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Acronym: AMAre

Project Title: AMARe - Actions for Marine Protected Areas

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

Specific Objective: 3.2
To maintain biodiversity and natural ecosystems through strengthening the management and networking of protected areas

https://amare.interreg-med.eu/

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National Marine Park of Alosissos Northern Sporades (NMPANS)

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Summary

List of abbreviations and terms

EBM – Ecosystem Based Management
GES – Good Environmental Status
MP – Management Plan
MPA – Marine Protected Area
MR – Marine Reserve
MSFD - Marine Strategy Framework Directive
NTZ - No take Zone
PA – Protected Area
PPZ - Partially Protected Zone
PUs – Planning Units
ROV – Remotely Operated Vehicle
SCI – Site of Community Interest
SST - Sea Surface Temperature
UPZ - Unprotected Zone
WFD – Water Framework Directive
SAC – Special Area of Conservation

Executive summary

An increasing number of EU legislations in the last decades have focused on 1) the sustainable exploitation and management of the natural capital and 2) the implementation of common conservation strategies for the biodiversity protection.

With this respect, for the implementation of a coordinated approach to the marine conservation at regional scale, the collection of fine-scale biological and ecological information across Mediterranean Marine Protected Areas (MPAs) is crucial, as current EU environmental policies stated. Among these, the Marine Strategy Framework Directive 2008/56/CEE, which seeks the EU Member States to attain and maintain the Good Environmental Status (GES) of marine resources by 2020. Furthermore, intense collaboration among member states is necessary to implement common and effective strategies to improve the conservation across member states. However, considerable gaps in spatially explicit ecological data exists, often restricting the achievement of international targets of conservation.

One of the aims of the InterregMed project AMAre was to assess the effectiveness of current protection measures, in order to provide a background for building a common management strategy. In the project, the MPAs have been used as pilot areas. Common conservation indicators have been selected among the Good Environmental Status descriptors of high ecological value and field surveys have been carried out through coordinated monitoring in order to describe the ecological status of the surveyed areas.

This document reports the results from pilot activities carried out at the Torre Guaceto Marine Protected Area, at the National Marine Park of Alonissos Northern Sporades (NMPANS) and at the Freus d'Eivissa i Formentera Marine Reserve (FEFMR) within the work-package 4 "Testing" of the AMAre InterregMed project. In addition, since Porto Cesareo became partner of the project during the second year, monitoring activities were carried out also in this MPA and results are reported. In detail, *Posidonia oceanica* meadows, the rocky subtidal–shallow infralittoral benthic assemblages and the coralligenous formations were chosen as the most ecologically relevant habitats and assemblages subjected to several threats at the surveyed MPAs (e.g. fishery, touristic frequentation, trampling, yachting, anchorage) and sampled within and outside the MPAs by shared methodologies based on non-destructive techniques. In addition, also monitoring of marine litter has been carried out, as it represents one of the main threats to the marine habitats. The achieved information provided a clear picture of the effect of current management strategies on the considered habitats and may be of support for an adaptive approach to the conservation of marine resource. Scaling-up this approach at the Mediterranean level is necessary in the perspective to design effective network of MPAs sharing the same conservation aims through coordinated strategies for the achievement of the Good Environmental Status.

1. AMAre Marine Protected Areas

Pilot activities have been implemented at four different MPAs across the west, the center and east sectors of the Mediterranean Sea (Figure 1). More specifically, the Italian Torre Guaceto and Porto Cesareo MPAs, the Spanish Freus d'Eivissa i Formentera Marine Reserve and the Greek National Marine Park of Alonissos Northern Sporades were involved as study areas were the surveys took place, with the inclusion of external controls as reference sites. These four MPAs are characterized by different features, varying in term of time since their institution, size, and zonation (Table 1).

Figure 1, AMAre MPAs involved in the pilot activity implementation.

Detailed information about each MPAs is provided below.

Table 1, Details about the four Marine Protected Areas involved in the pilot activities.

Marine Protected Area	Year of institution	Size (ha)	No-take Zone Size (ha)
Torre Guaceto Marine Protected Area	1991	2212	183
Porto Cesareo Marine Protected Area	1997	16741	1000
Freus d'Eivissa i Formentera Marine Reserve	1999	15378	442
National Marine Park of Alonissos Northern Sporades	1992	365137	155943

- **Torre Guaceto Marine Protected Area**

The MPA “Torre Guaceto” (40°42’N; 17°48’E, Southern Adriatic Sea, Italy) was formally instituted in the 1991, becoming effective in 2001 and covers a total surface of about 2207 ha, along 10 km of coastline. The MPA extends off shore to the bathymetry of 50 m and is divided in three zones where different restrictions regulate the anthropic activities. Among these, the C zone is a partially protected zone (PPZ), measuring 1808 ha and here a wide range of human uses is allowed; the B zone is the general reserve, which covers 149 ha, where human access is permitted although an increase of restrictions; the two A zones, or No-Take Zones (NTZ), include a total area of 183 ha, where the access to humans is totally forbidden with an exception for the MPA staff, scientists and policy forces. A map of the MPA with a representation of the C, B and A zones is provided by Figure 2.

The MPA includes part of the SAC (Special Area of Conservation) “Torre Guaceto & Macchia S. Giovanni”, which measures 7.978 ha and is spatially overlapped also to the Natural Terrestrial Reserve of Torre Guaceto (Figure 3). This terrestrial side of the Protected Area (PA) is a naturalistic oasis of European interest, included within the list of Special Areas of Conservation of the Habitat Directive, with a wetland area declared of national importance, according to the Ramsar Convention of 1971 (Fraschetti et al., 2013).

On the seaward, a gently sloping rocky bottom characterizes the whole area, declining from the sea level up to 10–12m of depth over coarse sand. Rocky bottoms alternate patches of sand and *Posidonia oceanica* meadows up to 12–20m depth. Between 25 and 35–40m depth, a mosaic of coralligenous formations and sand characterizes the bottom and at deeper stands sandy–muddy bottoms are the dominant substrate. Aerial photographs and direct observations suggest that habitat composition and patchiness within the MPA boundaries are typical of the whole surroundings (Guidetti et al., 2010).

The lack of adequate knowledge regarding the biodiversity distribution before the institution of this MPA prevented appropriate decision about reserve boundaries, with the consequence that habitats of high ecological value, such as biogenic formations, are not included in the A zones, in spite of their biological and ecological importance. Overall, before the MPA effective institution the barren ground was the most characteristic habitat in the area, due to the high sea urchin density. After the MPA establishment, the ecosystem shifted toward a macroalgae-dominated rocky bottom, due to the progressive increase of fish density and size and their pressure on grazer populations (Guidetti 2006).

A first detailed characterization of the habitats and the assemblages which the MPA comprises is available in Fraschetti et al. (2005). These authors reported a wide set of different habitats in the area, supplying a bathymetric map of the whole MPA and geo-referred information about the habitat distribution and extent.

Six major habitats were identified by the authors:

1) Sandy substrata which account for the 39% of the total area and are mostly in the partial reserve zone (B zone);

2) Muddy substrata have been found only within the general reserve zone (C zone), below 45 m depth and in a small percentage (about 7%);

3) Biogenic formations which are present only in the general reserve zone and characterize the seafloor between 14 and 40 m depth. Example of the main bioconstructor organisms of the area are: encrusting coralline red algae, such as *Peyssonnelia* spp., bryozoans, serpulids, massive sponges and anthozoans, such as *Eunicella singularis* and *E. cavolinii.*

4) The *Posidonia oceanica* meadow, mostly distributed within the general reserve zone, interspersed among sandy patches and dead matte. This habitat accounts for 20% of the total reserve area and covers the seafloor up to 17 m of depth. Only 0.5% of the *P. oceanica* meadow lies within the integral reserve zones, where small patches of the seagrass *Cymodocea nodosa* were also recorded.

5) Rocky substrata represent the dominant habitat within the integral reserve zones and account for about the 10% of the MPA seafloor, generally present from the shoreline up to 7–8 m depth.

6) The shallow infralittoral habitat, mainly characterized by the canopy-forming algae *C. amentacea* which covers a patchy algal-dominated assemblage characterized by turf-forming and other erect algae, such as dark filamentous algae, *Halimeda tuna* and *Halophytis incurvans*. Inside the A zones, rocky substrata were only occasionally represented by barren grounds.

Artisanal and recreational fishery, anchoring, trampling, diving frequentation and maritime traffic are severely regulated within the MPA buffers and completely excluded from the two fully protected zones, as these activities have been highly documented to directly and indirectly affect benthic habitats and assemblages (Claudet and Fraschetti, 2010). A detailed description of the restrictions in force within each MPA zone may be consulted at the official MPA web site (http://www.riservaditorreguaceto.it/index.php/it/l-ente-gestore/normative-e-modulistica/attivita-regolamentate).

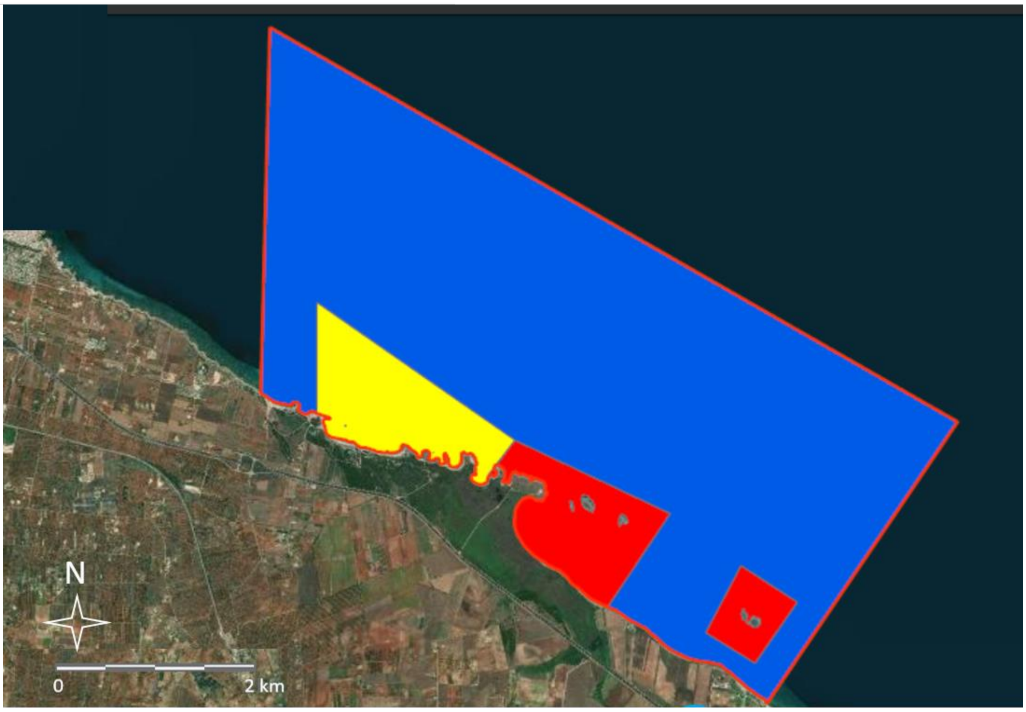


Figure 2, Map showing the Torre Guaceto MPA boundaries and zonation. Red: No-take zones; Yellow: Partially Protected Zone; Blue: Buffer zone.

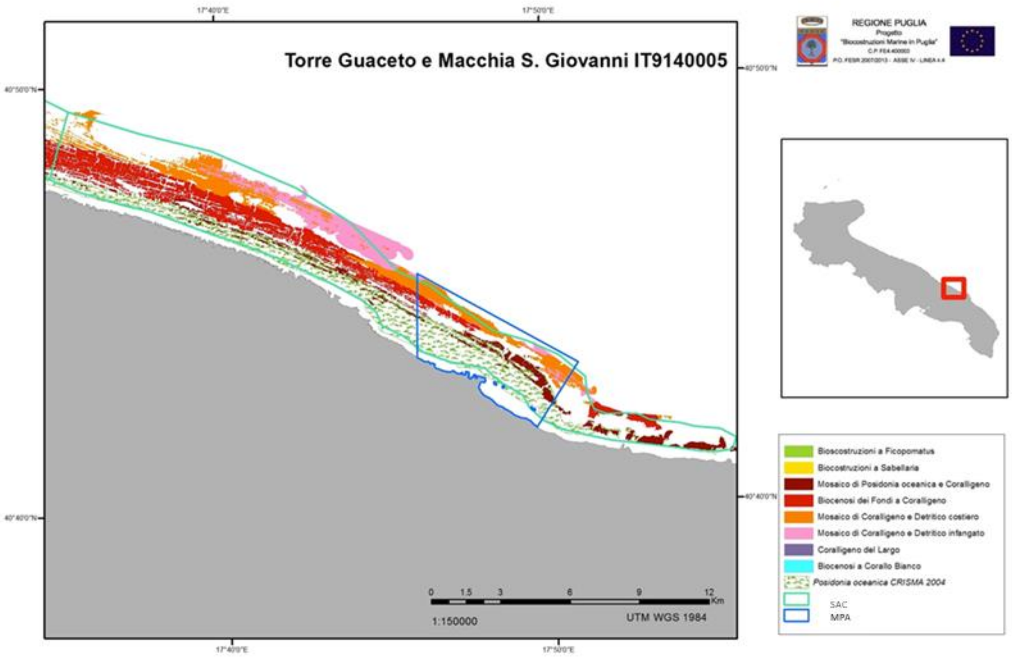


Figure 3, SAC "Torre Guaceto and Macchia S. Giovanni", administrative boundaries (green line) and habitat distribution.

- **Porto Cesareo Marine Protected Area**

Porto Cesareo Marine Protected Area (40°14’35”N—17°54’07”E) was instituted in 1997 and extends for 16654 ha (Guarneri et al., 2016), along 32 km of coast. This MPA also includes three SCIs (Site of Community Interest), covering a surface of 7169 ha and represents one of the largest Italian marine reserves. Two no-take zones and two partially protected zones (respectively measuring around 1000 and 3000 ha) are enclosed within a buffer zone, measuring in total about 14000 ha (Figure 4). The area is characterized by a gently sloping calcareous rocky reef and after 8‑12 m of depth a sandy bottom begins.

The main benthic invertebrates within the MPA are encrusting and massive sponges (*Crambe crambe*, *Phorbas paupertas*, Clionidae, *Chondrilla nucula*, *Sarcotragus* sp., *Ircinia* sp.), molluscs (vermetids, endolithic bivalves such as *Gastrochaena dubia* and *Lithophaga lithophaga*) and bryozoans (*Reptodeonella violacea*, *Schizoporella* sp.). The predominant macroalgae were the encrusting red calcareous algae (*Mesophyllum alternans*, *Peyssonnelia* sp., *Lithophyllum* sp.) and sparse patches formed by *Amphiroa* sp., *Dictyota* sp. and *Padina pavonica* (Guidetti et al., 2003).

The area is exposed to multiple stressors. The whole area has been historically depleted by the illegal fishing of the rock-boring mussel *Litophaga litophaga*, formally banned since 1988. However, the effects on the landscape of this highly impacting practice are still evident. The destruction of the rocky substrate due to the date-mussel collection, indeed, entailed the loss of physical complexity of the substrate with consequences both for the sessile biota (i.e. macroalgae and zoobenthos covering the carbonate rocks inhabited by date mussels) and for the fish assemblage, generating extensive damage on a regional scale (Fraschetti et al., 2001; Guidetti et al., 2003).

Furthermore, tourism represent another relevant pressure in the area and, data provided by the Italian Statistic Institute (ISTAT) in the 2012, confirmed that in the summer months the resident population increased from 159 to 2100 inhabitants km^2 (source: http://www.agenziapugliapromozione.it) (Guarneri et al., 2016).

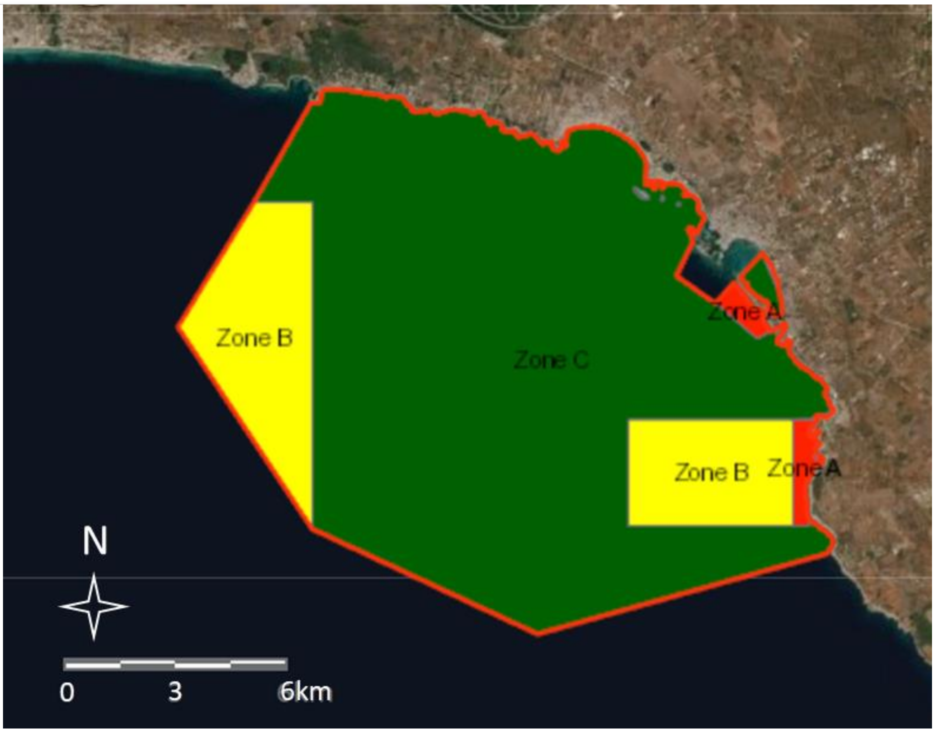


Figure 4, Map showing the Porto Cesareo MPA boundaries and zonation. Red: No-take zones; Yellow: Partially Protected Zone; Green: Buffer zone.

