual censuses of fishes are carried out in *P. oceanica* meadows.

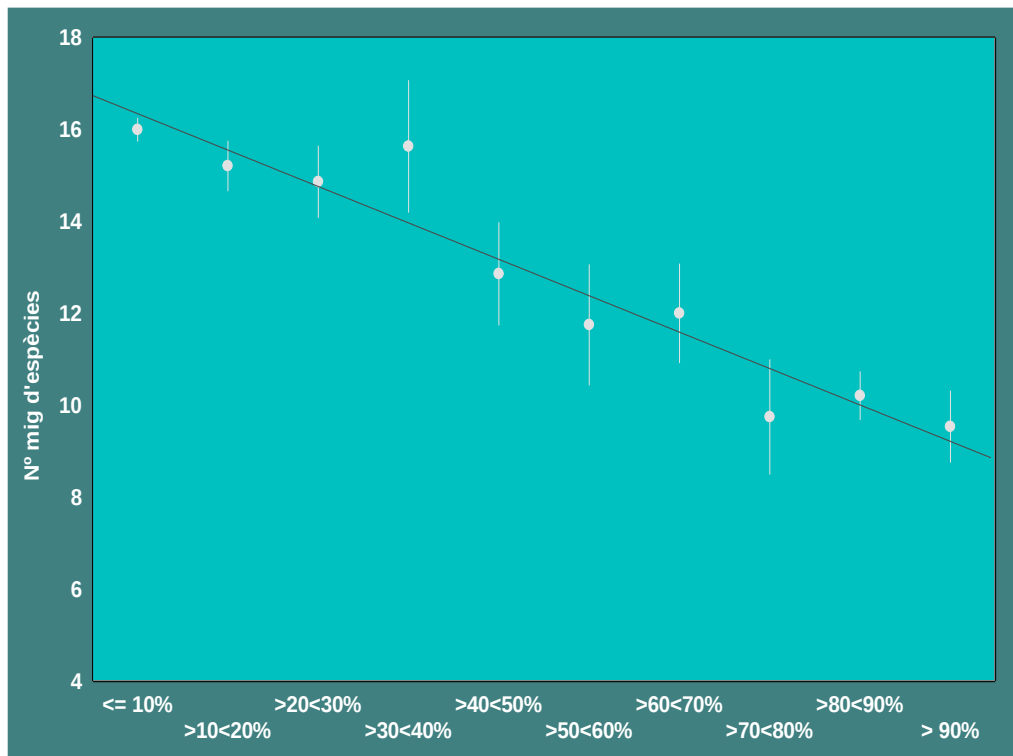


Figure 6. Relationship among mean species richness ($\pm$ s. e.) and % of *P. oceanica* cover on rocky bottoms (after Garcia-Rubies, unpublished data from Balearic Islands)

The visual transects are exactly the same as those carried out on rocky substrate, that is, 50 m long x 5 m wide, covering a total surface of 250 m², using a tape measure. On each transect the species are identified, quantified, and the size of the individuals is estimated with a margin of ± 2 cm.

A huge amount of information can be obtained from these data, including: specific richness, abundance, size and biomass of each species, by using the length- weight transformations available in the literature (Morey et al. 2003). The species can be then grouped into trophic groups (planktivorous, omnivorous, predatory non-piscivorous and piscivorous fish) or on the basis of their commercial value, or origin (“meridional” and exotic fish species).

Depth, and cover of the substrate must be taken into account as covariates in the model.

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- *Pinna nobilis*

Posidonia oceanica meadows host a rich animal community (Piazzi et al., 2016). One of the unique organisms inhabiting this habitat is *Pinna nobilis*, the largest bivalve endemic of the Mediterranean. Its populations have been altered since ancient times, both by mechanical disturbances (anchoring, fishing nets, etc.) and by catches with ornamental purposes. All that threats lead *P. nobilis* to a situation that some authors consider as vulnerable (Guallart and Templado, 2012), and it is currently included in Annex IV of the Habitats Directive (Directive 92/43 / EEC, of 21 May). The prohibition of anchoring on the prairies of *P. oceanica* has led to the recovery of the populations of *P. nobilis* in some MPAs (e. g. Cabrera National Park, Medes Islands Marine Reserve). The recent epidemic (caused by a haplosporidium) has completely decimated the populations of this species in the western Mediterranean in 2016, from Andalusia to the Ebro delta, on the Spanish Mediterranean coast, including the whole Balearic Islands (Vázquez-Luis et al., 2017). The epidemic has begun also to affect the Catalan waters, where a high mortality rate has been already observed on the South Catalonia coasts during the fall of 2017 (pers. obs), confirming that the disease is shifting quickly northwards. The imminent risk of total disappearance of this emblematic mollusc has caused the Spanish administration to consider this species as “vulnerable” in the Spanish Catalogue of Threatened Species (RD 139/11).

In order to save time, *P. nobilis* can be included in the same transects that have been proposed to evaluate the fish assemblage, but restricting the width to 1m.

2.1.3. Experimental design

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Main descriptors	Sampling units	N	Units
Lower limit	Observational (permanent marks)	4 per meadow	- Depth (m) - Shape
Cover (LIT)	Transects (50 m)	5 per Zone	- Cover living <i>P. oceanica</i> (%)
Cover (quadrats)	Random Quadrats (40 x 40 cm); 4 Sub quadrats (20 x 20 cm) per quadrat	10 per Zone	- N Sub Quadrat ⁻¹
Shoot density	Random Quadrats (40 x 40 cm)	10 per Zone	- Shoots m ⁻²
Canopy height	Random Quadrat (40 x 40 cm)	10 per Zone	- Max. Height in cm (in situ)
Fish	Transects (50 x 5 m)	5 per Zone	- Total S - Abundance - TL (cm) - Biomass (g m ⁻²)
Flowering	Quadrats (40 x 40 cm)	10 per Zone	- N flower m ⁻² - N flower shoot ⁻¹
<i>P. nobilis</i>	Transects (50 x 1 m)	5 per Zone	- N m ⁻²

Table 4 . Some indications for sampling *P. oceanica* meadows. All sampling units have to be replicated