- **The Marine Reserve Els Freus d'Eivissa i Formentera**

The "Reserve Marina d'Els Freus d'Eivissa i Formentera" (FEFMR, Figure 5) was instituted in 1999 and includes an area of 15353ha among the southern part of the island of Eivissa and the north of Formentera. This MPA is the second largest of the 11 Balearic Marine Reserves (MRs) and encompasses the same marine area than the Parc Natural de Ses Salines d'Eivissa i Formentera (SEFNP) that was instituted in 2001 (Figure 6).

The FEFMR aims concern the management of human activities, in order to ensure the maintenance of a sustainable fishery and a good state of the biological resources, while the SEFNP has the objective to protect both the terrestrial and the marine habitats and biodiversity, beside regulating human activities. The FEFMR zonation includes three different levels of protection, as Figure 5 shows: a special protection zone (SPZ) measuring 442.4 ha which is a no-take no-go zone, where any extractive activity, scuba-diving and anchoring are forbidden and only authorized scientific work is allowed; a marine reserve zone (MRZ), or partially protected zone, in which artisanal fishing is allowed to the vessels belonging to local areas; another zone of marine reserve where all kind of recreational fishing is prohibited, as a temporary closure respect selected species.

A detailed habitat map of the FEFMR is provided in Figure 7 (Ballesteros and Cebrián, 2003). Up to 35 benthic communities and 756 species have been observed within the FEFMR and an extensive *Posidonia oceanica* meadow roughly covers half of the MR (at least 7000 ha), and may also colonize extensive rocky bottoms. *Cymodocea nodosa* is widely present in the FEFMR and vast extensions of *Caulerpa prolifera* may dominate soft substrate.

Down to 35 m of depth brown algae are dominant and characterize the substrate (various species of the genus *Cystoseira* spp., *Sporochnus penduculatus*). The deepest areas of the reserve, starting at 40 m of depth, are characterized by the presence of the sea urchin *Spatangus purpureus*, the tunicate *Aplidium conicum* and maërl beds made by *Phymatolithon calcareum* and *Lithothamnion corallioides*, in an excellent state of conservation. All of these habitats are in a good state of conservation in the FEFMR, although they are susceptible to the destabilization of the coastal food chains due to overfishing and to pollution (Ballesteros and Cebrian, 2003).

Tourism produces a variety of pressures on the natural environment of the FEFMR and fishing activities, both commercial and recreational are regulated, while spearfishing is prohibited throughout the whole marine reserve. A scheme reporting the regulations within the MR is reported in Figure 8.

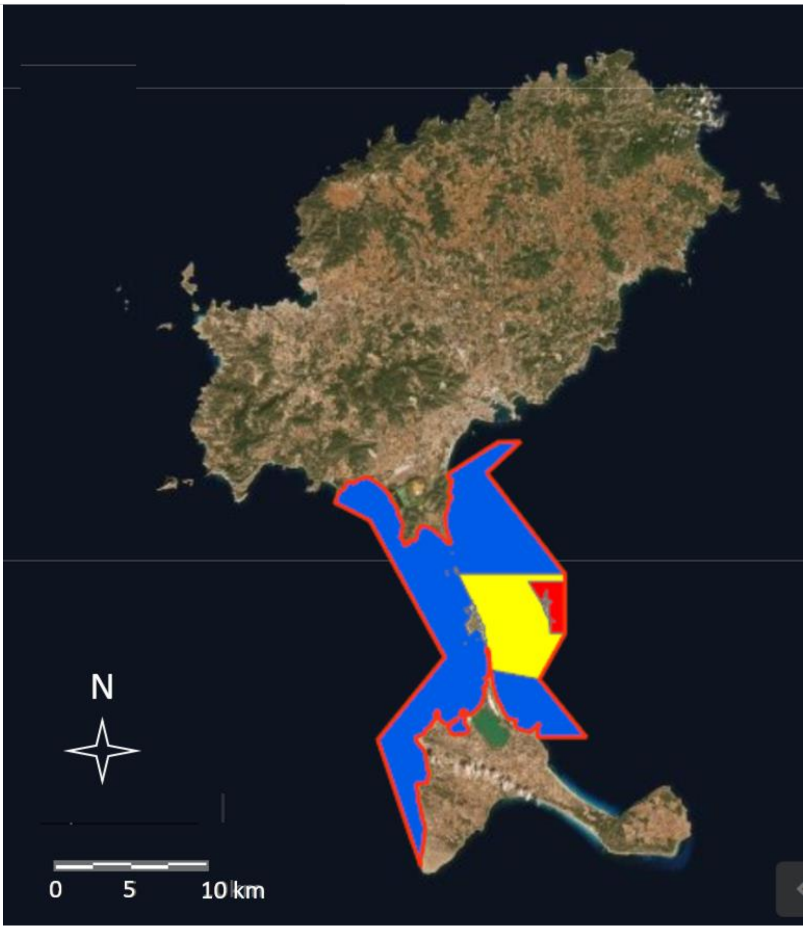


Figure 5, Map showing the Marine Reserve Els Freus d'Eivissa i Formentera boundaries and zonation. Red: No-take zone; Yellow: Partially Protected Zone; Blue: Buffer zone.

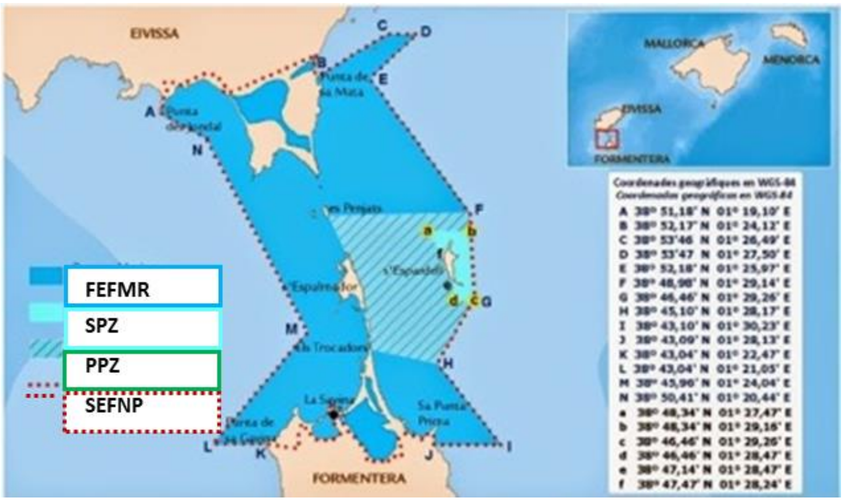


Figure 6, Els Freus d'Eivissa i Formentera Marine Reserve (FEFMR) and the Natural Park of Les Salines d'Eivissa i Formentera (SEFNP). PPZ: partially protected zone; SPZ: Special Protected Zone.

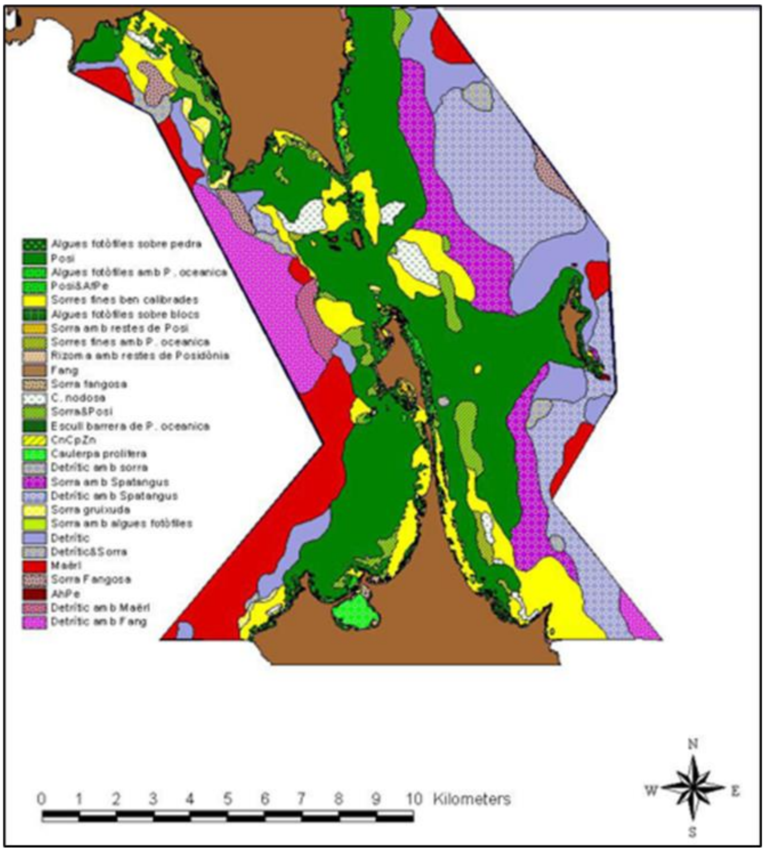


Figure 7, Map of the benthic communities at the Freus d'Eivissa i Formentera Marine Reserve. (Ballesteros and Cebrián, 2003).

Figure 8, Schematic representation of the regulations at the Freus d'Eivissa i Formentera Marine Reserve.

- **The National Marine Park of Alonissos Northern Sporades (NMPANS)**

The National Marine Park of Alonissos Northern Sporades (NMPANS, Figure 9) was instituted in 1992 and was the first Greek marine park. It covers an area of 365137 ha, including both land and sea ecosystems with great biological diversity, interesting geological structures and important archaeological elements (shipwrecks, old monasteries and churches dating back from the prehistoric era to the Byzantine Empire). Although the high ecological concern of the area, a draft of the first management plan (MP) has been prepared only in 2009 and it has never been officially approved by the competent authority (i.e. the Ministry of Environment). Subsequently in 2018, the geographical limits of the NMPANS have been extended over the adjacent NATURA 2000 areas by a Greek Government Law (4519/2018) and the design of the new MP is currently underway.

The NMPANS comprises a number of rocky offshore islets and islands, Alonissos is the only which is inhabited. The Park is divided into two main protection zones (A and B). The Zone A, measuring 155943 ha, which includes nine regions and aims to protect the biodiversity of specific sites, such as the islet of Piperi, through rigid conservation measures.

The Zone B, which covers 75787 ha, comprises four regions and is characterized by less strict protection measures. A detailed description of the existing restrictions can be found in the guide issued by the NMPANS Management Body (http://alonissos-park.gr/wp-content/uploads/2016/07/ENG-HD-.pdf).

Although the area is designated as a marine park, it includes terrestrial habitats with a wide variety of trees and shrubs that grow in the different islands, forming attractive landscapes. The combination of terrestrial and marine habitats creates the conditions for the conservation of important bird species nesting in the area, that include a variety of seabirds (e.g. *Larus audouinii*; *Phalacrocorax aristotelis desmarestii*, *Puffinus yelkouan*, *Calonectris diomedea*) with rare and endangered species, such as the impressive eagle *Hieraaetus fasciatus*.

Regarding the marine biodiversity, the first conservation objective of the NMPANS is the protection of the Mediterranean monk seal (*Monachus monachus*) and its natural habitat with active efforts started in 1986. The area, indeed, comprises important breeding and resting sites for this species. Recent data show that the population that finds refuge in the caves and steep rocky shores of the islands in the wider area of the Park is constantly increasing (Karamanlidis & Dendrinos, 2015; IUCN, 2015; IUCN 2017). For this reason, in 2014 the conservation status of the species has been reviewed and from “critically endangered” the species is now classified as “endangered” under the IUCN Red List, which probably reflects the efforts made in recent years through the establishment of the NMPANS.

Other conservation priorities concern significant populations of cetaceans (*Stenella coeruleoalba, Tursiops truncates, Delphinus delphis*) and two biogenic habitats of high ecological value which characterize several locations in the area: the *Posidonia oceanica* meadows and the coralligenous formations.

The seagrass meadows are almost everywhere from -3m down to -28-34 meters of depth, depending on the local seascape morphology and orientation. Coralligenous formations are common on vertical cliffs (Dendrinos et al., 1999b) and usually appear from the depth of -25m, as a pre-coralligenous habitat, down to deep waters and show a great variability in the hosted biodiversity, mainly represented by: gorgonian forests, great diversity of sponges and bryozoans and large walls covered by coralline algae. High number of important commercial fish species rely in the MPA sustaining the local economy, among these: *Oblada melanura*, *Boops boops*, *Pagrus pagrus*, *Dentex dentex*, *Mullus surmuletus*, *Spondyliosoma cantharus*, *Scorpaena scrofa*, *Merluccius merluccius* (Tsikliras et al. 2018). Local fishers are also involved in targeting other invertebrates, such as the lobsters *Palinurus elephas* and the squids *Loligo vulgaris*. Also, famous and very important for the local economy is the Alonissos tuna (*Thynnus alalunga* and *Thunnus thynnus*) which is processed by traditional methods and marketed inside and outside the country.

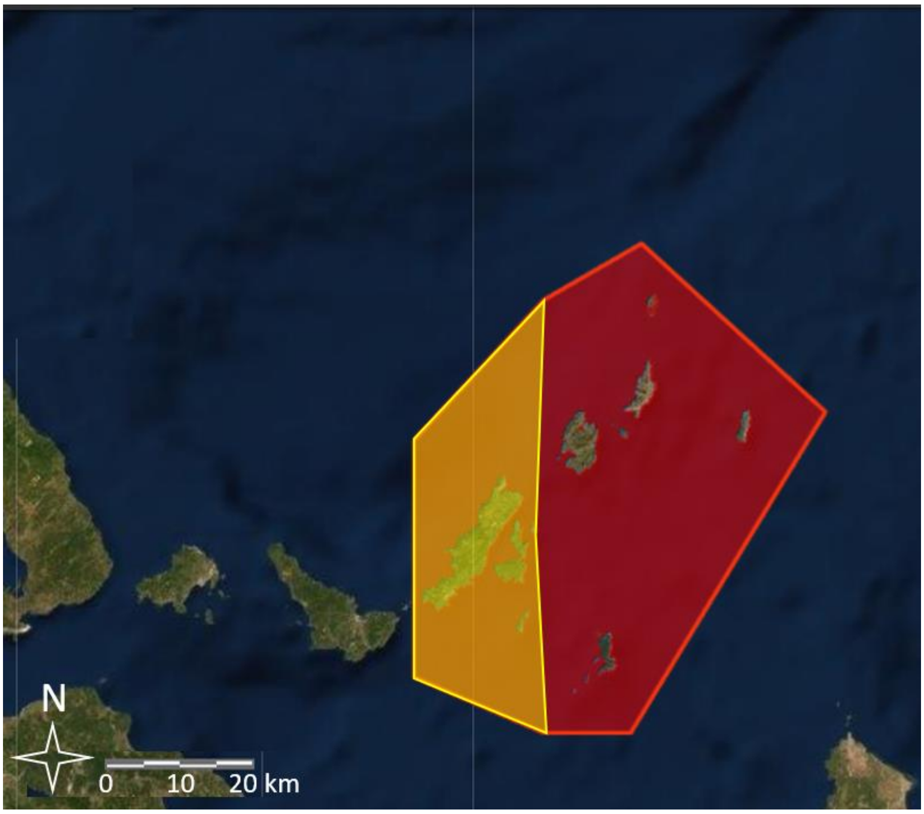


Figure 9, Map showing the National Marine Park of Alonissos Northern Sporades boundaries and zonation. Red: No-take zone; Yellow: Partially Protected Zone.

2. Conservation objectives and strategies to achieve them

In the Mediterranean Sea, the MEDPAN network represents an important organization grouping the managers of a number of MPAs, coordinating their strategies, in order to enhance policies of effective environmental conservation. However, the difficulty to involve the MPA management bodies in the governance evaluation is recognized as a great limit to design coordinated concrete management actions. With this respect, the ISEA framework, established by a project funded by the Italian Ministry of the Environment (http://www.progettoisea.minambiente.it/), represents a tool designed to facilitate the interaction with the MPA managers and among them. This framework supports the MPA networks by designing standard management schemes for the implementation of a systematic approach to achieve the conservation targets at the basin scale.

This approach firstly defines the conservation targets for a specific area, critically examining both the most ecologically relevant biological components of the environment and the direct and indirect threats acting on them, so that conservation actions can be designed in order to achieve the established targets of conservation. Furthermore, the conservation effectiveness assessment is an essential step of the ISEA schemes, informing about the suitability of each management plan. With this regard, monitoring is the tool which provides qualitative and quantitative data on the conservation status of specific descriptors, by comparing their status among protected and unprotected areas and among different levels of protections.

Each scheme was designed considering the characteristics of each MPA, including the ecological and the socio-economic context analyses and provided relevant indications for the definition and the achievement of specific management targets. The ISEA schemes for the four AMAre MPAs are reported in the Figure 10, 11, 12, and 13.

The adaptation of the ISEA scheme to MPAs that are very different each other can be considered an experiment to extend this management framework to the whole Mediterranean Sea.

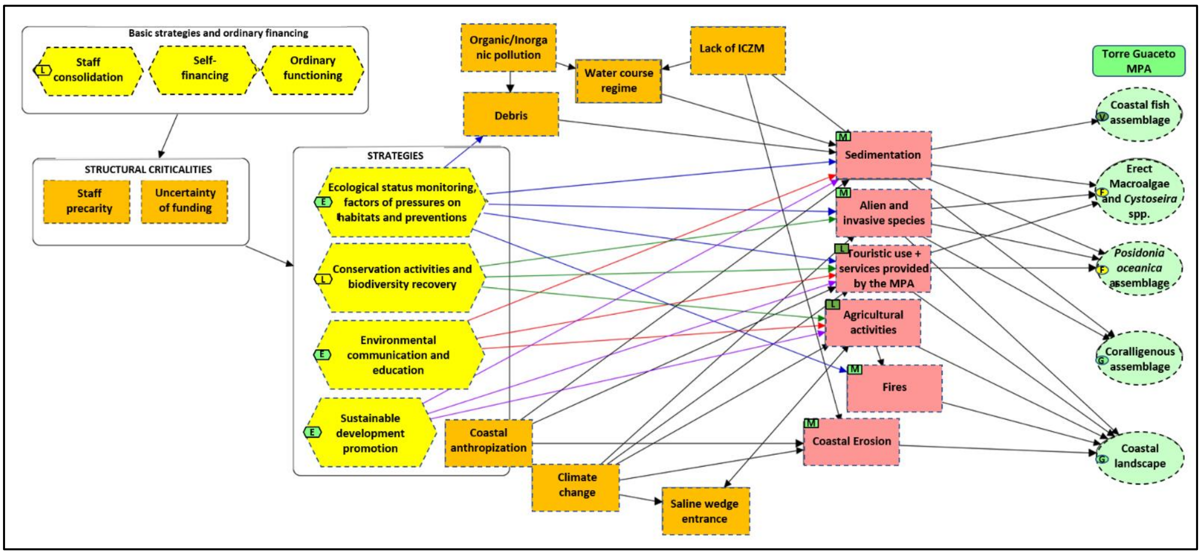


Figure 10, ISEA scheme for Torre Guaceto MPA.

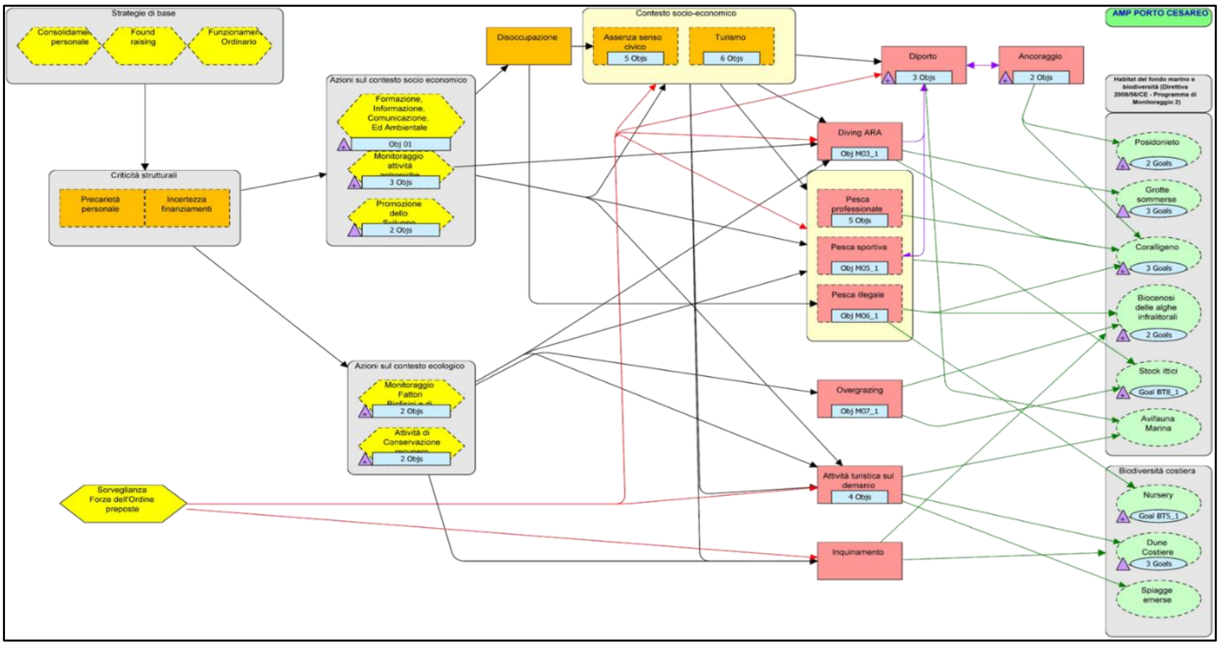


Figure 11, ISEA scheme for Porto Cesareo MPA.

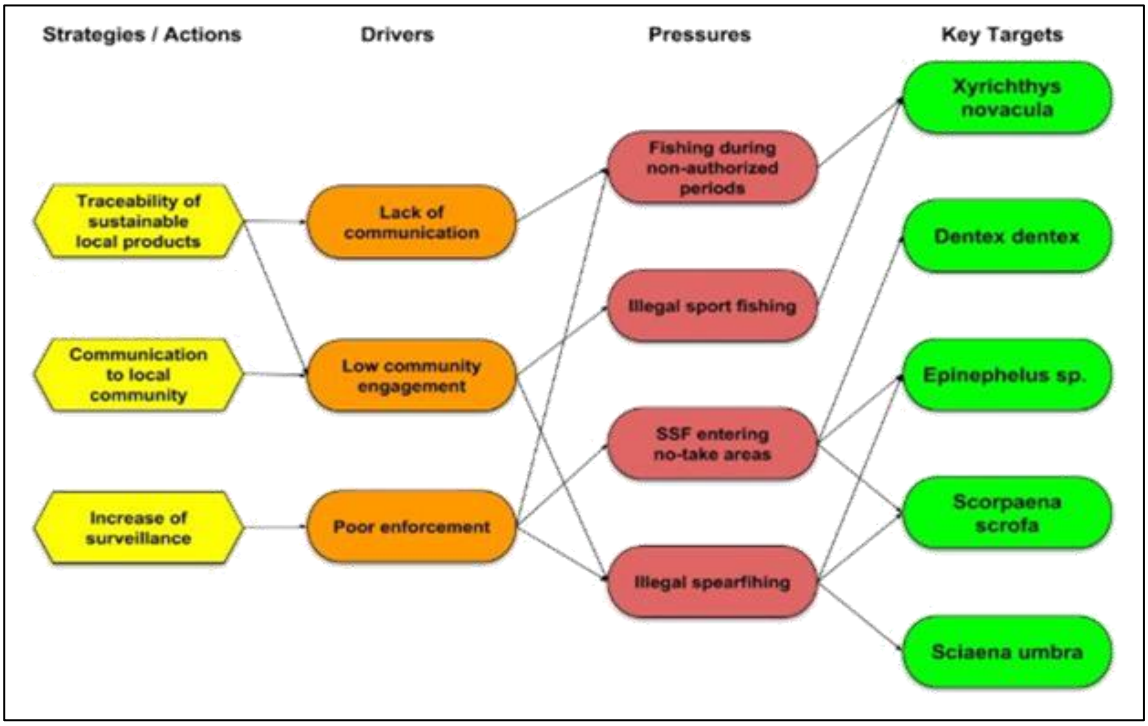


Figure 12, ISEA scheme for the Freus d'Eivissa i Formentera MPA.

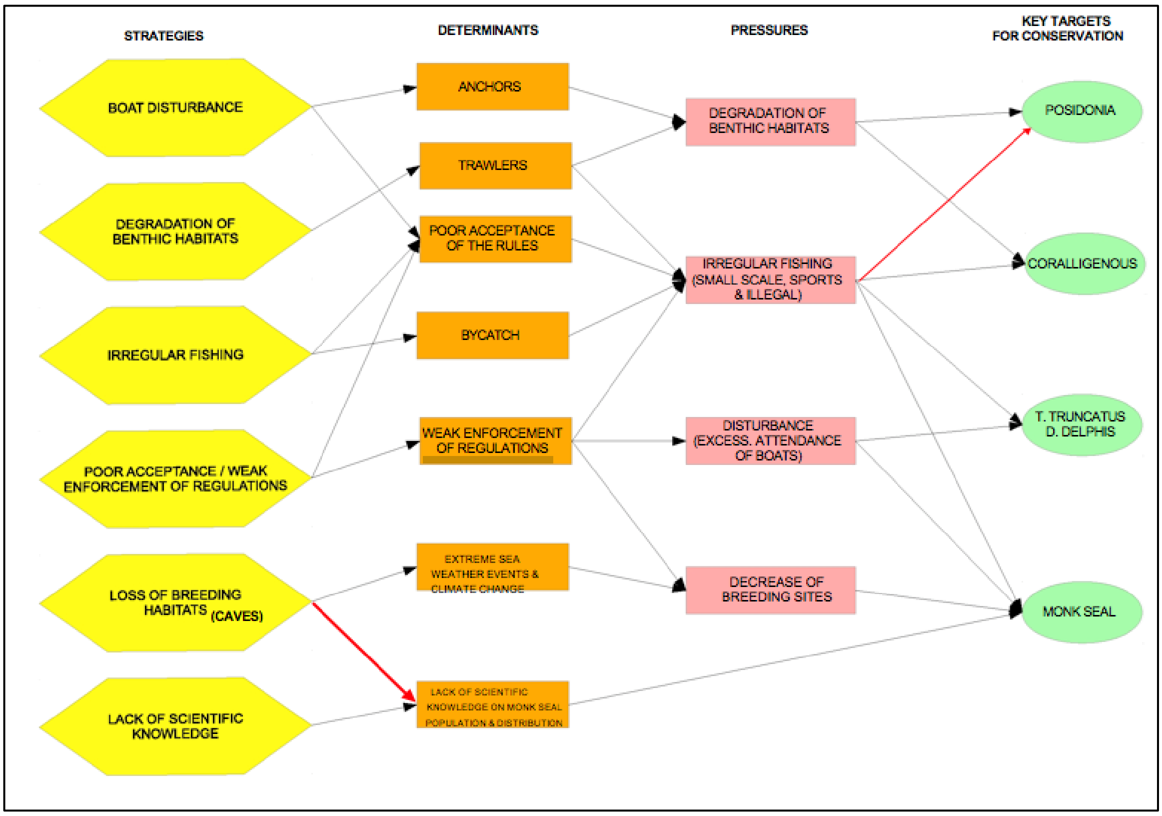


Figure 13, ISEA scheme for the National Marine Park of Alonissos Northern Sporades.

3. Planning of Pilot Activities

The availability of georeferenced spatial information on the distribution of human-uses and ecological data in the Mediterranean Sea is very limited and heterogeneous, although, the quantification of the overlap between biological resources and cumulative threats is one of the main concerns of the studies relative to maritime spatial planning (Micheli et al., 2013). Ecosystem-based management requires an assessment of the cumulative effects of human pressures and environmental change. The operationalization and integration of cumulative effects assessments (CEA) into decision-making processes often lacks a comprehensive and transparent framework.

In order to address this issue, one of the aims of AMAre was the implementation of multilayer maps showing both human uses and the biological features of each MPA and the surroundings. More specifically a full list of threats for the MPAs included in the project is accessible at the AMAre spatial GeoPortal (http://gismarblack.bo.ismar.cnr.it:8080/mokaApp/apps/AMAV3tg/index.html).

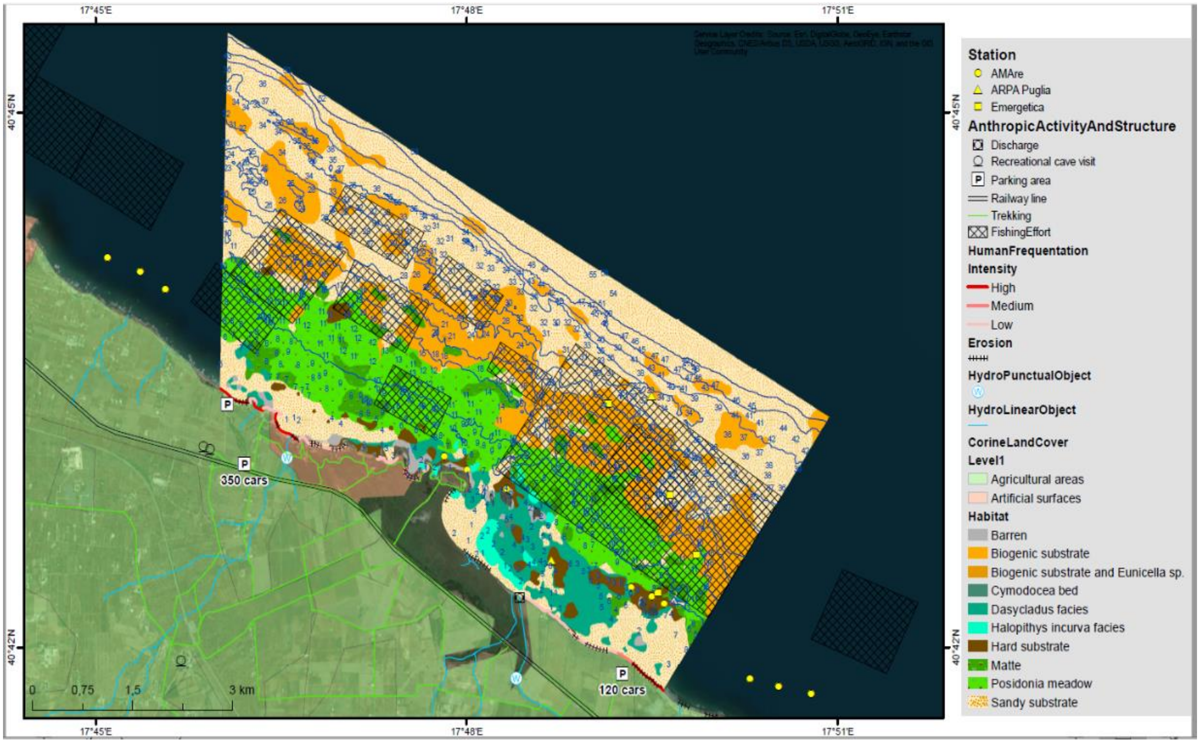
The GeoPortal is a web-based tool developed during the WP 2 "Project Communication", which stores all the spatial data regarding the MPAs included in the project (administrative, socio-economic, environmental and biological). This database can be currently updated with new data and provides the information about pressures within each protected area, classified in: human pressures, system modification and fishing activities.

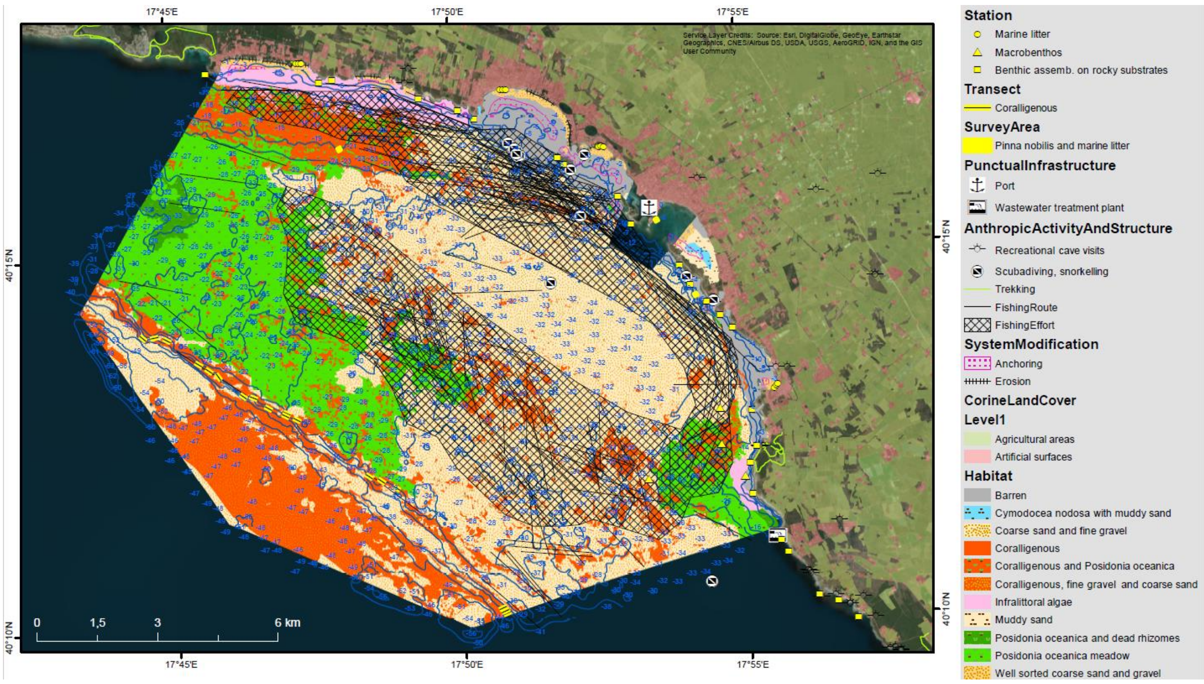
In light of an integrated marine management (Stephenson et al., 2019), these thematic maps represent important tools providing useful indications for the implementation of monitoring activities. These spatial data represent valuable information to MPA managers to assess the effects of cumulative impacts within their MPAs and identify areas where the management of human uses should be improved. Furthermore, this information is instrumental to analyze spatial scenaria aiming to reduce unsustainable impacts for marine spatial planning purposes (Stelzenmüller et al 2020).

In the context of the AMAre project, the production of multiple-layer maps coupling the information about biodiversity and human uses allowed to direct the identification of sampling sites where the interaction between biodiversity and threats was more pronounced (Figure 14).

Furthermore, sampling activities at the NMPANS have been organized also on the basis of Cumulative Impact Assessment (CIA) analysis, used to identify areas that may be of concern depicting how the ecosystem components under study respond to human pressures.

The aim of these pilot activities was to assess the ecological status of selected biological targets for a further planification of aligned strategies to achieve the GES.