The experimental design proposed here is based on the assumption that in general there are no data from “Before” the implementation of the MPAs. To assess the effects of protection we stress the need to compare the selected response variables of the meadow inside an outside the MPA. Replicated control areas are compulsory. A good example of well replicated experimental design is available in Fraschetti et al. (2013). Here, sampling was carried out in July, once per year, from 2006 to 2009. Due to the lack of seagrass beds within the no take zones, the density of shoots was sampled in close proximity of the protected locations but in the buffer zone, where the seagrass forms extensive meadows but human activities potentially affecting this habitat (i.e. anchoring, trawling) are also banned. Two unprotected locations outside the MPA were sampled. At each location, two patches (100–300 m apart) were randomly chosen within beds at 8–10 m depth. In each patch, the density of shoots was estimated in situ within five 1 m² random quadrats. The status of *P. oceanica* beds was evaluated on the basis of the number of shoots per square meter following Pergent et al.

At the smallest scale, replicated line intercept transects (LITs) might be laid to estimate linear cover of *P. oceanica*; the same transects can be used to count fishes (50 x 5m), and

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Pinna nobilis (50 x 1m). Additional quadrats for the herbivores (sea urchins *P. lividus* and *A. lixula*) and detritivores (holothurians) can also be counted.

2.2. Coralligenous assemblages

The coralligenous is an endemic Mediterranean habitat of biogenic origin that is the result of the accumulation of calcareous encrusting organisms that dwell in dim light conditions. All plants and animals thriving in coralligenous assemblages are, thus, sciaphilic. The main criterion used to define the coralligenous is the presence of a bioherm (a body of rock composed by calcareous organisms) of coralline algae grown at low irradiance levels (from 0.05 to 3% of irradiance at the surface and in relatively calm waters (Ballesteros, 2006), usually below the thermocline. This bioherm is always very complex in structure and allows the development of several kinds of communities (Laborel, 1961; Laubier, 1966), including those dominated by living algae (upper part of the concretions), suspension feeders (lower part of the concretions, wall cavities and overhangs), borers (inside the concretions) and even soft-bottom fauna (in the sediment deposited in cavities and holes). Two main typologies were defined by Ballesteros (1992): banks, growing on more or less horizontal substrata, and rims, growing on vertical cliffs and in the outer parts of marine caves. Therefore, the coralligenous assemblages should be considered more as a submarine landscape or a community puzzle with a high biodiversity (more than 1666 species, after Ballesteros, 2006), and a complicated trophic net.

Coralligenous assemblages include some highly valuable species including fish, crustaceans and red coral (*Corallium rubrum*). It also forms a fascinating underwater seascape that is specially appreciated for recreational scuba-divers.

In spite of its ecological and economical importance, and its vulnerability, the European legislation does not refer directly “Coralligenous Habitat” and it is only included as generic “Reefs” in Annex I of the Habitat Directive (HD, 92/43/EEC) in the list of habitat of community interest, although it has been recently included in by EU Red List of marine habitats (Gubbay et al., 2016).

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2.2.1. Main Threats

Coralligenous assemblages develop under conditions of a high environmental stability, of reduced (or predictable) hydrodynamics, and usually below the thermocline (<20°C). These habitats are therefore very sensitive to any environmental change, so they are threatened by a number of direct and indirect human drivers. Some of the main threats to the coralligenous are fishing (extraction of highly valuable species such as crustaceans, fishes and red coral), the excess of sediment input (caused by the resuspension due to nearby trawling), or pollution (which increases turbidity and sedimentation). Other threats imply direct mechanical destruction, such as artisanal or sport fishing (both active or lost nets, fishing lines and longlines), the submarine works (i.e. laying of submarine cables), anchoring, and heavy diving (Coma et al. 2004; Ballesteros, 2006).

Most of these activities can be reduced or regulated, (though not always), in the MPAs. Other threats cannot be controlled in MPAs, including sporadic extreme storm events (Teixidó et al., 2013), invasive species (Piazzi et al., 2012; Cebrian and Rodriguez-Prieto 2012; Piazzi et al. 2012), mucilage events (Schiaparelli et al., 2007) and, mainly, the deleterious effects of climate change (Garrahou, et al., 2009) (Pairaud et al., 2014). In fact, climate change in the Mediterranean Sea translates into widespread massive mortality events, which can be currently considered as the most serious threat to the coralligenous habitats, only comparable to widespread episodes of coral bleaching in the tropical seas (Crisci et al., 2017).

2.2.2. Monitoring coralligenous assemblages.

Unlike *P. oceanica* meadows, a correct and comprehensive monitoring of the coralligenous habitat presents numerous difficulties. In this particular case, trained divers with a sound knowledge of the environment are required, since the underwater work must be carried out at important depths and a maximum amount of information should be extracted in the

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minimum diving time. Moreover, coralligenous habitats show an extremely high heterogeneity, from small (e.g. 1–100 m) to large (0.1–100 km) scales, and there is still a lack of sound knowledge about these habitats (Gubbay et al., 2016).

Conservation status	Surveillance	Information and descriptors
Area	Mapping	- Detailed GIS map including the habitat extent and bathymetric range. - Direct and indirect surveys (Scuba/Side scan/ROV) - Local Stressors
Structural (basic indicators)	Monitoring	- Species/Categories composition/abundance - o Indicators of the degree of complexity - o Indicators on coralligenous functioning ▪ Bioeroders ▪ Bioconstructors • Algal builders • Animal builders - o Species of particular importance - Qualitative or semiquantitative indicators of disturbance - o Fishing nets - o Invasive species - o Sedimentation
Environmental data	Monitoring	- Main local threats (from GIS Map) - Water Transparency (satellite) - SST (satellite) - SST (data loggers)
Experimental design	Monitoring	- Analysis: - o Protection: P (inside vs. outside each MPA) - o Location: L(P) (each MPA) - o Site: S(L(P)) - o Area: A(S(L(P))) - o Time: T

Table 5. Summary of the coralligenous habitats monitoring protocol

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As many other habitats, coralligenous has been largely affected by human activities across the Mediterranean Sea. As a consequence changes may be assumed using baselines that do not longer represent a 'pristine' condition (Gatti et al., 2015). The wide range of depths that coralligenous habitats can reach makes difficult the comparison between distant zones. In spite of all those problems, indexes of environmental status have proliferated under MSD and MSFD. Also in this case, it has to be stressed that replicated control locations have to be always used when assessing the effect of protection also with the use of indicators.

The Ecological Status of Coralligenous Assemblages index (ESCA; Cecchi and Piazzzi 2010; Cecchi, Gennaro et al. 2014) and the Coralligenous Assemblages Index (CAI; (Deter et al., 2012)) both adopt the state of coralligenous habitats as an ecological status indicator of coastal waters according to the old WFD. Both are based on data collected by photographic sampling and subsequent image analysis using the free software (CpCe Coralligenous Assemblages Version, Image J). The Rapid Visual Assessment (RVA method) and the associated COARSE index (Gatti et al., 2012, Gatti et al., 2015)) aim at assessing the quality of coralligenous reefs as an indicator of seafloor integrity according to the MSFD; this method is based on a seascape approach through direct diving surveys. The Index-COR (Sartoretto et al. 2014) integrates the sensitivity of species to fine sedimentation and organic matter input; the taxonomic richness; the structural complexity of the assemblages, and the level of necrosis of gorgonians. Ruitton et al. (2014) developed an ecosystem approach in the Ecological Quality Biological Index (EQBI), based on the identification and weighting of the functional components at different trophic levels, whereas the OCI index (Paoli et al., 2016) combines structural and functional measures to evaluate the overall ecological complexity of coralligenous ecosystems (Ballesteros, 2006). Not of all these indices lead to same results, making it difficult to know which one gives the most objective estimate of GES (Bavestrello et al., 2016).

The calculation of these indexes requires a considerable amount of fieldwork, as well as highly specialized personnel in identifying a large number of species *in situ* or through photographs. All these indexes can be considered good enough for the evaluation of the environmental status of the coralligenous at a given moment, or also to verify the variations

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that can occur at medium or long term. However, we consider rather unrealistic its application in a high frequency systematic annual or biannual monitoring protocol. Table 5 shows a simplified monitoring program.

- Mapping

Mapping of coralligenous habitats (Figure 6) is very complex because of its patchy

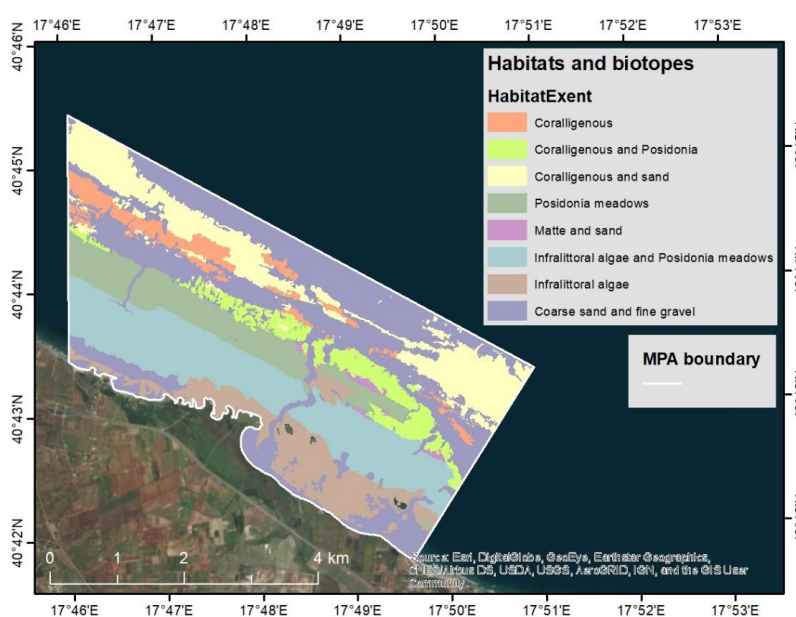


Figure 6 . Map of the benthic habitats of Torre Guaceto MPA

distribution, sometimes limited extensions and occurrence of some of the assemblages on vertical walls. These features make its mapping expensive and time consuming and will demand the combined use of the most modern technologies and new analytical methods (use of new devices such as

side-scan sonar or multibeam echosounder) for field surveys and post-data treatment. Methodological problems associated with mapping of coralligenous habitats, such as the acquisition and interpretation of side-scan sonar images for horizontal substrata, or multibeam echosounder images suitable both for horizontal substrata as well as vertical cliffs (the latter are one of the most characteristic substrata where coralligenous outcrops develop), need to be addressed to provide significant advances in mapping activities.

Mapping and monitoring should be combined in a way to increase the knowledge on the distribution of the coralligenous habitats. The monitoring areas could then be chosen with

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Algal builders	
Rhodophyta • Coralline algae • Encrusting <i>Peyssonnelia</i> spp.	
Animal builders	
Bryozoans • <i>Myriapora truncata</i> • <i>Schizotheca serratimargo</i> • <i>Pentapora fascialis</i> • <i>Smittina cervicornis/Adeonella pallasii</i> • Encrusting bryozoans²	Serpulids • <i>Filograna implexa/Salmacina dysteri</i> • <i>Serpulidae</i> • <i>Protula</i> sp. Scleractinians • <i>Caryophyllia inornata</i> • <i>Caryophyllia smithii</i> • <i>Hoplangia durotrix</i> • <i>Leptopsammia pruvoti</i> • <i>Madracis pharensis</i>
Agglomerative' animals	
Sponges • <i>Faciospongia cavernosa</i>	Bryozoans • <i>Beania</i> spp.
Bioeroders	
Sponges • <i>Cliona</i> sp. Echinoids • <i>Echinus</i> sp. • <i>Sphaerechinus granularis</i>	Molluscs • <i>Gastrochaena dubia</i> • <i>Lithophaga lithophaga</i>
Species of particular importance (particularly abundant, sensitive, architecturally important or economically valuable)	
Rhodophyta • Uncalcified <i>Peyssonnelia</i> spp. Sponges • <i>Crambe crambe/ Spirastrella cunctatrix</i> • <i>Petrosia ficiformis</i> • <i>Hexadella racovitza</i> • <i>Aplysina cavernicola</i> • <i>Axinella cannabina</i> • <i>Axinella polypoides</i>	Gorgonians • <i>Paramuricea clavata</i> • <i>Eunicella singularis</i> • <i>Eunicella cavolini</i> • <i>Corallium rubrum</i> Alcyonarians • <i>Alcyonium acaule</i> Zoantharians • <i>Parazoanthus axinellae</i>
Invasive species	
Clorophyta • <i>Caulerpa cylindracea</i>	Rhodophyta • Red turf algae
Table 6. Example list of species occurring within the coralligenous habitat along the Croatian part of the Adriatic Sea (From RAC/SPA - UNEP/MAP, (2014))	