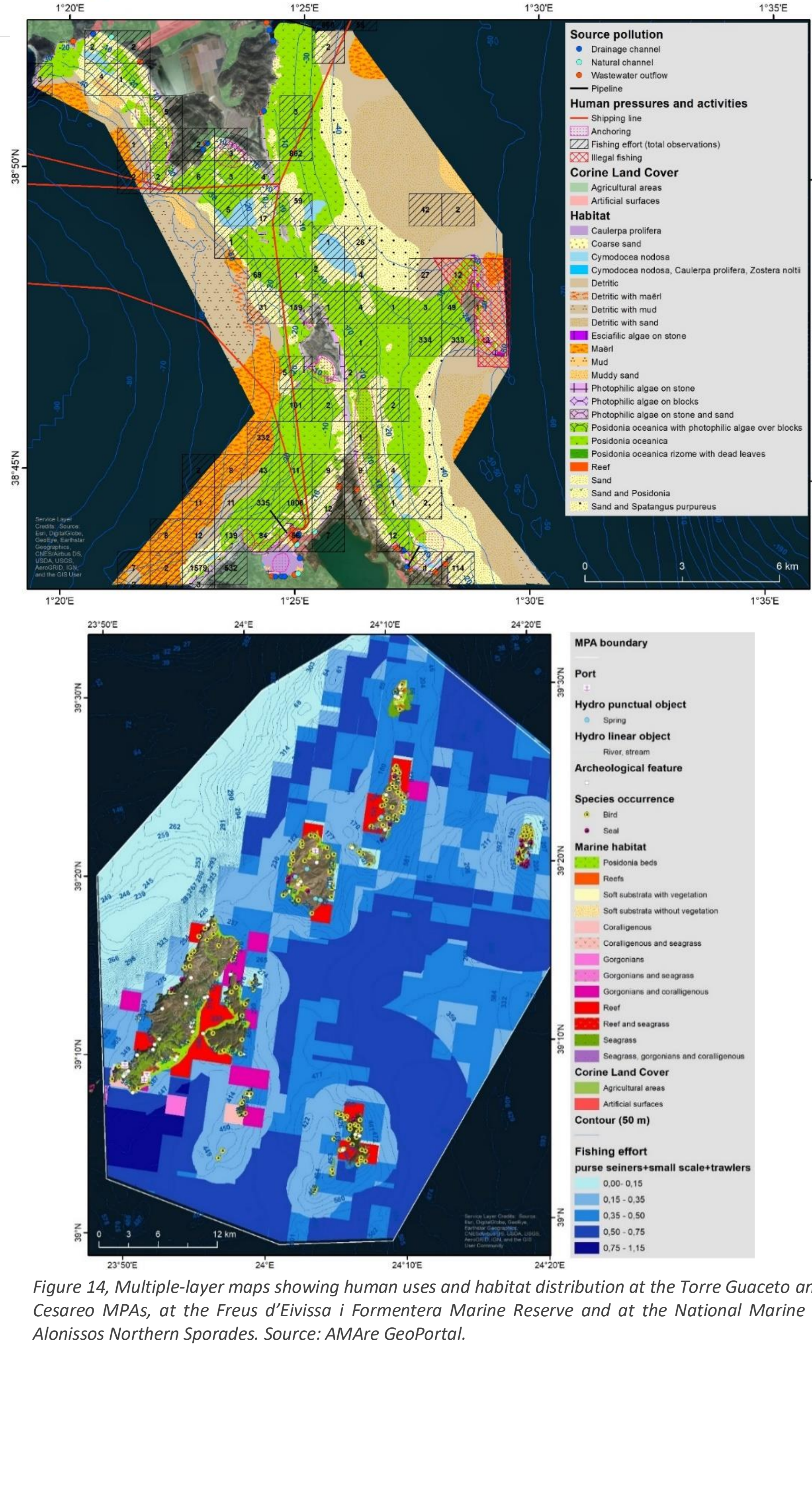


Figure 14, Multiple-layer maps showing human uses and habitat distribution at the Torre Guaceto and Porto Cesareo MPAs, at the Freus d'Eivissa i Formentera Marine Reserve and at the National Marine Park of Alonissos Northern Sporades. Source: AMAre GeoPortal.

- **Selection of the conservation targets**

Overall, the research on the effectiveness of MPAs has principally focused on the most directly exploited taxa (e.g. fish and few invertebrates), considering management of fisheries as a synonym to conservation of biodiversity as a whole. In addition to this, a number of studies also reports that the removal of human pressures may have direct or indirect effects on benthic assemblages and their structure and functioning variously respond to specific management measures (Betti et al., 2019; Fraschetti et al., 2013; Sala et al., 2012; Bevilacqua et al., 2006; Casu et al., 2006; Pinnegar et al., 2000). Furthermore, the coastal zone is deeply subjected to the overlap of multiple stressors, affecting nearshore habitats such as seagrass meadows, coralligenous assemblages, infralittoral communities.

The idea of AMAre was to focus on those benthic habitats considered of critical importance by the EU, representing valid indicators of MPA performance.

In detail, *Posidonia oceanica* meadows, the rocky subtidal-shallow infralittoral benthic assemblages (5-12m of depth) and the coralligenous formations (20-30m of depth) were assessed during the pilot activities carried out at the four surveyed MPAs within the Work Package 4 "Testing" of AMAre (Figure 15). A detailed description of each habitat is contained in the deliverable 4.4.1. These habitats have been chosen among the indicators of GES suggested by the MSFD as targets of conservation efficacy. Due to their high accessibility to humans and exposure to potential threats, indeed, the selected benthic habitats are susceptible to a variety of impacts, allowing to test the effectiveness of current conservation efforts against different forms of individual and cumulative impacts, from overexploitation to trampling, tourism, diving activities, anchoring.

In addition to this, also the presence of marine litter at the study areas has been monitored. Marine litter, indeed, represents one of the main global environmental concerns which harms a wide range of marine biota and ecosystems (Bergmann et al., 2015). However, current knowledge on its environmental impact is still limited (CIESM, 2014; UNEP, 2015; Galgani et al., 2015; Ioakeimidis et al., 2017; Galgani et al., 2018). Marine litter is cited by the MSFD as an indicator of good environmental status and its density in the Mediterranean Sea is among the greatest in the world (UNEP/MAP, 2015a). However, measures for its management and mitigation still need to be implemented and coordinated at regional level. MPAs lack official standardized protocols to collect spatial and temporal series of data for further monitoring, which may inform about the conservation performance and reveal weakness in the management strategies.

Figure 15, Monitoring during the pilot activities at the four AMAre MPAs. Clockwise order: P. oceanica *meadow; rocky subtidal shallow-infralittoral benthic assemblage; marine litter; coralligenous formations.*

4. Pilot activities

- ***Posidonia oceanica* sampling**

In order to describe the ecological status of the *P. oceanica* meadows comparing protected and control conditions, sampling activities focused on shoot density. Density reductions lower than 50% are indicative of the meadow degradation (Pergent et al., 1995; de los Santos et al., 2019; Montefalcone et al., 2019; MSFD 2008/56/EC). Overall, the shoot density was measured between 8 and 10 m of depth and recorded by *in situ* visual estimates within replicated frames of 40x40 cm^2 (Figure 16), subsequently standardized on 100 cm^2. Only at Porto Cesareo a deeper meadow was also surveyed. Multiple sampling sites (100-300 m apart) were randomly selected along a gradient of protection at each MPA. Furthermore, this sampling was also conducted at Malta, since the Natura 2000 Site of the Island was involved in a sub-set of the project activities.

The ecological status of the seagrass meadows at each surveyed site was assessed by comparing the revealed shoot densities to the values reported by Pergent et al. (1995) which indicate the quality of the meadow at each range of depth (Table 2). In detail, at 10 m depth, a shoot density < 237 shoots/ m^2 describes a very disturbed meadow, >237 and< 349 describes a disturbed meadow, >349 and <573 is typical of a not disturbed meadow and > 537 describes an excellent meadow. In addition, since control locations were also included, a formal comparison between protected and not protected locations was also carried out. Details concerning the sampling design adopted within each MPA are provided below.

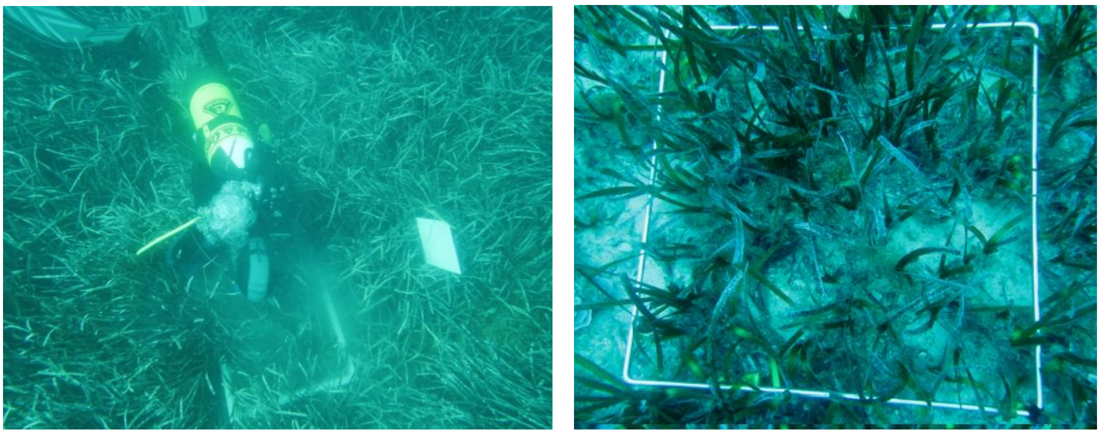


Figure 16, Field survey on a P. oceanica *meadow.*

Table 2, Classification of the status of P. oceanica *meadows (from Pergent et al., 1995).*

	Very disturbed	Disturbed		Not disturbed		Excellent	
Depth (m)							
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	
40			¬		«	**221**	

- **Torre Guaceto**

Posidonia oceanica accounts for about the 20% of the infralittoral within the Torre Guaceto MPA (Fraschetti et al., 2013). Sampling activities on the seagrass meadows were carried out in September 2018. Due to the lack of seagrass beds within the no-take zones of the MPA, the density of *P. oceanica* shoots was sampled in close proximity of these two zones. Here the seagrass forms extensive meadows (Fraschetti et al., 2005), but human activities potentially affecting this habitat are banned (i.e. anchoring, trawling).

Sampling was carried out in two locations close the no-take zone, in two locations within the buffer zone and in other two locations outside the MPA (respectively named P1 and P2, B1 and B2, C1 and C2 (Figure 17, a). At each location, two patches 100–300 m apart were randomly chosen within the beds at 8–10 m depth. In each patch, the density of shoots was recorded by *in situ* visual estimates within six 40x40 cm^2 randomly allocated quadrats.

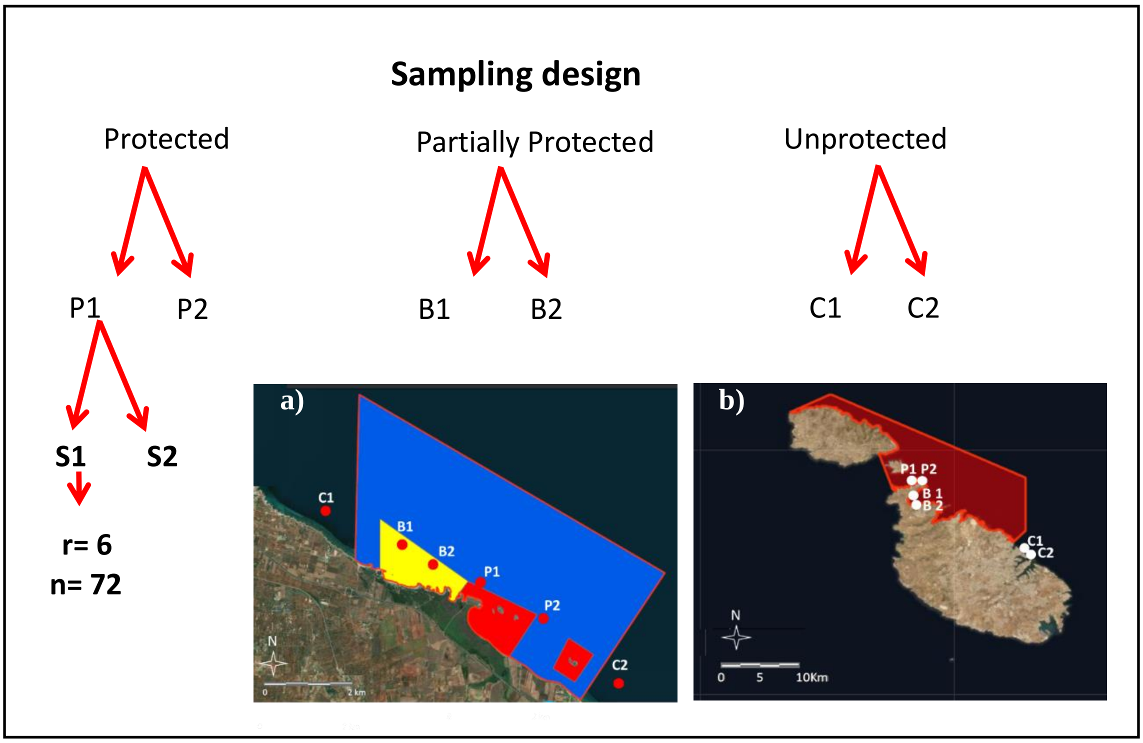


Figure 17, Sampling design and site map describing the P. oceanica *survey at a) Torre Guaceto and b) Malta Island. The dots represent the surveyed locations. P: Protected locations; B: partially protected locations; C: external controls. S: surveyed sites; r: replicates of shoot density collected at each site; n: total number of*

The same experimental design has been adopted in Malta (Figure 17, b). At the protected zone of the North-East Marine Protected Area *P. oceanica* forms a continuous meadow unaffected by anchoring and far from land discharge. Surveyed meadow at the partially protected zone of the MPA are placed along a large sandy bay and are close to one of the most popular beaches of the islands, where a great number of hotels and other touristic amenities are present. Here, the *P. oceanica* meadow is subject to anchoring, especially during the summer season. External controls were selected offshore from the main touristic area of the Maltese coastline and are exposed to intense vessel traffic and moderate anchoring pressure.

- **Porto Cesareo**

The survey on the seagrass meadows was carried out in June 2019.

Sampling was executed at two different depths (shallow: >10< 20 m; deep: >20<27 m). *P. oceanica* shoot density/m^2 was measured at five protected locations and three controls outside the MPA. Within each location, two sites were selected and at each site five replicates of seagrass shoot density were collected by visual estimates on 40 x 40 cm^2 frames, for a total of 80 replicates (Figure 18).

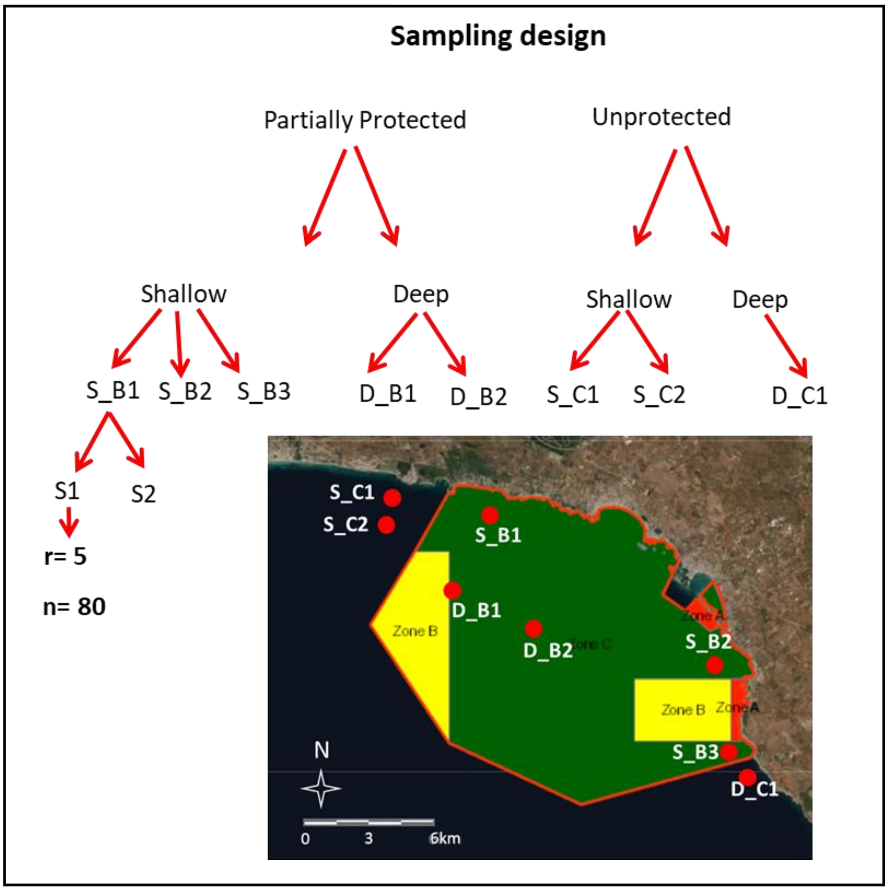


Figure 18, Sampling design and map describing the P. oceanica *survey at Porto Cesareo. The red dots represent the surveyed locations. B: partially protected locations; C: external controls; S: shallow meadow; D: deep meadow. S: surveyed sites; r: replicates of shoot density collected at each site; n: total number of replicates.*

- **Freus d'Eivissa i Formentera Marine Reserve**

The ecological status of *Posidonia oceanica* meadows has been measured in the no-take area, in the partially protected area and in the unprotected area across Formentera Island. The shoot density/m^2 has been measured at 8-10 m of depth within 40 x 40 cm^2 frames randomly allocated along transects 25 m length. Surveyed sites and sampling design are reported in the Figure 19.

The sampling was carried out in two locations for each protection level. Three sites have been replicated within each location, far apart 100 -300 m and three transects (25 m long x 1 m) have been placed within each site, spaced 50-100m apart Along each transect five quadrats 40 x 40 cm^2 have been randomly allocated.

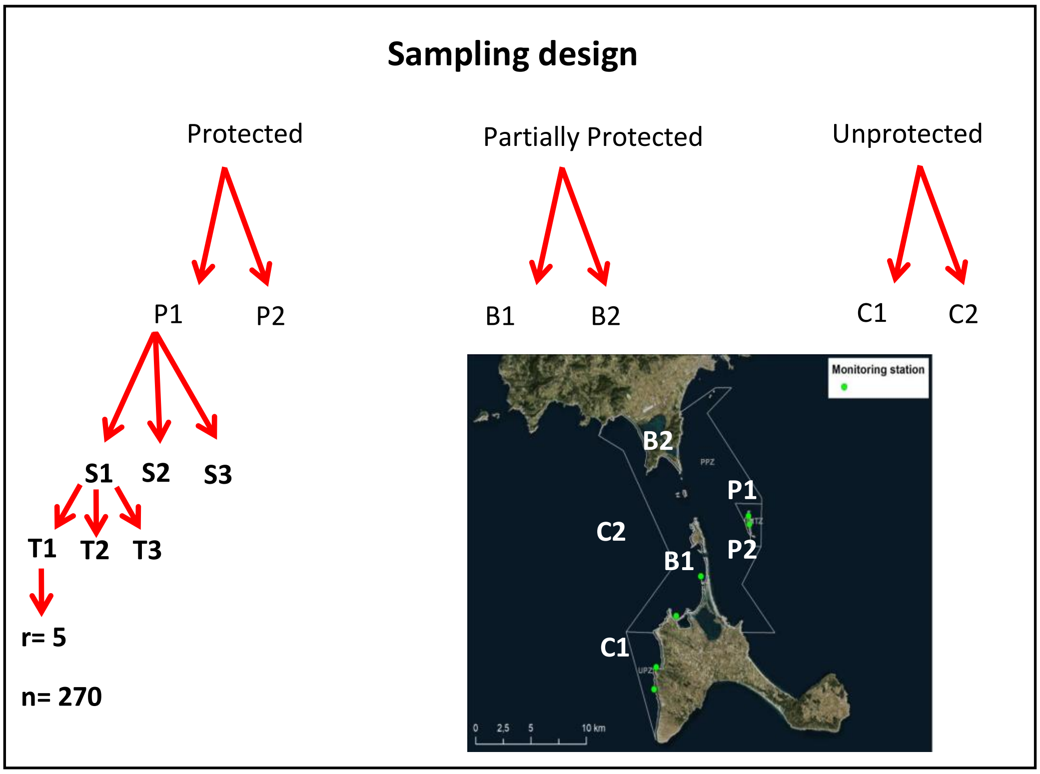


Figure 19, Sampling design and site map describing the P. oceanica *survey at the Freus d'Eivissa i Formentera Marine Reserve. The green dots represent the surveyed locations. P: Protected locations; B: partially protected locations; C: external controls. S: surveyed sites; T: transect replicated at each site; r: replicates of shoot density collected along each transect; n: total number of replicates.*

Moreover, invasive species presence, flowering events and the density of living *Pinna nobilis* (individuals/m^2) were also recorded during the surveys. Visual estimates were also carried out on the fish assemblage associated to the seagrass meadow, as the most exploited organisms by fishing activities and sensitive to protection measures as well. Details about this survey are provided by the deliverable 4.8.1.