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regards to different environmental conditions, providing a broader view on the status of the habitat (RAC/SPA., 2013)

-Sampling the coralligenous

The depth at which coralligenous habitats develop is a limiting factor for scuba sampling time. The sampling must optimize the sampling time, in such a way that the maximum amount of information possible should be obtained in a short time. This is achieved through the use of photography, widely used in coralligenous studies. In this case we suggest photosampling of minimum of three areas of 10 m² in which 10 photos of 50 x 50 cm were taken within the same site at the same depth range. These photographs were then analysed using the free software ImageJ (Cecchi et al., 2014), to identify and quantify (cover in%) each species or morphological groups (if the species are not easily identifiable).

In addition to obtaining the number of species, and their abundance, some functional parameters can also be obtained. Thus, the organisms can be classified into bioeroders and bioconstructors, if they contribute to the growth or erosion of the habitat. It is also important to distinguish species of special interest, given that they the most sensitive to suffer massive mortalities due to thermal anomalies and invasive species (Table 6). It is also worthwhile to note the presence of nets or other fishing gear during the dives.

2.2.3. Experimental design

Also in this case, we introduce as an example a study (Piazzi et al., 2015) in which the effects of protection were assessed by a simple design. The main factor "Protection" (P) is fixed with two levels (protected vs. unprotected); the factor "Locality" (L (P), is random, nested in "Protection" with as many levels as MPAs; the factor "Site" (S (L (P), is random, nested in "Locality" with two levels, and finally the random factor "Area" nested in "Site" (A (S (L (P). In each area 10 photos are randomly taken in 3 areas of 10m². Localities were situated 10

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km apart; sites were 1000 m apart and areas 10m apart. This design should be replicated once / two times every year.

2.3 Mass Mortality events

The massive mortalities of filter-feeder invertebrates in the Mediterranean have been documented since 1973, when a mortality of *Eunicella stricta* (= *singularis*) was observed in Banyuls-sur-Mer by (Weinberg, 1975). The author attributed the mortality to an excessively hot and prolonged summer, due to the absence of the prevailing winds (from north and south), which used to break the water stratification after the summer. Weinberg (1975) ruled out that the mortality were due to a decrease in oxygen, although he did not give alternatives to mortality other than high temperature. Actually all the colonies that died were located in areas that exceeded 24°C.

A massive mortality event was documented later in the Ligurian Sea affecting sponges and cnidarians in 1983. From then on, more than 240 mass mortality events have been frequently observed from 1992 to 2009 (Rivetti et al., 2014), including two severe peaks in 1999 (Cerrano et al., 2000; Perez et al., 2000) and 2003 (Garrahou et al., 2009), which affected more than 30 benthic macro- invertebrates along 1000km of coastline in the NW Mediterranean Sea. The two peaks of mortality (1999 and 2003) coincided with positive temperature anomalies that, in all cases, were between 3 and 4 °C above the usual values (Rivetti et al., 2014). The authors excluded the independence of massive mortality events and abnormally high temperature episodes with 99% probability both on a large scale (1000 km), and on a local scale (300 km). However, these results should be taken with caution (Rivetti et al., 2014) because:

- 1) Mortalities of benthic organisms have been observed occasionally without a systematic follow-up focused on these events. That implies that these events have not been properly documented, or that some have not been documented at all.

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- 2) The singularity of mortality events makes its statistical treatment impossible, since only positive results are obtained (when mortality actually occurs), but not negative ones (when a thermal anomaly does not translate into mortality).
- 3) Different organisms exhibit different levels of sensitivity or resilience at different temperatures (Table 7).

High temperatures and progressive low food availability for invertebrate suspension feeders, characterize summer conditions in the Mediterranean Sea. In the last 33 yrs. the global warming, produced around 40% lengthening of the summer conditions that represent prolonged exposure to warmer summer conditions coupled with reduced food resources (Coma et al., 2009). By means of a simulation of the effects of these conditions on a model organism Coma et al. (2009) demonstrated a biomass loss of >35%, which was similar to that noted in field observations during mass mortality events. These results indicate that temperature anomalies are the underlying cause of the mortality events, with energetic constraints serving as the main triggering mechanism, either due to starvation or to secondary infections due to the weak state of the organisms.

In experimental conditions, (Crisci et al., 2011) demonstrated that the mortalities of *Paramuricea clavata* due to abnormally high temperatures were produced based on two causes:

- 1) Exposure during a short period of time to temperatures much higher than usual. Under these conditions, *Paramuricea clavata* exhibited the first signs of necrosis after one week of exposure at 25°C. When the colonies were exposed to higher temperatures ($\leq 26^\circ\text{C}$) necrosis began from the very first day, and extensive damage was observed after in less than 5 days. In experimental conditions 25°C was also the critical temperature for other octocoral species, such as *Corallium rubrum* (Torrents et al., 2008) and *Eunicella cavolini* (Pivotto et al., 2015).
- 2) Exposure for a long period of time to temperatures slightly higher than usual (from 23°C to 24°C). In these conditions *P. clavata* showed the first stages of necrosis after 49 days.

Crisci et al., (2017) revealed that *Paramuricea clavata* could present slight differences in

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resistance to the exposure to high temperatures between different populations from three locations in the Western Mediterranean (Medes, Calanques and Scandola), at two depths (20 and 40m). The thermo-resistance of *P. clavata*

did not coincide with local phenotypic adaptations, but with differences due to the genetic drift of the different populations. In any case, it was shown that the superficial populations (20m) were slightly more resistant than the deep ones (40m) in all cases, in spite of their provenance.

The resistance or resilience also varies between different species, being those of deeper distribution the more vulnerable to long exposures at high temperatures. That is also true within the same species with a broad depth distribution such as *Corallium rubrum*. Torrents et al. (2008) contrasted resistance to high temperatures of two populations of *C. rubrum*, from the same zone at two different depths (11 and 40m). They demonstrated that exposure during 24 days at 24°C only caused a beginning of mortality among the colonies of *Corallium rubrum* from the deep population (from 40m depth), while an exposure to 25°C caused mass mortality in both populations. Table 7 shows a summary of the thermotolerance of various Mediterranean sessile organisms under experimental conditions.

The mortalities can be due both to short exposures to very high temperatures, as well as to long exposures to temperatures slightly higher than usual (Rivetti et al., 2014). That implies that the depth the thermocline reaches and the time it remains during summer conditions are fundamental factors to explain the mass mortality events. Mortality can be spatially very localized, depending on the characteristics

of local hydrodynamics, or generalized, if the positive thermal anomaly is extensive. That is why not all mortality events are always widespread at a regional level (Rivetti et al., 2014). In any case, the formation of the thermocline, the water temperature reached above the thermocline, the depth the thermocline reaches (thickness) and the period of time it remains, are the key factors to trigger, or not, massive mortality events. Since mass mortalities have been related to the anomalous positive temperature conditions, and these

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conditions are expected to be more frequent under the current global warming trend, mass mortality events might increase during next decades (Garrahou et al. 2009).

2.3.1 Monitoring Mass Mortality events

Mass mortalities of benthic invertebrates in the Mediterranean Sea are incontestably related to positive thermal anomalies of the upper water column (Rivetti et al., 2014). Mass mortality events that occurred in 1993, 1999 and 2008 were directly associated to sudden deepening of the thermocline (Cerrano et al., 2000; Linares, 2005; Coma et al., 2009; Garrahou et al., 2009; Bensoussan, et al., 2010; Cebrian et al., 2011; Huete-Stauffer et al., 2011). However, the thermal anomalies along the water column are difficult to obtain, since there are only a few coastal sites in the NW Mediterranean collecting high-resolution temperature series (Salat and Pascual 2002). Models (such as OPA Parallélise; (Madec 2008)) and satellite measures of SST are good indicators of water column temperatures within the entire infralittoral zone (42m depth) only during the winter and spring seasons, that is, before and after the thermocline formation (Deidun et al., 2016), so the only way to accurately measure the main thermocline parameters is the installation of data-loggers (autonomous sensors) encompassing the depth the thermocline can reach and the time that remains in place.

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Species	Treatment T (°C)	Upper T °C (tolerance limit)	Number of exposure days before showing the first signs of necrosis	N Popul.	References
Gorgonians					
<i>Corallium rubrum</i>	24, 25, 27,30	25	14(25), 3(27), 1(30)	2	(Torrents, Tambutté et al. 2008)
<i>C. rubrum</i>	14 to 25	25	3	1	(Previati, Scinto et al. 2010)
<i>Paramuricea clavata</i>	24, 25, 26	24	5(24), 3(25), 2(26)	1	(Bally and Garrabou 2007)
<i>P. clavata</i>	23,5	23,5	89	1	(Coma, Ribes et al. 2009)
<i>P. clavata</i>	14 to 25	25	3	1	Previati et al. (2010)
<i>P. clavata</i>	25, 26, 27, 28	25	4-21(25), 3-5(26), 2-4(27),2-3(28)	8	(Crisci, Bensoussan et al. 2011)
<i>P. clavata</i>	25	25	1-10	4	(Kipson, Linares et al. 2012)
<i>P. clavata</i>	25 to 27	25	15(25), 3(25+1day)	1	La Rivière (2013)
<i>Eunicella singularis</i>	24, 26	26 sublethal	1	2	Ferrier- Pagés et al. (2009), (Pey, Zamoum et al. 2011)
<i>E. singularis</i>	24, 26, 27, 28, 30	27-28	1	4	(Pey 2012)
<i>E. singularis</i>	26, 27, 28, 29	28-29	1-2	2	Linares et al. (2013)
<i>E. singularis</i>	18 to 26	24 sublethal	No necrosis	1	(Ezzat, Merle et al. 2013)
<i>E. singularis</i>	14 to 25	>25	No necrosis	1	Previatti et al. (2010)
<i>Eunicella cavolini</i>	14 to 25	25	3		Previatti et al. (2010)
Anthozoans					
<i>Acyonium acaule</i>	26, 27, 28, 29	26	12(26), 8(27), 4(28), 2(29)	1	ClimCares experiment
<i>Leptosamia pruvoti</i>	26, 27, 28, 29	26(> 21 days) 27	13(27), 2(28), 4(29)	1	ClimCares experiment
Zooxanthellate corals					
<i>Oculina patagonica</i>	24, 26, 28	28	34		Rodolfo-Metalpa et al. (2006)
<i>Cladocora caespitosa</i>	24, 26, 28	26	48(24), 34(26), 34(28), 21(28)	1	Rodolfo-Metalpa et al. (2005, 2006))
<i>C. caespitosa</i>	26, 27, 28, 29,30	>30	No necrosis	2	Linares et al. (unplubished data)
Sponges					
<i>Ircinia fasciculata</i>	23, 27	27	4	1	Cebrian et al (2011)
<i>Crambe crambe</i>	26, 27, 28, 29	26	3(26), 3(27), 2(28), 2(29)	2	ClimCares experiment
<i>Petrosia ficiformis</i>	26, 27, 28, 29	26	9(26), 7(27), 2(28), 2(29)	1	ClimCares experiment
<i>Dysidea avara</i>	26, 27, 28, 29	26	9(26), 7(27), 2(28),1(29)	1	ClimCares experiment

Table 7. Thermotolerance experiments compiled in Garrabou et al. (2013) and references therein.