- **National Marine Park of Alonissos Northern Sporades**

According to the policy restrictions for the protection of the NMPANS underwater cultural heritage, a first *P. oceanica* survey took place in the B zone of the park in September 2018 and six sites were sampled. After acquiring a special license for research and scientific diving in the Park by the Ephorate of Underwater Antiquities, 18 new locations in both zones A and B have been included in the survey, completed in June 2019.

Sampling was conducted at 10 m of depth, overall, 24 locations have been surveyed and *P. oceanica* shoot density/m^2 was sampled at 3 sites for each location (Figure 20). Visual estimates of shoot density were replicated 5 times at each site (n=360).

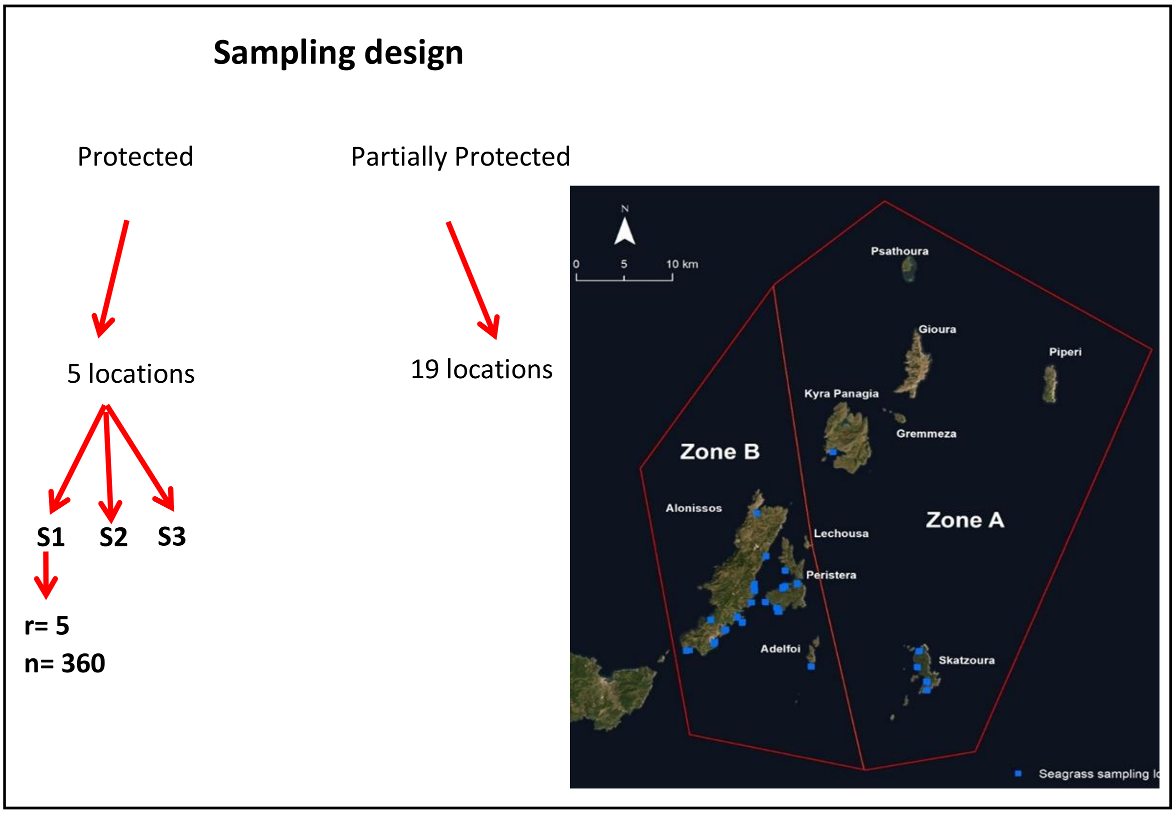


Figure 20, Sampling design and site map describing the P. oceanica *survey at the National Marine Park of Alonissos Northern Sporades. The blue dots represent the surveyed locations. S: surveyed sites; r: replicates of shoot density collected at each site; n: total number of replicates.*

Also, at four random locations, shoot density has been collected over the full depth range of the meadow and for each depth interval the shoot density/m^2 has been calculated and compared against reference values, in order to classify the ecological status of the whole meadow at each depth.

In order to characterize the ecological status of each location based on the shoot density, the majority of the classifications have been considered (e.g. if 3/ 5 depths were classified as good, then the site was classified as good).

- **Rocky subtidal – shallow infralittoral benthic assemblage sampling**

This field activity was conducted at the Torre Guaceto and Porto Cesareo MPAs and at the Freus d'Eivissa i Formentera Marine Reserve and the aim was to evaluate the reserve effect on the rocky subtidal - shallow infralittoral benthic assemblage, as this consists of sensitive organisms to protection and pressures as well.

Visual estimates by different techniques were carried out to perform the surveys and a comparison between protected and unprotected locations was carried out. Detail on the sampling design and methods adopted within each MPA are provided below.

- **Torre Guaceto and Porto Cesareo**

At both the MPAs, a comparison between two protected and two unprotected locations has been carried out by underwater photo sampling during June 2018. Three sites far apart approximately 100 m have been selected at each location and at each site. Ten photographic replicates (16 x 24 cm^2) have been randomly taken between 5 and 7 m of depth. Overall, 120 replicates have been collected. The sampling design is represented in Figure 21 for both the MPAs. A grid of 24 sub-quadrats was superimposed on each photo by using the software Microsoft Power Point and the cover of each species was calculated and expressed as a percentage value (Figure 22). Organisms not identified at species level were lumped into higher taxonomic groups.

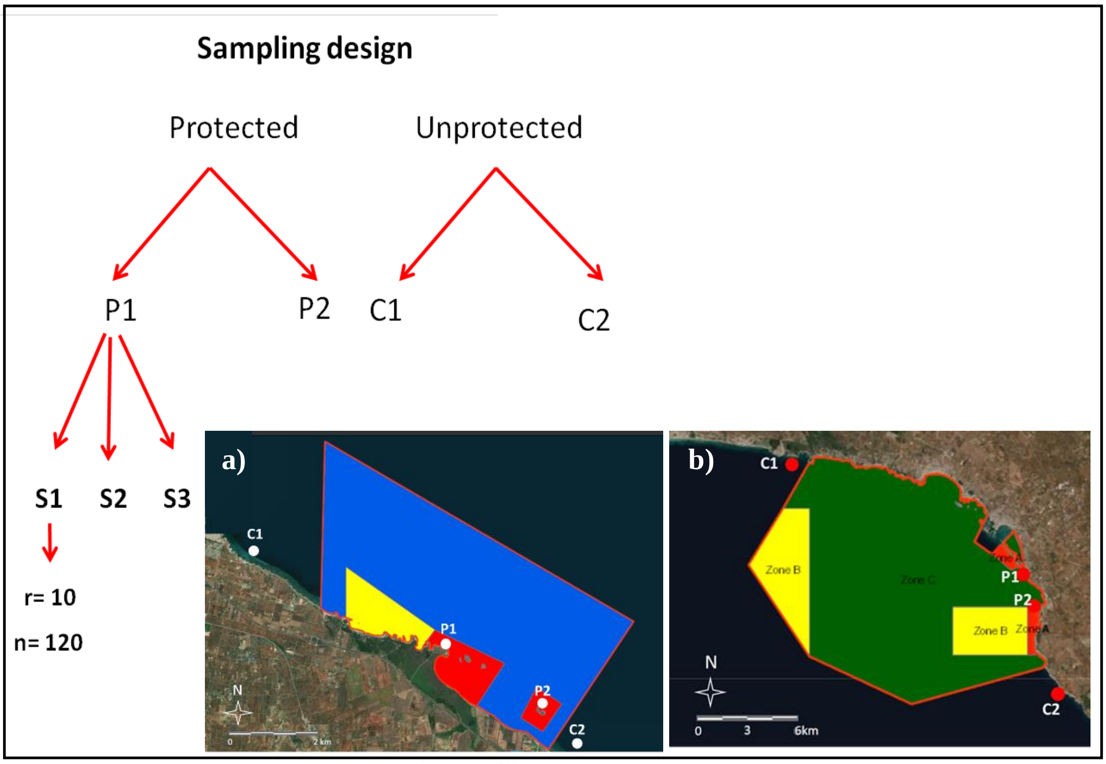


Figure 21, Sampling design and map describing the rocky shallow-infralittoral benthic assemblage surveys at Torre Guaceto (a) and Porto Cesareo (b). P1 and P2: Protected locations; C1 and C2: Unprotected locations; S: sites replicated within each location; r: number of pictures collected at each location; n= total number of pictures.

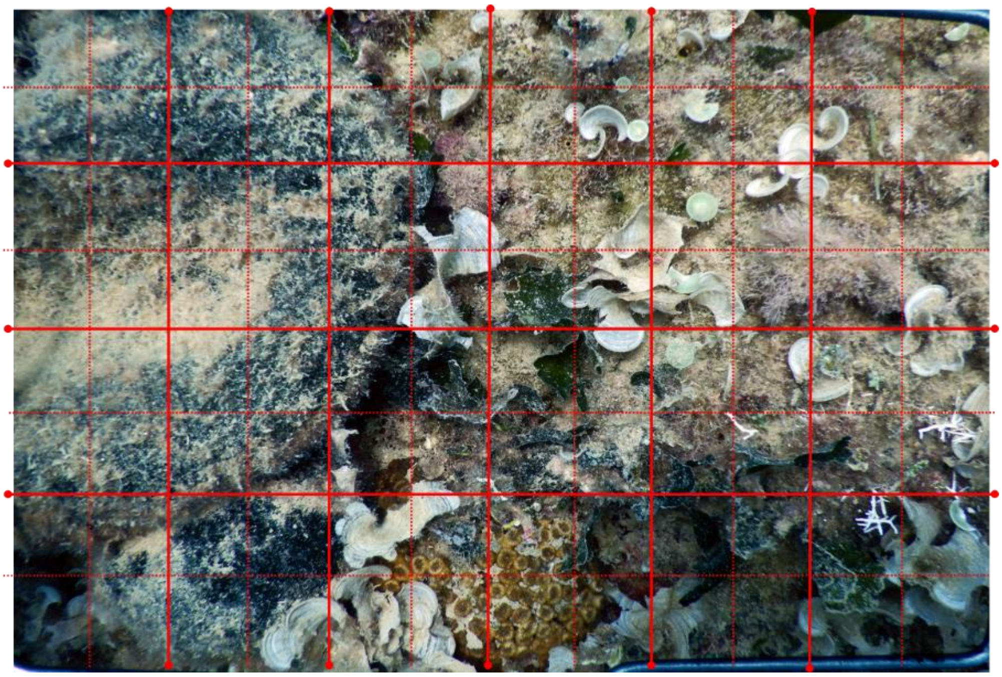


Figure 22, Examples of the approach adopted for assessing the % cover of each species within a photo-frame.

- **Freus d'Eivissa i Formentera Marine Reserve**

Rock-subtidal benthic assemblages between 8-12.5 m of depth have been assessed by *in situ* underwater visual estimates, through the line-point intercept method on 25 m long transects (Kohler and Gill, 2006; Sala et al., 2012) (Figure 23). This method consists of counting how many times a given organism is found every 20cm along the transect.

Two locations were selected within the no-take zone of the MPA, two locations in the partially protected zone and two locations within the external controls. At each location, 4 transects far 50 - 100m apart were randomly placed. Along each transect 125 contact points were censused (n=3000, Figure 24). Species were lumped into higher operational taxonomic units (OTUs) and, for each transect, the total score for each OTU was assessed, considering also the presence of bare rock and of sediment.

Moreover, visual estimates were also carried out on the fish assemblage and details about this survey are provided by the deliverable 4.8.1.

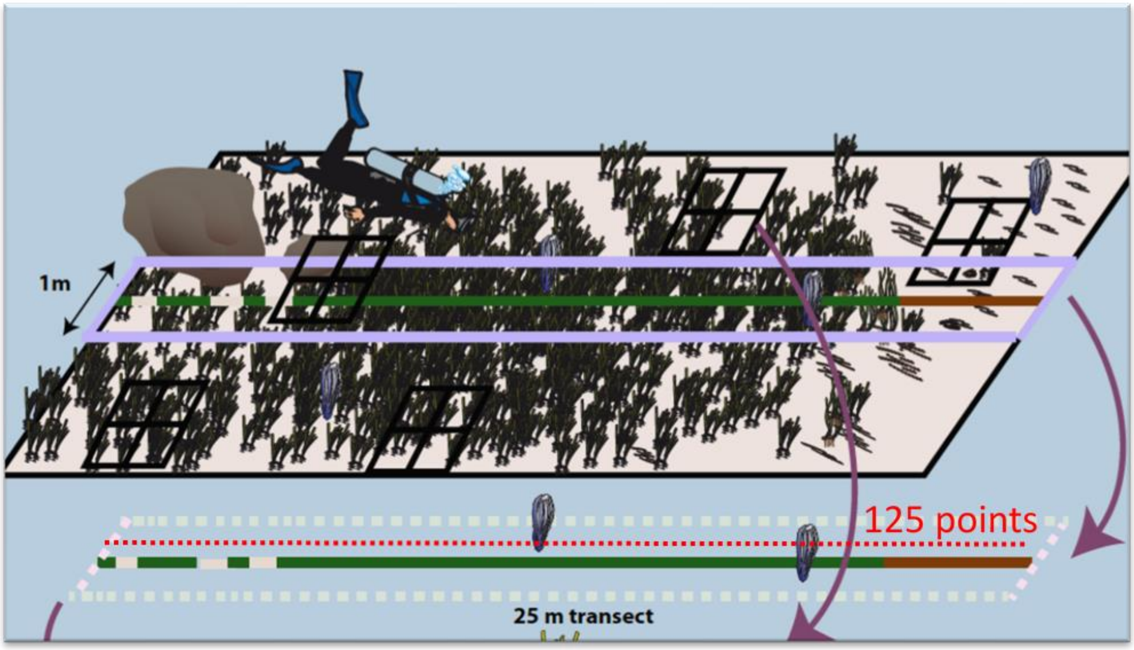


Figure 23, Schematic representation of the line point intercept method.

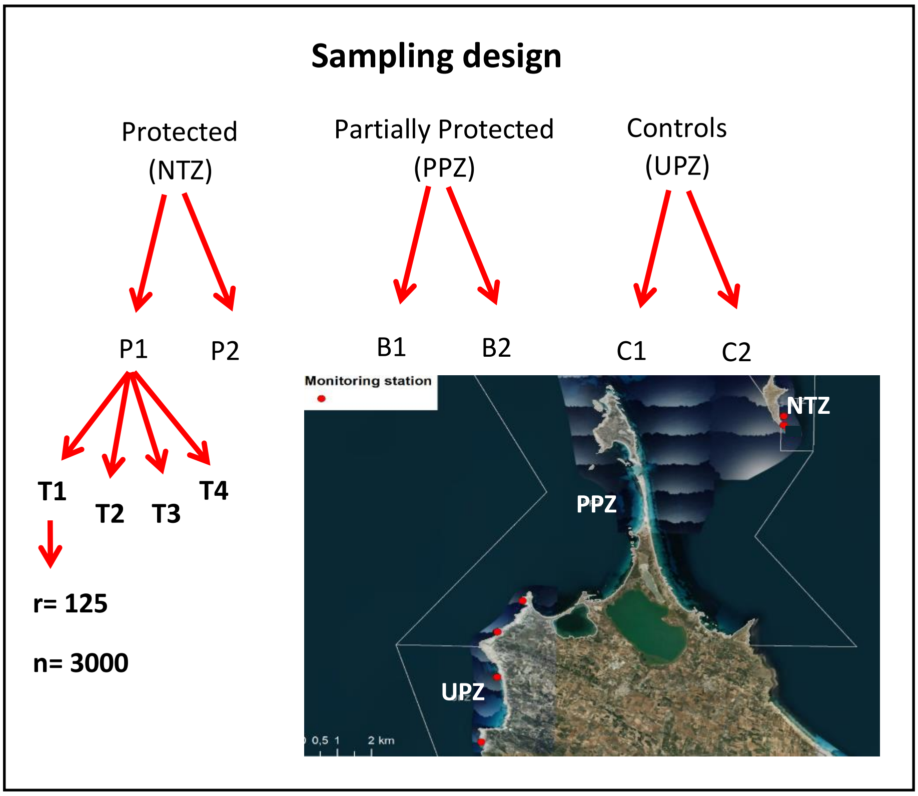


Figure 24, Sampling design and site map describing the rocky subtidal benthic assemblage survey at the Freus d'Eivissa i Formentera Marine Reserve. Red dots: surveyed locations; T: transects replicated at each location; r: number of points sampled on each transect; n= total number of sampled points.