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- Sampling

To monitor the status of the populations of large invertebrate filter feeders, the first condition is to know their distribution in the area. Priority will be given to areas that harbour populations of gorgonians, such as *Paramuricea clavata*, *Eunicella cavolini*, *Eunicella stricta*, *Lophogorgia ceratophyta* and *Corallium rubrum*. Once located, these places must be georeferenced and their extension and density estimated (number of colonies m^{-2}), as well as the upper and lower limits, based on visual inventories. It would be very desirable if more than one species were taken into account, if possible.

The second step is to estimate the current status of the population, that is, the number of colonies affected or not by necrosis and the degree of necrosis of the colonies (see Figure 6). It is considered that an affected colony is the one that presents 10% necrosis. It should also be assessed whether the partial mortality is recent (nude axis or colonized by pioneering species such as hydrozoans) or old (axis densely populated by long-lived species such as calcareous algae or bryozoans). The combination of recent or old necrosis implies that there has been more than one episode of mortality in a relative short time.

The monitoring of these populations should be at random, counting a minimum of 100 colonies or by randomized 10x1m transects (density is expressed as n colonies m^{-2}). The number of transects will depend upon the extent of the population.

Random transects can also be combined with fixed transects. Fixed transects (signalled by plastic screws stuck to the bottom, which are the basis for extending an elastic grid of 10 quadrats of 1m² each). On these transects the colonies are counted and measured one by one. That allows getting an exact idea of the dynamics of the colonies inside each quadrat; that is, growth, necrosis and disappearance (by uprooting) or recruitment (appearance of new young specimens) (Coma et al. 2000).

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Figure 7. Estimation of the extent of necrosis in a colony. Colonies affected with more than 10% of necrosis are considered as affected. If necrosis affects more than 75% the colony is considered dead (Perez et al., 2010)

Data loggers should be placed near the sampling sites. They collect hourly high precision records (0.2°C and a resolution of 0.15 to 0.02°C). The sensors were placed at 5m intervals from 5 to 40m at least. The data loggers should be ideally attached to plastic screws sealed along rocky walls (Pairaud et al. 2014), and they have to be laid before and recovered after the period of summer stratification.

Counting should be done annually after the summer period of stratification. Population sampling should coincide with this period and be able to relate the state of the population with the temperature registers obtained during this period. Whatever the results obtained in each MPA should be put in common.

Estimates of mucilaginous aggregates or invasive algae cover can be made, also, in each quadrat to cope with the potential heterogeneity, however usually this phenomenon is quite homogenous, at least at the scale of the 10 m^2 transects, and it could be easier to provide a single estimate for the whole transect (Garrabou et al. 2013).

2.3.2 Experimental Design of Mass Mortality events

Also in this case, replicated sampling inside and outside the MPA should be done. As an example Micheli et al. (2012), to assess the efficacy of reserves in recovering abalone

populations from fishing impacts, between 2006–2010 they monitored the abundance, size structure, reproductive output, and post-larval recruitment of pink abalones (*Haliotis corrugata*) within the reserves and in adjacent fished areas with similar habitat characteristics. Their data precede the 2009 mortality event, allowing them an unprecedented view of its demographic effects, both within reserves and in fished areas. Prior to 2009, fishers had not witnessed sudden and widespread benthic invertebrate mortality. Table 8 for some indications about response variable and tools to be used in the framework of monitoring the effects of climate change.

Conservation status	Surveillance	Information and descriptors
Area	Mapping	- Detailed GIS map including the habitat affected and bathymetric range. - Direct and indirect surveys (Scuba)
Structural (basic indicators)	Monitoring	- Species affected - o Density - o % Necrosis -
Environmental data	Monitoring	- SST (satellite) - SST (data loggers)
Experimental design	Monitoring	- Analysis: - o Protection: P (inside vs. outside each MPA) - o Locations: replicated in protected and not protected conditions - o Sites replicated within locations

Table 8. Summary of the mass mortality monitoring protocol

However, some aspects should be taken into account:

- 1) Not all the most susceptible species to thermal anomalies are likely present in all MPAs.
- 2) Most of these species show a contagious distribution, that is to say, colonies or individuals can be found in certain areas being absent in others.

Under these circumstances a reasonable approach should be:

- 1) Species whose thermo-tolerance is known (Table 7) must be the priority to monitoring.

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- 2) Try to find the places where these species are abundant. Check what density presents there and estimate the status of the population (% necrosis), based on transects of 10x1m or counting a minimum of 100 individuals or colonies.
- 3) Install the temperature data loggers in the chosen area, where the population selected for monitoring is located.

2. 4. Rocky reef fish assemblages

When appropriately designed, MPAs favour the recovery of harvested fish populations in the Mediterranean Sea and elsewhere (Bell, 1983; Garcia-Rubies and Zabala, 1990; Harmelin et al., 1995; Claudet et al., 2011; Fenberg et al. 2012). The main reason for these protection effects is the drastic reduction in overall mortality: when fishing mortality is removed or reduced, stock recovery is the most logical expected consequence (Bell, 1983). The more vulnerable to fishing a species is, the more it will respond to cessation of fishing mortality (Macpherson et al. 2000). Therefore, the ecological benefits derived from these conservation units are essential for the sustainability of exploited ecosystems and effective management of MPAs is a key issue in an age of changing oceans (Ribeiro and Olsen, 2013). Total recovery of protected populations in MPAs has only recently been described in the Mediterranean Sea (Coll et al. 2012; Garcia-Rubies et al., 2013), in spite of the large number of Mediterranean MPAs. This is probably due to the scarcity of well-protected areas and/or the relatively young age of many of these MPAs; however, it can also be attributed to the lack of long-term studies on the changes of protected fish populations in most MPAs.

The recovery of fish stocks in no-take marine reserves is simply spectacular in terms of biomass. According to (Sala and Giakoumi 2017) the biomass of fish in the no-take areas is, on average, 617% higher than in the fished areas. Other, more subtle result, of fish recover, is the inversion of the trophic chain, due to the progressive recovery of large predators, which can reach half of the fish biomass in strictly protected areas (Sala et al., 2012). In any case, the recovery of the most vulnerable fish populations can be an extremely long process,

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which may exceed 25 years (Garcia-Rubies et al., 2013). Obviously, the recovery of fish populations produces secondary side effects on the benthic communities. The most well-known has been the function of predatory fish to keep at bay the proliferation of sea urchin, thus avoiding overgrazing and the subsequent the formation of barrens in infralittoral rocky reef habitats (Sala and Zabala 1996; Guidetti and Sala 2007).

But not all well protected no-take areas produce the same spectacular results. Environmental factors acting at both, small and medium spatial scales, are determining factors in the final fish biomass that a given no-take area can reach. The environmental “rugosity” (Luckhurst and Luckhurst 1978), the depth (between 0-15m) on a small scale, the exposure of the area to waves and currents, and a sharp nearby depth change, on a medium spatial scale, are positively related to fish biomass. Taking into account these environmental features, one can predict what would be the maximum biomass of fish a given area can get if it were effectively protected (Coll et al., 2013). Knowing all these environmental factors, the difference between the observed and the expected biomass gives us a very realistic idea of the effect of fishing.

2. 4. 1. Main threats

Fishing is currently considered one of the major human disturbances in coastal marine ecosystems (Jackson et al., 2001). The Mediterranean is a sea that has been exploited since ancient times (Lotze et al., 2011). At present, 93% of the stocks can be considered overexploited. The effect of overfishing is very tangible in the rocky bottoms near the coastline, where commercial artisanal fishing converges with recreational fishing, which has experienced a notable boom in recent years, along with the popularization of nautical activities.

In the Balearic Islands, for instance, the recreational fishing is practised by up to 70000 people, around of 10% of the total population, that catch 1209 tn year⁻¹ (roughly a 43% of the commercial catch) (Morales-Nin et al., 2005, Morales-Nin, et al. 2010). In general, the most notable effects of fishing in littoral rocky reef fish assemblage, translate into a notable

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decrease in the abundance, size, biomass. Lower frequency of occurrence of certain highly vulnerable species to selective fishing, such as spear fishing, is also a good descriptor of fishing intensity. In fact, spear fishing it is the only modality in which the fisherman can choose exactly the prey that will be captured (Coll et al., 2004). This implies that valued species and individuals of large sizes will be always the preferred preys (Dalzell, 1996). The dichotomy between the effectiveness of hook or spear gears, related to their density dependence, can be inferred from the study by (Hansen et al., 2000), which demonstrated that spear fishing increased the proportion of the population caught (not the total catch) per unit effort when fish density declined. The populations of big, high-trophic level, long-lived sedentary species (such as *Epinephelus* spp., *Labrus viridis*, *Sciaena umbra*) can virtually disappear from large portions of coastline waters even under a moderate spearfishing pressure.

More subtle effects of fishing are the change in the behaviour of target species (Alos et al., 2012), or seriously alter the sex ratio in proterandric and/or proteroginic species (Lloret et al., 2012), compromising the reproduction and the viability of the populations at medium and long term fishing may alter life histories and favour trait combinations that collectively reduce adult body size (Alos et al., 2014).

2. 4. 2. Monitoring rocky reef fish assemblages

This sampling method has been carried out in the marine protected area of the Freus d'Eivissa and Formentera marine reserve, from the year 2000 to the present (Coll et al., 2012, Coll et al., 2013) to monitor the commercial fish populations from the year 2000 to the present. The same sampling can be applied to any MPA to evaluate the entire fish assemblage and the effects of protection.

A number of indicators can be derived from abundance and size, such as species richness, abundance and biomass. The species can be grouped according their trophic level (Fishbase; www.fishbase.org/), their commercial interest, or their origin ('meridional' species or NIS)

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- UW Fish censuses

Fish and habitat data were collected through visual censuses carried out by scuba divers. The sample unit was a transect of 50 x 5 m (250 m²) marked out on the seafloor with a tape as the diver swam forward. Each transect was sampled by a single diver that identify all the species, and estimates the abundance and the size of each specimen. All transects were haphazardly distributed, always on rocky substrata between 10 and 15m depth and a distance greater than 20 m between contiguous transects in the same site.

- Small scale environmental variables

In each transect the percent cover of six habitat types is directly measured on a tape: small blocks (Ø, 1 m), medium blocks (1, Ø, 2), large blocks (Ø, 2), homogeneous rock, sand and *Posidonia oceanica*. The depth of transects is kept as constant as possible, and the minimum and maximum depths were recorded (from 10 to 15m). The proxy of “rugosity” is a semi-quantitative variable that is scaled from a minimum of 1 to a maximum of 4 based on a scale that has been used previously by other authors (Ordines et al., 2005). The slope is a semi-quantitative variable ranked locally on the transversal inclination of each transect: 1, inclination less than 30°; 2, from 30 to 60°; 3, from 60° to 90°; and 4, for caves and overhangs.

2. 4. 3. Experimental design

Also in this case, the effects of protection should be compared comparing protected vs non protected locations (Table 9). There are many examples in the literature dealing with this issue.

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After tested for collinearity, environmental variables (at small scale) act as covariates of response variables (species richness, abundance, and biomass).

Conservation status	Surveillance	Information and descriptors
Area	Mapping	- Detailed GIS map sampling zones - Local stressors
Structural (basic indicators)	Monitoring	- Fish - o Species ▪ Trophic level ▪ Commercial ▪ Meridional ssp ▪ NIS - o Abundance - o Biomass
Environmental data	Monitoring	- Small Scales Environmental factors - o Substrate Cover - o Rugosiy - o Slope

Table 9. Summary of the rocky reef fish monitoring protocol

2.5. Algal canopies

The well-lit rocky bottoms of all temperate seas are characterized by the dominance of big macroalgae (Fucales and Laminariales). These bioengineer species form highly diverse coastal habitats, which are typical of pristine environments (Dayton, 1985). Algal canopies are very important habitats providing habitat, shelter, food, and nursery areas for a multitude of organisms including fishes (Cheminée et al., 2013).