- **Coralligenous formations**

Coralligenous sampling has been carried out at the Torre Guaceto and Porto Cesareo MPAs and at the National Marine Park of Alonissos Northern Sporades. Sampling adopted two different methodologies: a Remotely Operated Vehicle (ROV) was used in Torre Guaceto and Porto Cesareo, while a photographic method by diving was carried out at the NMPANS to compare the assemblage structure of the coralligenous formations along a gradient of protection. For each protection level, the potentially different fishing pressure was also considered. Fishery, indeed, has been recognised to have a potential effect on the coralligenous formations (Ballesteros, 2006; UNEP-MAP-RAC/SPA 2008; Martin et al., 2014), due to the physical contact of recreational and professional fishing tools with the structuring taxa (Betti et al., 2020; Otero et al., 2016; Bavestrello et al., 1998).

- **Torre Guaceto and Porto Cesareo**

Remotely operated vehicle (ROV) piloted from a vessel (Figure 25) has been used to describe the ecological status of coralligenous formations under protected and control conditions at the two MPAs. Sampling locations have been chosen considering the habitat map showing the coralligenous distribution at both the areas. At Torre Guaceto two locations were selected within the MPA, and two outside the MPA, one at the north and one at the south of its boundaries. Within each location, three transects measuring at least 200m were covered by the ROV. Overall, 12 transects were replicated (Figure 26). At Porto Cesareo three locations were selected within the MPA and two outside; three sites were replicated at each location and at each site three 200m long transects were covered by the ROV. Overall, 45 transects were replicated (Figure 27).

For this activity the Marine Strategy Framework Directive protocol (MSFD), section 7 (Art. 11, D.lgs. 190/2010) has been applied. During the ROV navigation, the vehicle was placed at 1.5 m from the bottom, advancing at speed lower than 1 knot. *A-posteriori* video analysis consisted of the assessment of the total number of megabenthic sessile taxa associated to the hard substrate along the transects, of the abundance of structuring species and of the abundance of fishing tools and debris.

Due to their tridimensionality, structuring species are considered as the most sensitive organisms to physical damage, being vulnerable to those activities which may generate a mechanical impact on the sea bottom, such as fishing and dredging. These organisms, moreover, are highly exposed to the risk of entrapment by ghost fishing or other objects (plastic objects, general litter). The slow growing rate which characterizes the life cycle of these organisms, moreover, reduce their ability to restore once physical damages occurred, with adverse ecological consequences for the whole community.

A list of species and the presence of structuring taxa, abundance and mean size have been produced for each video. For these analyses, species from the genus *Eunicella* sp. and *Leptogorgia* sp. were grouped at the class level within "Anthozoa" and the massive dark sponges were lumped all together in the group "MDS".

Figure 25, ROV Equipment used during the coralligenous survey at Torre Guaceto and Porto Cesareo.

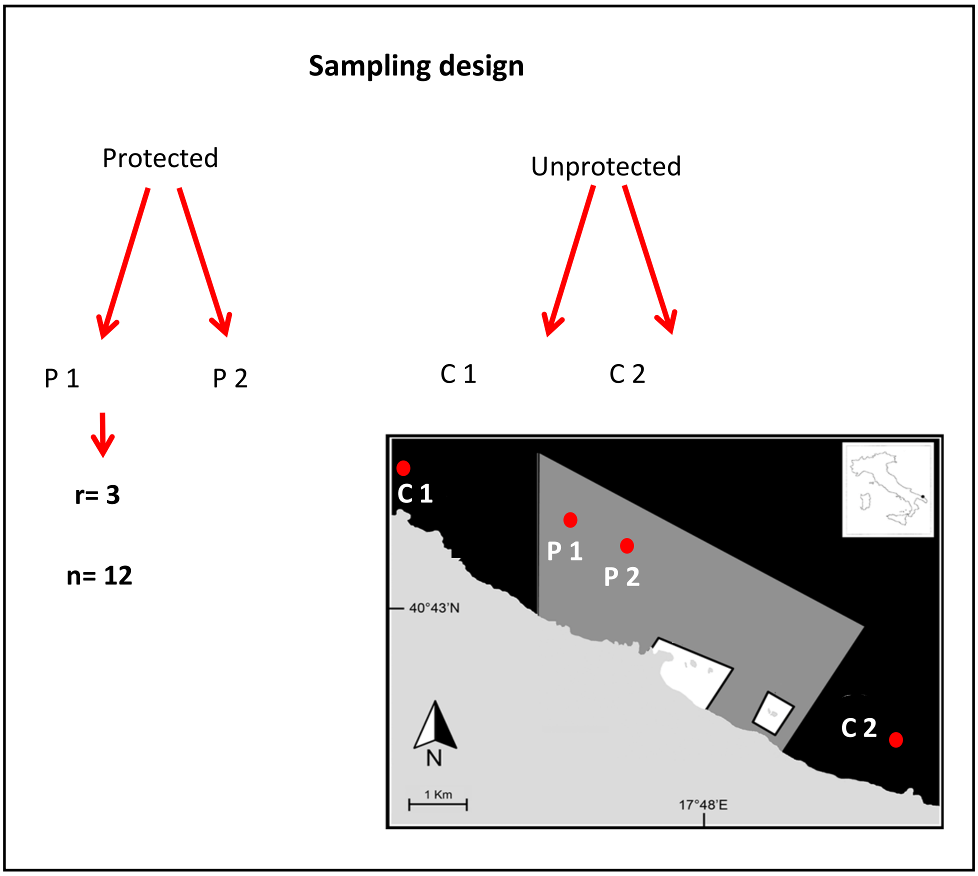


Figure 26, Coralligenous formation sampling design and map of surveyed locations at Torre Guaceto. C 1 and C 2: Unprotected locations; P 1 and P 2: Protected locations; r: number of transect replicated at each location; n= total number of transects.

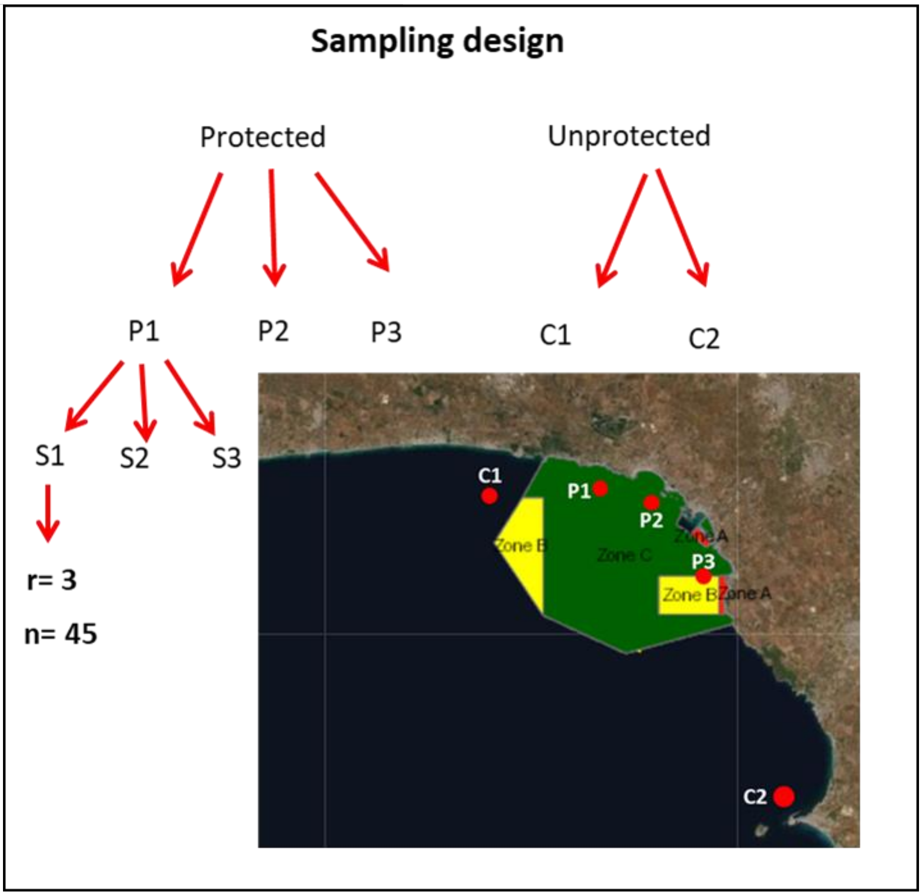


Figure 27, Coralligenous formation sampling design and map of the surveyed locations at Porto Cesareo. C1 and C 2: Unprotected locations; P 1, P 2 and P3: Protected locations; r: number of transect replicated at each location; n= total number of transects.

- **Survey at the National Marine Park of Alonissos Northern Sporades**

At the National Marine Park of Alonissos Northern Sporades an underwater photo-sampling was conducted in two locations within the B zone of the MPA, subjected to a different degree of fishing pressure. Three sites were randomly sampled at each location, with 5 photographic replicates of 25x25 cm^2 at each site (Figure 28). The distribution and the % abundance of structuring taxa were described by visual estimates and compared among the two levels of fishing pressure.

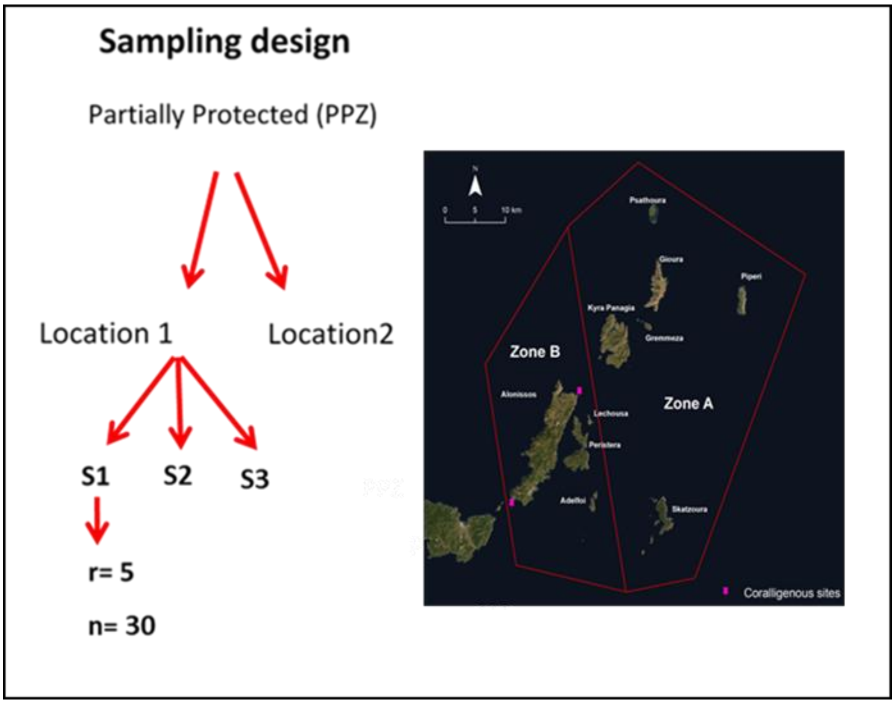


Figure 28, Coralligenous formation sampling design and map of the surveyed locations at the National Marine Park of Alonissos Northern Sporades. Pink dots: surveyed locations; S: surveyed sites at each location; r: number of photo-frames replicated at each site; n= total number of replicates.

- **Marine litter surveys at Torre Guaceto and Porto Cesareo MPAs and at the National Marine Park of Alonissos Northern Sporades**

Initial information on the marine litter at the Torre Guaceto and Porto Cesareo and at the National Marine Park of Alonissos Northern Sporades has been obtained by the description of the litter found during the coralligenous surveys (e.g. fishing objects, plastics and other debris typology, Figure 29). Furthermore, data from the locations at the NMPANS where the *P. oceanica* survey have been carried out have been also included in this first marine litter assessment.

Litter typology, distribution and abundance have been described and compared among different levels of protection at the three MPAs.

Figura 29, Examples of fishing tools found at Torre Guaceto and Porto Cesareo.

- **Stakeholders engagement**

Due to the interdependency that exists between the ecosystem resources and its users, successful implementation of ecosystem-based management depends on the identification and understanding of different stakeholders, their practices, expectations and interests (Pomeroy and Douvere, 2008).

Local people and stakeholder involvement in the MPA management plan production (i.e. co-management), indeed, is recognised as an important step for effective marine ecosystem conservation and multiple goals achievement, with ecological, economical and socio-cultural returns (Mascia 2003; McClanahan et al. 2006; Gelcich et al. 2008). Moreover, the dissemination and the direct perception of MPA positive effects by the locals are crucial to increase the MPA acceptance among the common people and their support (e.g., Pollnac et al. 2001; Kritzer 2004; Gelcich et al. 2008). Environmental communication, education, sensibilisation, public involvement since the outset of the development of an MPA (e.g. selection of site and habitats to protect, planning of the conservation strategy, zoning and management) and an adaptive co-management approach are, thereby, necessary to favor a positive local perception of the MPA, enhancing the conservation performance and success.

In the frame of the pilot actions of the AMAre project, key stakeholders at the national, regional and local level have been identified to exchange information, views, and promote dialogue on issues related to the biodiversity conservation and sustainable use of the marine resources.

Special effort was exerted to interact with local stakeholders (Environmental Policy makers, MPA officials, fishers, diving centers, tourism represents and regional authorities) to raise awareness about the project's objectives and results, and gather their perceptions of conservation issues.

A number of events has been organized within the framework of the AMAre project, involving the Management Body of Torre Guaceto MPA, the NMPANS, focusing on the participation and interaction with a wide variety of stakeholders.

Unfortunately, the Freus d'Eivissa i Formentera Marine Reserve was not able to organize any event aimed at the stakeholder involvement, since this MPA resigned the project partnership.

Furthermore, another relevant stakeholder workshop has been organised in Malta as part of the of the European Maritime Day 2019.

5. Results

- *Posidonia oceanica* surveys

- **Torre Guaceto**

The average densities of *P. oceanica*/m^2 (± standard error, SE) for each level of protection are reported in Figure 30.

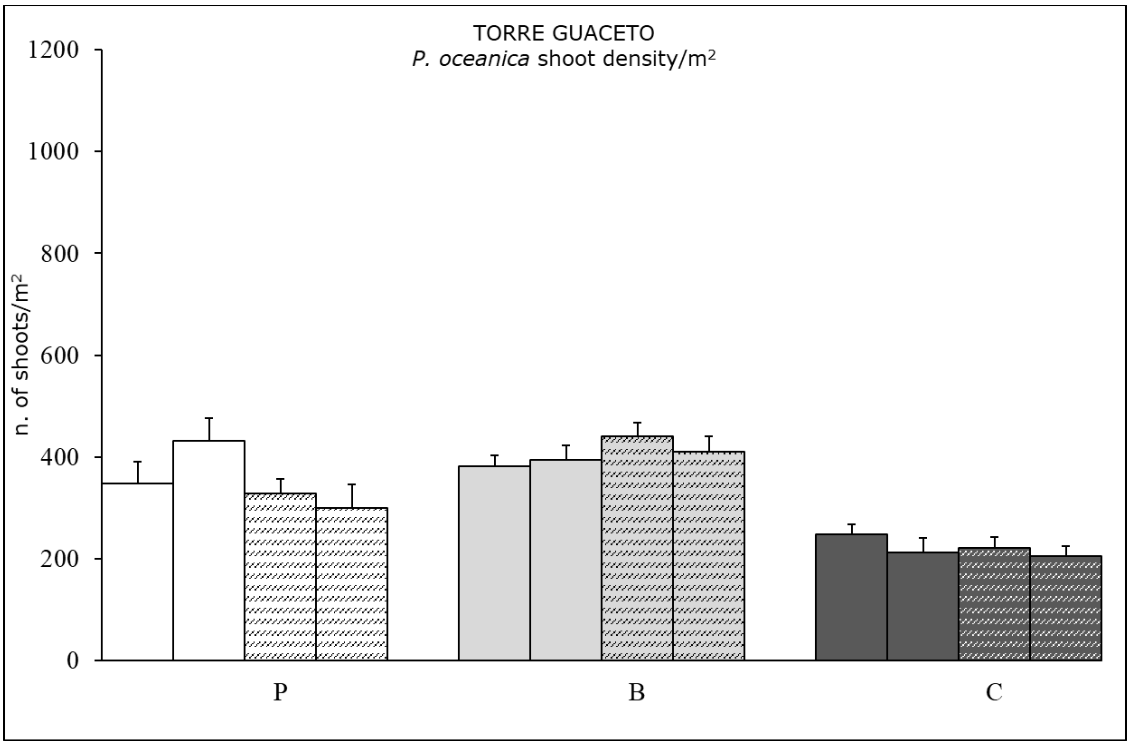


Figure 30, Average densities ± standard error of P. oceanica *within protected sites (P), partially protected sites (B) and control sites (C) at Torre Guaceto. Different bar textures: different surveyed locations.*

The average shoot density/m^2 measured at each sampling site at Torre Guaceto was also compared to the values reported by Pergent et al. (1995). Referring to these values, the *P. oceanica* bed in the area of Torre Guaceto resulted very disturbed, disturbed and in equilibrium (Figure 31).

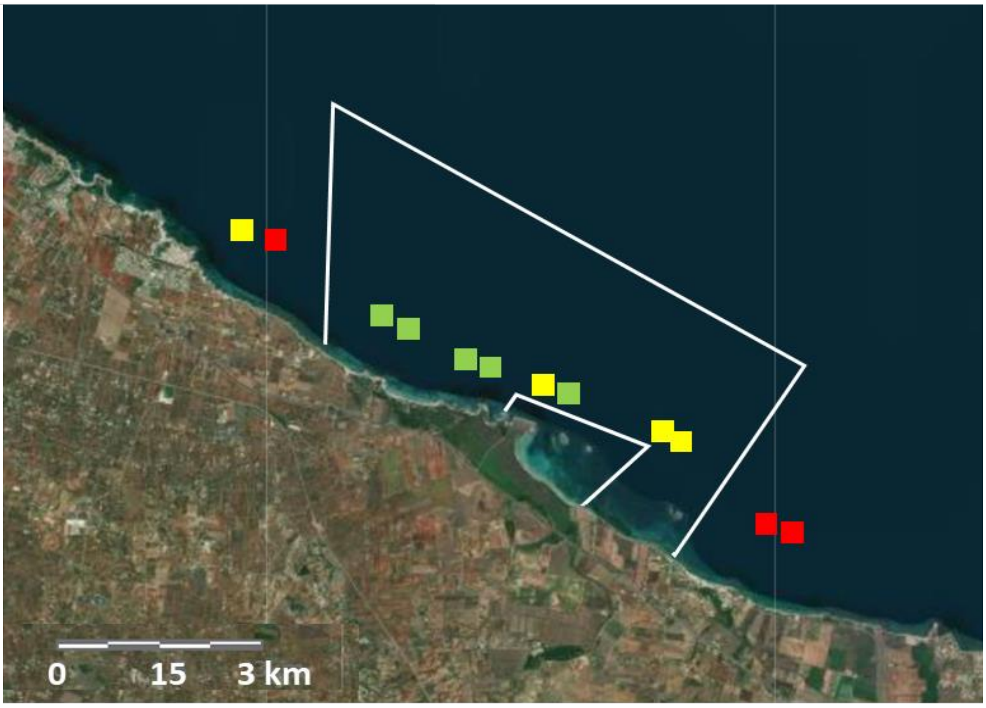


Figure 31, Classification of the ecological status of the P. oceanica *meadows at Torre Guaceto. In red: very disturbed conditions; in yellow: disturbed conditions; in green: not disturbed conditions.*

Three out of 4 four totally protected sites showed disturbed meadow conditions, while not disturbed conditions, according to the literature, were recorded in one totally protected site and at all the partially protected sites. Outside the MPA, shoot density values showed very disturbed meadow except the northernmost site, revealing a seagrass meadow under pressure.