In the Mediterranean Sea the genus *Cystoseira* C Agardh dominates the well-lit rocky bottoms of the Mediterranean, from the very littoral fringe at 0m depth, down to more than 30m depth in the sub-littoral zone.

Of the 51 species of the *Cystoseira* genus, 36 are present in the Mediterranean Sea, and 30 are endemic to this sea. Three species are typical of the littoral fringe, from 0 to 1m depth (*Cystoseira amentacea*, *C. mediterranea*, *C. tamarascifolia*), while the rest are typically sublittoral distributed in function of light, temperature, and herbivory. It is admitted that

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the shallow populations of *Cystoseira* are limited by bottom-up mechanisms, while the sublittorals are top-down controlled (Hereu 2008; Cardona et al., 2013).

2.5.1. Threats

All *Cystoseira* species are in clear regression in the Mediterranean and some have totally disappeared from large areas (Thibaut et al 2005, Thibaut et al 2014), which has caused all species to be included as Community Interest Habitats by the EU and, except *C. compressa*, all of them are included in Annex II of the Barcelona Convention (2010). *Cystoseira amentacea*, *C. mediterranea*, *C. sedoides*, *C. spinosa* and *C. zosteroides* are considered as species strictly protected by the Berne Convention (1979).

These habitats are becoming rare at local, regional and basin scales at an alarming rate (Airolidi and Beck, 2007). In the past decades, algal canopies have suffered widespread and apparently irreversible loss. The phenomenon of the disappearance of the algal canopies has been documented, mostly in the Western/ Central Mediterranean and the Adriatic Sea but it has gone largely unnoticed along most of the Mediterranean littoral.

In the Eastern Mediterranean, the magnitude of overgrazing by invasive lessepsian fish species *Siganus luridus* and *S. rivulatus* (Sala et al., 2012; Vergés et al 2014, Giakoumi, 2014) has not yet been properly quantified.

The most important stressors (Cebrian et al, 2014) are poor water quality, coastal development and/or littoral urbanization, overgrazing by sea urchins, increase sediment inputs, and severe storms. Other, such as substratum instability, changes in water circulation, heavy metal pollution and aquaculture seems to be more restricted to local pressures. Human trampling and high water temperature affect mainly the shallowest distributed species.

Lost algal forests tend to be replaced by assemblages of lower structural complexity, such as turf-forming, filamentous or other ephemeral seaweeds, mussels or “barrens” (Mangialajo et al., 2008; Connell et al., 2014; Strain et al., 2014). Canopy algae and turfs have been suggested to represent alternative states in shallow temperate rocky coasts

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under different disturbance and stress regimes (Airolidi *et al.*, 2009). Although some of these changes can be reversed, if the conditions improve (such as water quality, for example), others are much more resilient. Given that most *Cystoseira* species have a limited ability of propagate, if the original conditions recover it is possible to consider artificial re-forestation of depleted bottoms from natural healthy populations (Gianni et al., 2013), and see the project MERCES (Marine Ecosystem Restoration in a Changing Sea; www.merces-project.eu/):

The case of barrens ground caused by overgrazing of sea urchins is a typical example of a discontinuous catastrophic regime shift: there is an abrupt change from one state (algal forest) to the other (barren ground) that occurs once the the sea urchins exceed a certain threshold of density or biomass; the new state is very stable and hysteretic, as sea urchin biomass is maintained after the shift, preventing any reversion towards the previous state. An obvious temporal asymmetry is also introduced since the sea urchin biomass can be maintained after the establishment of a barren at the cost of urchins having to eat encrusting algae, sessile invertebrates, and newly settled macroalgae preventing any recolonization (Ling et al., 2015).

No-take zones in MPAs are useful in maintaining high densities of sea urchin predators, mainly sea breams *Diplodus vulgaris* and *D. sargus* (Guidetti, 2006) and Guidetti and Sala (2007) demonstrated that a minimum of 15 adult sea bream per 100m², were necessary to reduce interannual variations in sea urchin densities by preventing abnormally high annual recruitments (Hereu et al 2012) that can produce the shift in the coming years.

2.5.2. Monitoring algal canopies

An extensive exploration should be carried out to locate the forests and identify the species. Once located, to map and georeferenced the forests is needed.

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Conservation status	Surveillance	Information and descriptors
Area	Mapping	- Detailed GIS map of algal canopies /barren distribution - Local stressors
Structural (basic indicators)	Monitoring	- Algal canopies - o Species ▪ Cover - o Sea urchins ▪ Density ▪ Biomass - o Barrens
Environmental data	Monitoring	- SST (satellite) - SST (data loggers) - Water transparency
Experimental design	Monitoring	- Analysis: - o Protection: P (inside vs. outside each MPA) - o Locations: replicated in protected and not protected conditions - o Sites replicated within locations

Table 10. Summary of the algal canopies monitoring protocol

Sampling

The fastest and most efficient method is to roughly estimate the coverage of *Cystoseira* with respect to the cover of other benthic communities or habitats (other algae, sand patches, turfs, barren grounds...) on a 50m long transect. Changes must be recorded at small scale (each 25 to 50cm along the transect). Up to 4 transects should be carried out per site. Ten 20 x 20 cm quadrats per site should be randomly located within the canopy to measure density and height of the individuals, as estimates of the health status of the canopy.

Density of sea urchins should be also assessed in 5 randomly placed 10 x 1m transects per site. Only adult specimens (diameter > 3cm) must be taken into account. It could be also interesting to map and georeferenced the presence and dimensions of barren grounds in order to quantify changes in time.

2.5.3. Experimental design

A possible suggestion is to follow what has been done in Frascchetti et al. (2005) two locations corresponded to the two A zones and two locations were selected as reference areas (controls) outside the A zones (Fig. 1). Controls were chosen at random from a set

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of possible locations, to provide comparable habitats to those occurring at the fully protected locations (in terms of type and slope of the substratum and exposure to waves). Three sites (approximately 100–300 m apart from each other) were randomly selected at each of the four locations.

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