Univariate PERMANOVA revealed significant differences among levels of the fixed factor Protection (p= 0.0073; C<P=B). No significant differences were found among locations within the same protection level and among the sites within the same location (Table 3).

Table 3, Summary of univariate PERMANOVA results testing for the effect of protection on the P. oceanica *meadows at Torre Guaceto. "Pr": protection; "Loc": locality; "Si": site.*

	Torre Guaceto		
Source of variation	**MS**	*Pseudo-F*	*P(MC)*
Pr	5373,6	20,16	0,0073
Loc(Pr)	266,51	2,01	0,19
Si(Lo(Pr))	132,13	0,97	0,44
Res	135,32		

The significantly higher *P. oceanica* shoot density recorded at the protected and partially protected locations compared to unprotected ones may be a direct consequence of protection measures on the seagrass.

- **Malta**

In Malta, the average *P. oceanica* shoot density/m^2 ranges among 492 (±4 SE) at one of the protected sites and 385 (±15 SE) at one of the controls (Figure 32).

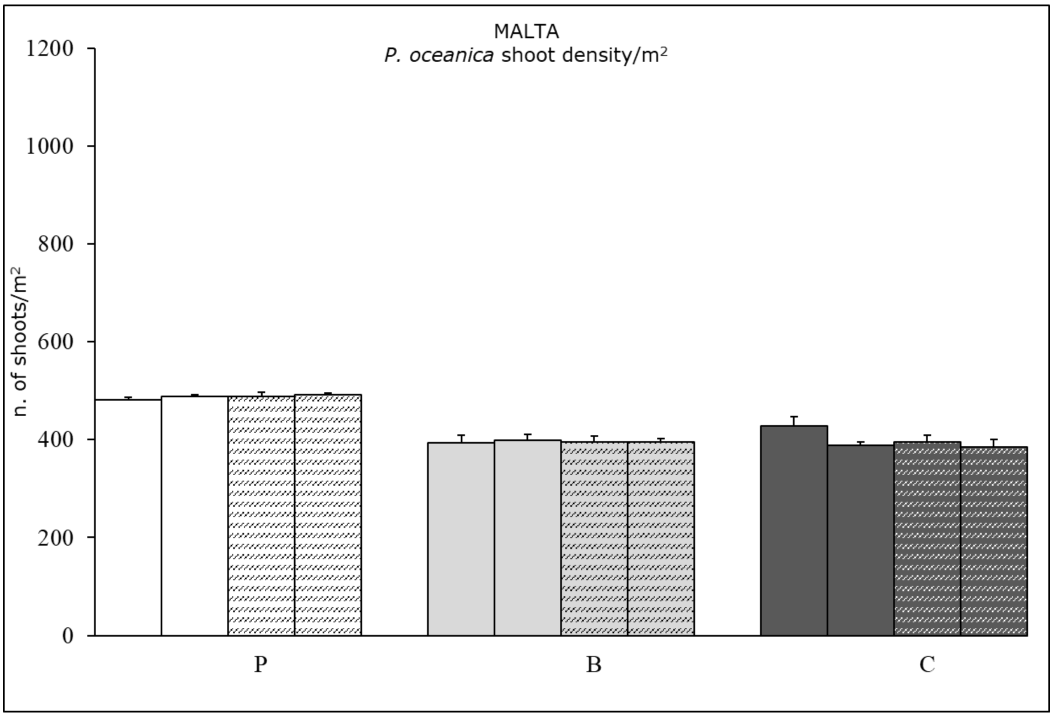


Figure 32, Average densities ± standard error of P. oceanica *within protected sites (P), partially protected sites (B) and control sites (C) at Malta Island. Different bar textures: different surveyed locations.*

A high homogeneity of the values of shoot density/m^2 among sites within the same location is also evident (Figure 32) and the meadow ecological status features as not disturbed at all the surveyed sites. Statistical analyses revealed significant differences in the seagrass shoot density among the three different conditions of protection (Table 4), showing a positive effect of the full protection on the meadow, compared to partial protection and unprotected controls.

Table 4, Summary of univariate PERMANOVA results testing for the effect of protection on the P. oceanica *meadows at Malta. "Pr": protection; "Loc": locality; "Si": site.*

	Malta		
Source of variation	**MS**	*Pseudo-F*	*P(MC)*
Pr	845,26	80,87	0,0017
Loc(Pr)	10,45	0,84	0,52
Si(Lo(Pr))	12,44	1,06	0,39
Res	11,76		

- **Porto Cesareo**

At Porto Cesareo, both the shallow and the deep *P. oceanica* meadows do not exhibit marked differences of shoot density among protected and control conditions. The average seagrass density of shoot/m^2 (± standard error, SE) for both the depth ranges is reported in Figure 33.

The *P. oceanica* ecological status has been assessed at each surveyed site and is shown in Figure 34. The majority of the surveyed sites showed not disturbed conditions. The northernmost area was characterized by a not disturbed meadow, in spite of the southern sites which exhibited, on average, a disturbed status. Furthermore, management measures currently in force within the MPA do not seem to enhance the seagrass ecological status respect to unprotected conditions.

PERMANOVA carried out on shoot density, indeed, does not show statistically significant differences among protected and control conditions (Table 5).

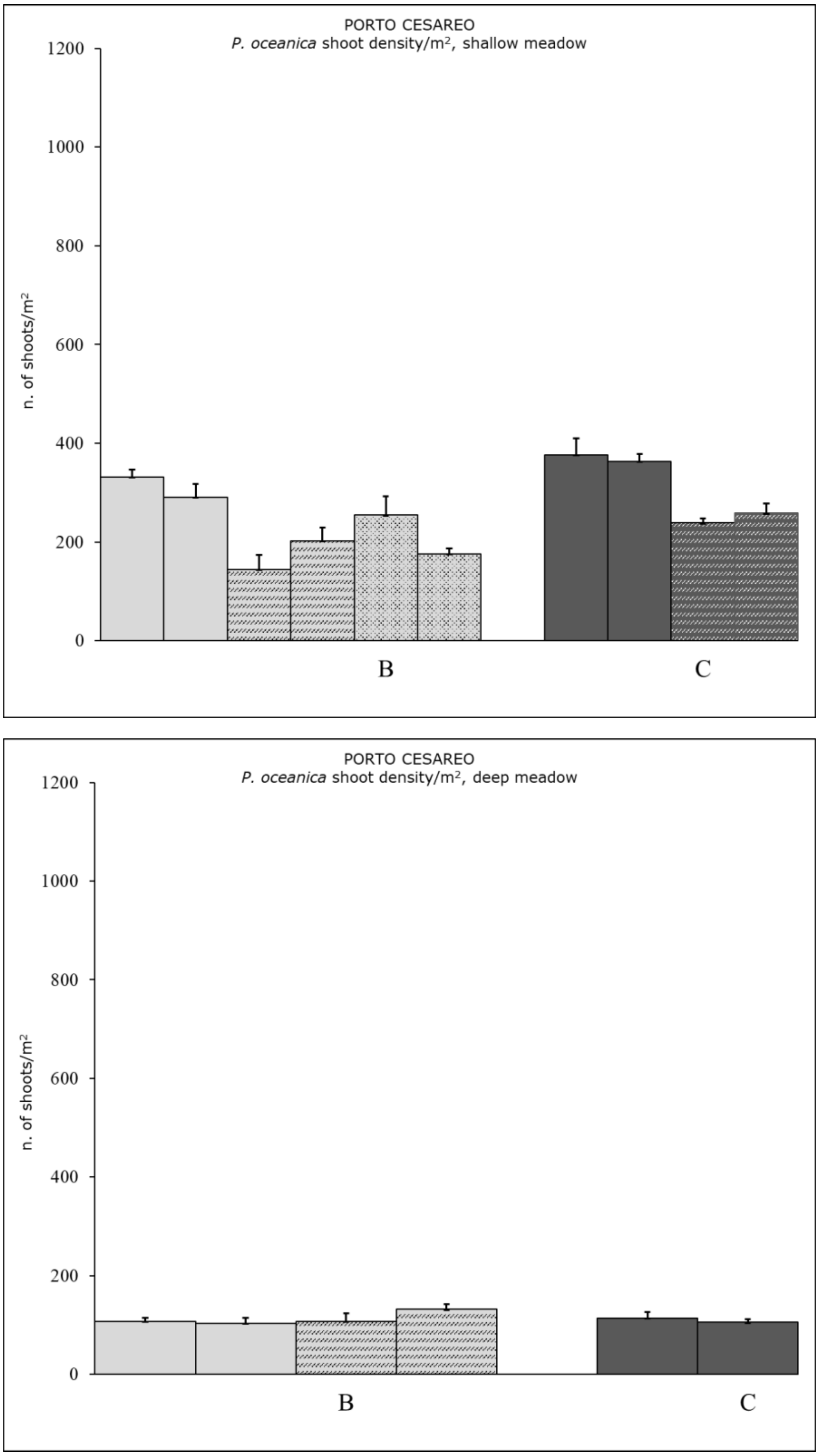


Figure 33, Average shoot densities ± standard error of P. oceanica *at the shallow (graph above) and deep meadows (graph below) at Porto Cesareo. B: partially protected sites; C: control sites (C). Different bar textures: different surveyed locations.*

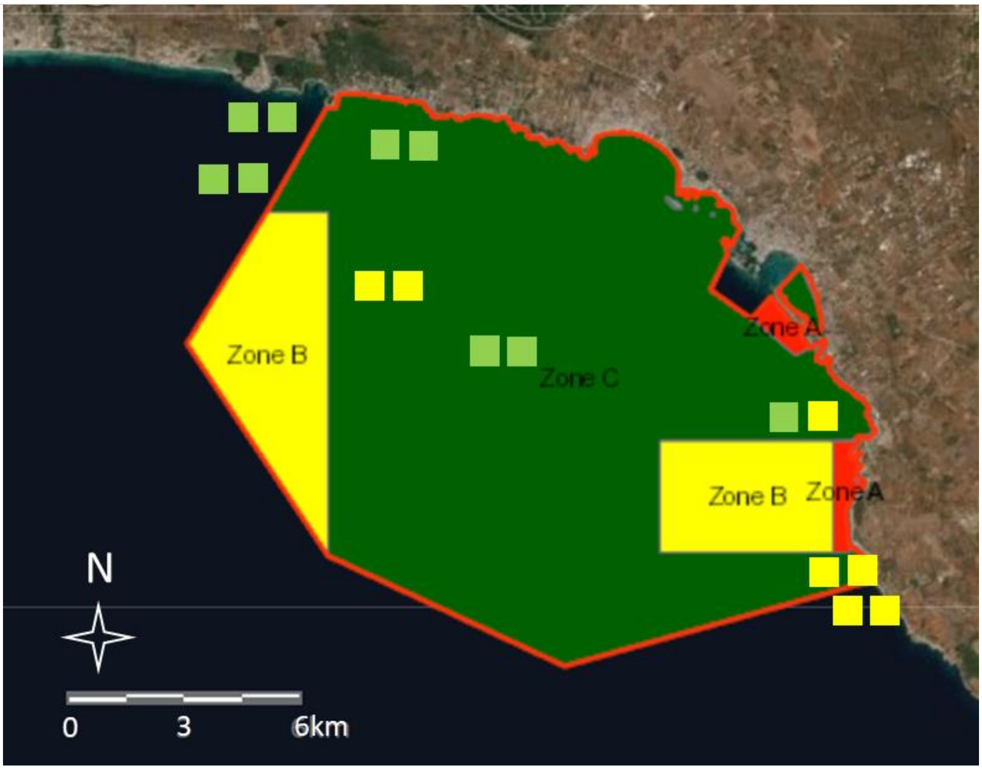


Figure 34, Classification of the ecological status of the P. oceanica *meadows at Porto Cesareo. In yellow: lower subnormal densities; in green: normal densities*

Table 5, Summary of PERMANOVA results testing for the effect of protection on the P. oceanica *shallow and deep meadows at Porto Cesareo. "Pr": protection; "Loc": locality; "Si": site.*

	Porto Cesareo					
	Shallow meadow			Deep meadow		
Source of variation	**MS**	*Pseudo-F*	*P(MC)*	**MS**	*Pseudo-F*	*P(MC)*
Pr	2489,4	1,21	0,35	8,24	0,05	0,91
Loc(Pr)	2060,2	6,64	0,02	162,88	1,26	0,33
Si(Lo(Pr))	310,18	2,09	0,06	129,34	0,97	0,42
Res	148,16			132,99		

- **Freus d'Eivissa i Formentera Marine Protected Area**

P. oceanica shoot density was featured by high values at all the surveyed locations, with higher average values recorded at the totally protected locations (P) and at the controls (C) (Figure 35).

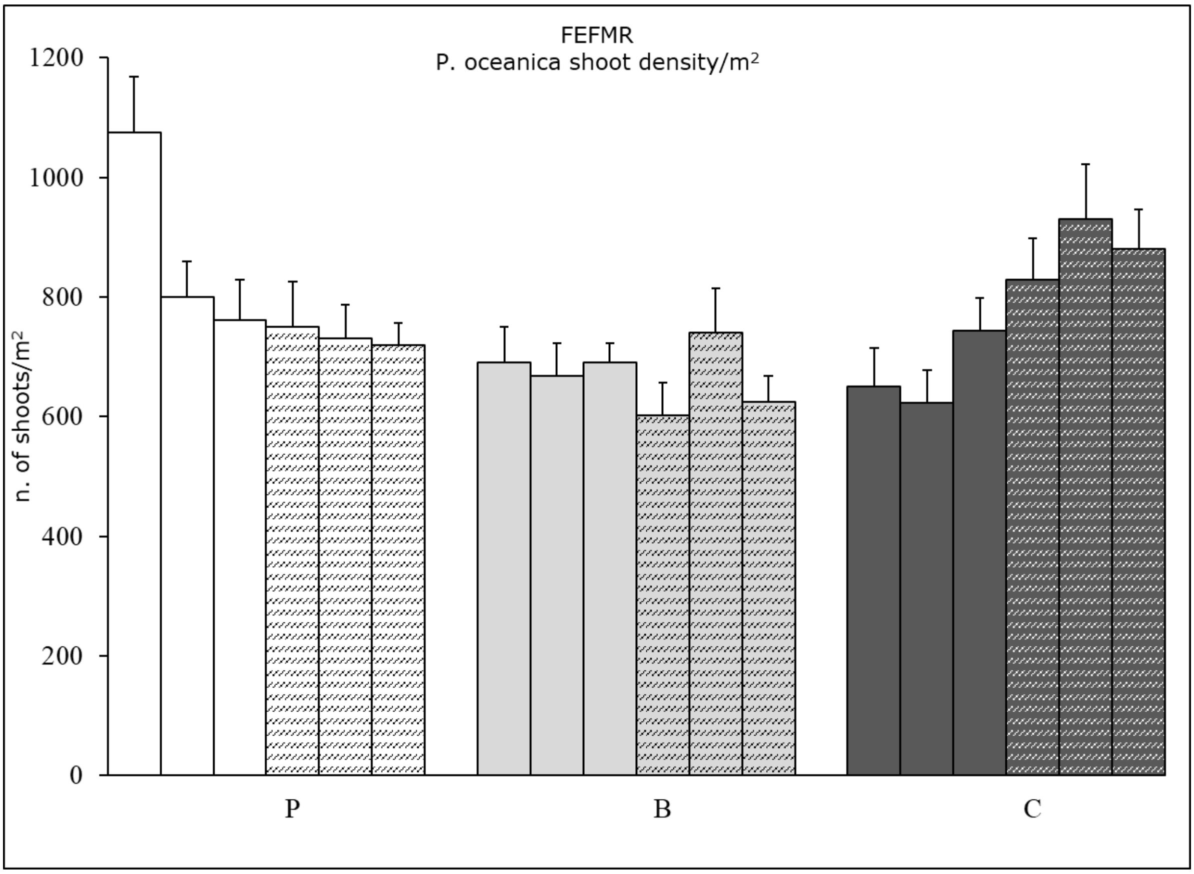


Figure 35, Posidonia oceanica *shoot density/m² at the Freus d'Eivissa i Formentera Marine Reserve. P: protected sites; B: partially protected sites; C: control sites. Different textures: different surveyed locations.*

According to the scale of Pergent et al. (1995), the mean values obtained in all the transects describe meadows not disturbed by human activities (Figure 36).

Furthermore, differences in the seagrass shoot density among protection levels were confirmed by statistical analysis (Table 6).

Moreover, no flowering event occurred and invasive species were observed in the meadow and all the observed *Pinna nobilis* were found dead.

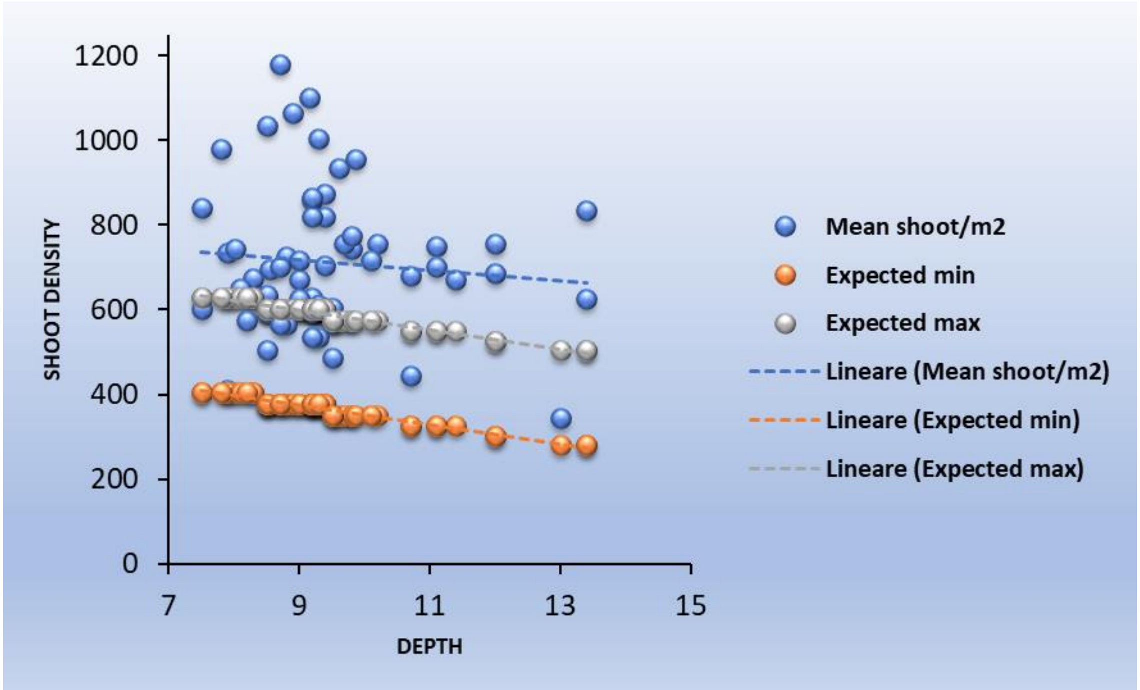


Figure 36, Classification of the ecological status of the P. oceanica *meadows at the Freus d'Eivissa i Formentera Marine Reserve.*

Table 6, Summary of univariate PERMANOVA results testing for the effect of protection on the P. oceanica *meadows at the Freus d'Eivissa i Formentera Marine Reserve. "Pr": protection; "Loc": locality; "Si": site.*

	FEFMR		
Source of Variation	**MS**	*Pseudo-F*	*P(MC)*
Pr	89237,89	6,34	0,005
Loc(Pr)	104955,18	7,46	0,001
Si(Lo(Pr))	19923,14	1,41	0,21
Res	14066,67		

- **National Marine Park of Alonissos Northern Sporades**

The average *P. oceanica* shoot density/m^2 measured at each sampling site at the NMPANS are provided in Figure 37.

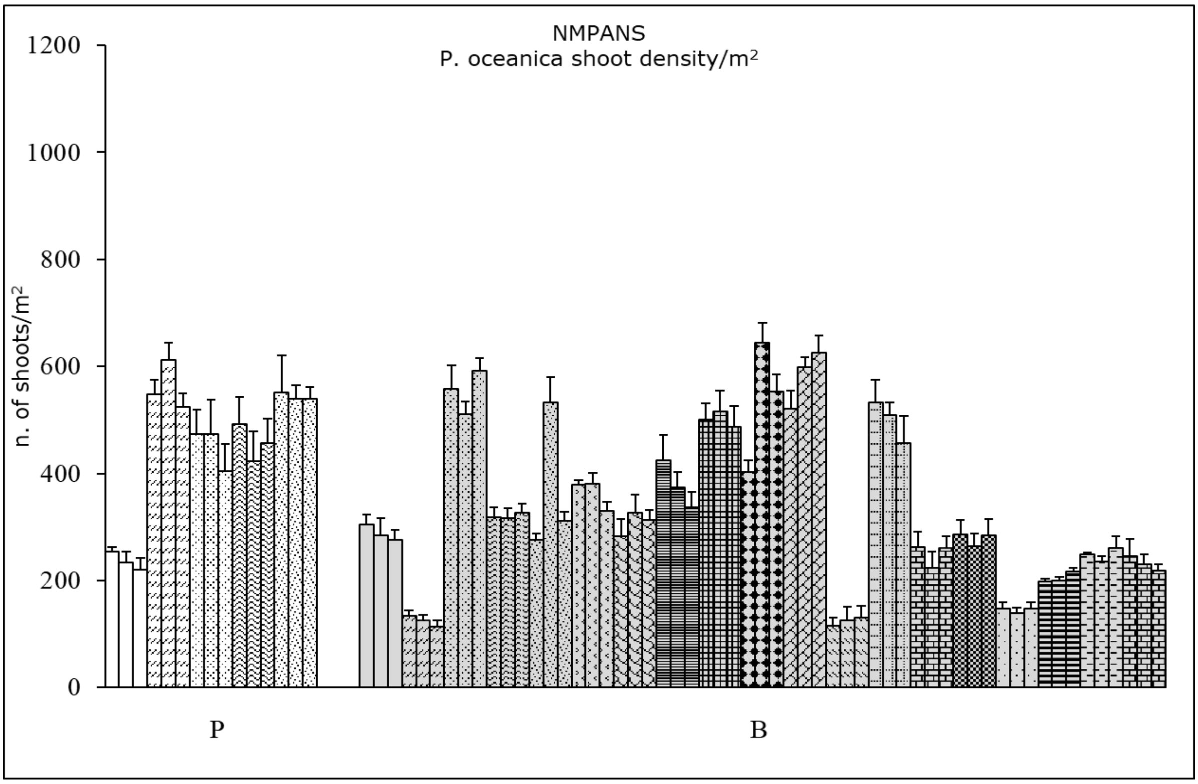


Figure 37, Posidonia oceanica *shoot density/m² at the National Marine Park of Alonissos Northern Sporades. P: protected sites; B: partially protected locations. Different textures: different surveyed sites.*

Overall, a high spatial variability of the shoot density of the *P. oceanica* meadow across the surveyed locations and sites within locations has been revealed. The Figure 38 reports the ecological status assessment of the surveyed NMPANS meadows referring to the values reported by Pergent et al. (1995). Not disturbed meadow conditions have been revealed at all the sites within the no-take zone of the NMPANS, with one exception, while the partially protected zone of the marine park showed a high variability of the seagrass ecological status (Figure 38).

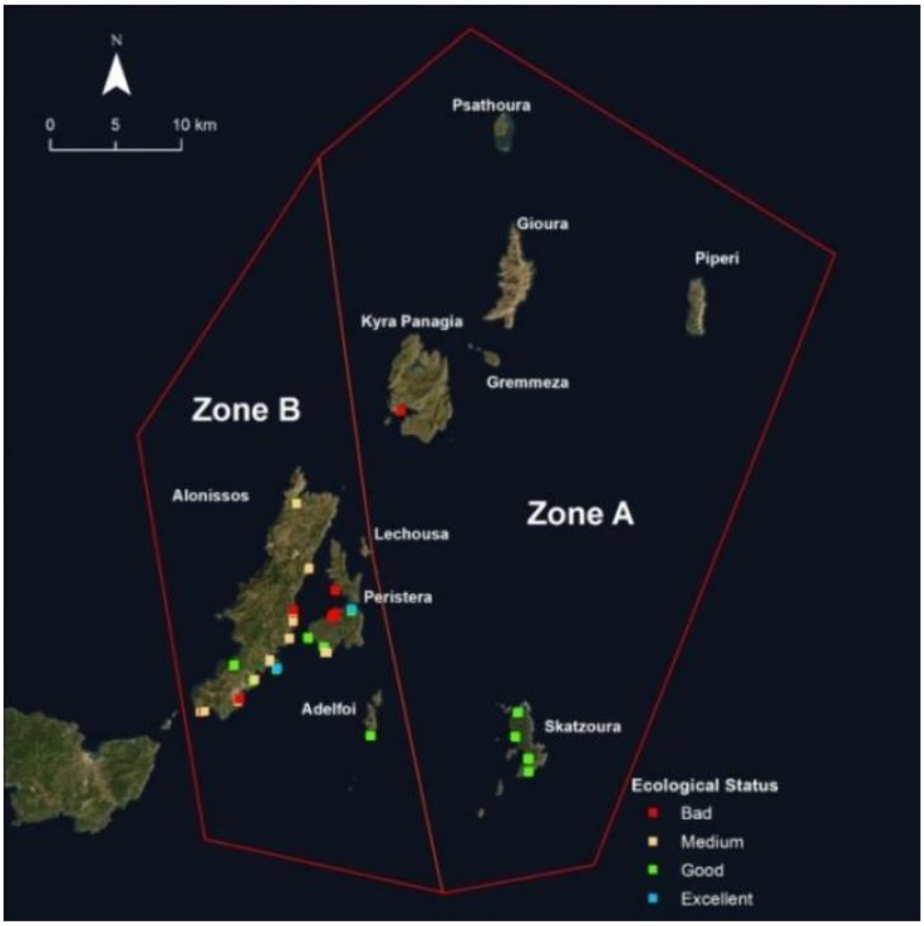


Figure 38, Classification of the ecological status of the P. oceanica *meadows at the National Marine Park of Alonissos Northern Sporades surveyed sites. Red: very disturbed; yellow: disturbed; green: not disturbed; blue: excellent.*

In detail, comparing the Zones A and B, Zone A exhibited a good status in most of the cases, except the location at Agios Petros, probably for reasons related to human uses. The location, indeed, is used as temporary anchorage location by visiting boats during the summer, having as starting point the Alonnisos island for day trips.

Looking at the Zone B and particularly at Peristera island, the two sides of the island appeared very different. At Vasiliko bay, the meadows have been classified into a bad ecological status. The bay is used by several types of boats, from small leisure boats to large yachts and fishing boats. Due to the absence of permanent moorings that could mitigate the impacts on the seabed, the repeated anchoring from daily visits of locals and visitors has caused significant degradation to the meadows. On the other side, the location at the outside part of the island, named Klima shows an excellent ecological status with lush meadows growing from the 5 down to 35 meters, thanks to the limited access to the area.

PERMANOVA did not revealed significant differences among protection levels. By contrast, high diversity among locations and sites has been confirmed by the analysis (Table 7), confirming the high heterogeneity of this habitat at the study area.

Table 7, Summary of PERMANOVA results testing the effect of protection on the P. oceanica *meadows at the National Marine Park of Alonissos Northern Sporades. "Pr": protection; "Loc": location; "Si": site.*

	NMPANS		
Source of Variation	**MS**	*Pseudo-F*	*P(MC)*
Pr	4238	0,65	0,48
Loc(Pr)	6542	37,08	0,0001
Si(Lo(Pr))	176,43	1,82	0,0013
Res	97,01		

Measurements from the deep limit up to the shallow meadow limit conducted on a subset of the surveyed locations collecting shoot density/m^2 every 5 meters, showed, in general, that seagrass meadows are at a good ecological status with the exception of one specific sites (Steni Vala). This site was classified under a medium ecological status, showing a higher influence of pressures within 15m of depth and (Table 8). Ecological status classification for each site was based on the majority of the interpreted measurements as medium, good or excellent (Table 8).

Table 8, Mean P. oceanica *shoot density/m^2 per 5 m intervals. Yellow: disturbed densities (medium ecological status); green: not disturbed densities (good ecological status); blue: excellent densities (excellent ecological status).*

Locations	**10m**	**15m**	**20m**	**25m**	**30m**	**35m**	**Ecological Status**
SteniVala	268	220	129	169	70		
Peristera - Klima	615	500	480	345	275	225	
DyoAdelfia	540	435	385	430	310	325	
Peristera Wreck	510	370	305	205	220		

Moreover, during the June 2019 diving survey, extensive parts of the sea bottom were covered by a marine mucilage which has become an increasing problem in many areas of the Mediterranean Sea (Sartoni et al., 2008) and has been also documented in other areas of Greece, entailing necrosis of gorgonian forests and impacts on benthic assemblages (Skoufas & Poulicek, 2001, Skoufas et al., 2015).

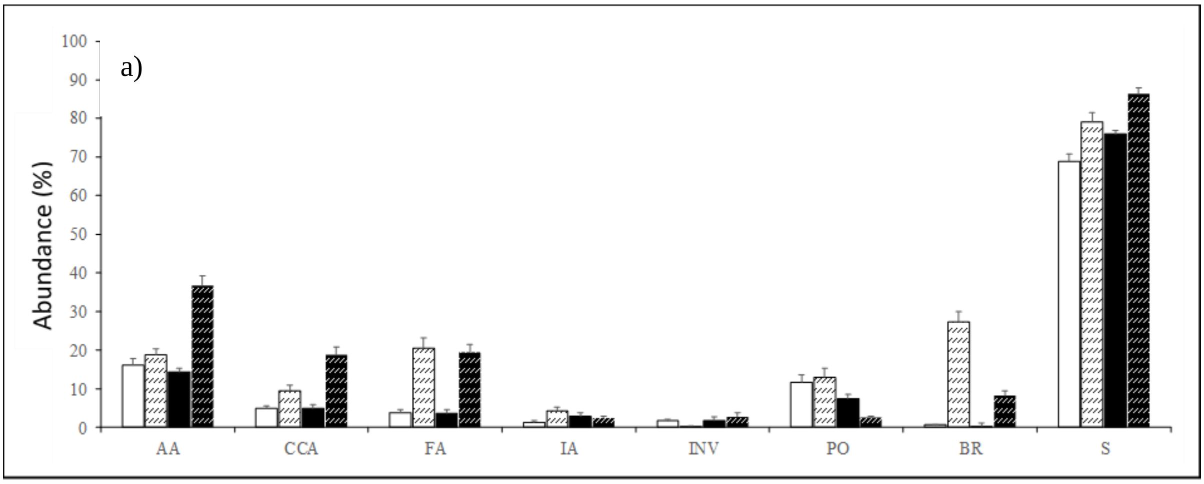
- **Rocky subtidal – shallow infralittoral benthic assemblage survey**
- **Torre Guaceto and Porto Cesareo**

A taxonomic list of species and operational taxonomic units (OTUs) recognised by visual identification of photo-sampling collected during the survey at Torre Guaceto and Porto Cesareo is reported in Table 9.

Table 9, List of taxa and species found in the surveyed rocky shallow infralittoral benthic assemblages at Torre Guaceto and Porto Cesareo

BENTHIC ASSEMBLAGE TAXONOMIC LIST FOR TORRE GUACETO (TG) AND PORTO CESAREO (PC)					
	TG	PC		TG	PC
Rhodophyceae			Porifera		
Articulated Corallines (AC)	x	x	*Acanthella acuta*	-	x
Amphiroa sp.	x	x	*Aplysina aerophoba* Nardo, 1843	x	x
Laurencia complex	x	x	*Agelas* sp.	-	x
Liagora viscida (Forsskål) C.Agardh, 1822	-	x	*Chlatrina* sp.	-	x
Peyssonnelia sp. Decaisne, 1841	x	x	*Chlatrina* sp.	-	x
Sphaerococcus coronopifolius Stackhouse, 1797	x	-	*Chondrilla nucula* Schmidt, 1862	x	x
Tricleocarpa fragilis (Linnaeus) Huismans & R.A. Townsend, 1993	x	x	*Chondrosia reniformis*	-	x
Wrangelia penicillata (C. Agardh) C. Agardh, 1828	x	x	*Cliona* spp.	x	x
			Encrusting Red Sponges (ERS)	x	x
Phaeophyceae			*Hemimycale columella* (Bowerbank, 1874)	x	x
Cystoseira sp. C.Agardh, 1820	x	x	*Ircinia variabilis* (Schmidt, 1862)	x	x
Dictyotales	x	x	Massive Dark Sponges (MDS)	x	x
Dumontiaceae	-	x	*Petrosia ficiformis* (Poiret, 1789)	x	x
Padina pavonica (Linnaeus) Thivy, 1960	x	x	*Phorbas fictitius* (Bowerback, 1866)	x	x
Stypocaulaceae	-	x	*Terpios fugax* Duchassaing & Michelotti, 1864	x	-
Chlorophyceae			Hydrozoa	x	-
Acetabularia (Acetabularia) acetabulum (Linnaeus) P.C.Silva, 1952	x	x			
Anadyomene stellata (Wulfen) C. Agardh, 1822	x	x	Anthozoa		
Caulerpa cylindracea Sonder 1845	x	x			
Caulerpa prolifera (Forsskål) J.V. Lamouroux	x	-	*Actinia* sp.	x	x
Codium bursa (Linnaeus) C. Agardh, 1822	x	x	*Balanophyllia europaea* (Risso, 1826)	x	x
Colpomenia sinuosa	-	x	*Caryophyllia smithi* Stokes & Broderip, 1828	x	x
Dasycladus vermicularis (Scopoli) Krasser, 1898	x	x	*Cladocora caespitosa* (Linnaeus, 1758)	x	x
Flabellia petiolata (Turra) Nizamuddin	x	x			
Green Filamentous Algae (GFA)	x	x	Polychaeta		
Halimeda tuna (Ellis & Solander) J.V. Lamouroux, 1816	x	x	Calcareus Tube Worms (CTW)	x	x
Palmophyllum crassum (Naccari) Rabenhorst	x	-			
Valonia macrophysa	-	x	Bivalvia		
			Gastrochaena dubia (Pennant, 1777)	x	x
Other algal groups					
Dark Filamentous Algae (DFA)	x	x	Gastropoda		
			Vermetidae	x	x
Ascidiacea					
Didemnidae	x	x	Cirripedia		
Diplosoma listerianum (Milne-Edwards, 1841)	x	-	Balanidae	x	x
Microcosmus sp.	x	-			
Bryozoa					
Encrusting Bryozoans (EB)	x	x			

The species reported in the list above were arranged within the following groups: Arborescent algae; Crustose Coralline Algae (CCA); Erect Algae; Filamentous Algae; Invasive Species; Invertebrates; Porifera. Further analyses were performed considering these operation taxonomic units (OTUs). The % of bare rock and sediment were also assessed. Average % cover (±SE) for each considered OTU at both Torre Guaceto and Porto Cesareo are shown in the Figure 39.



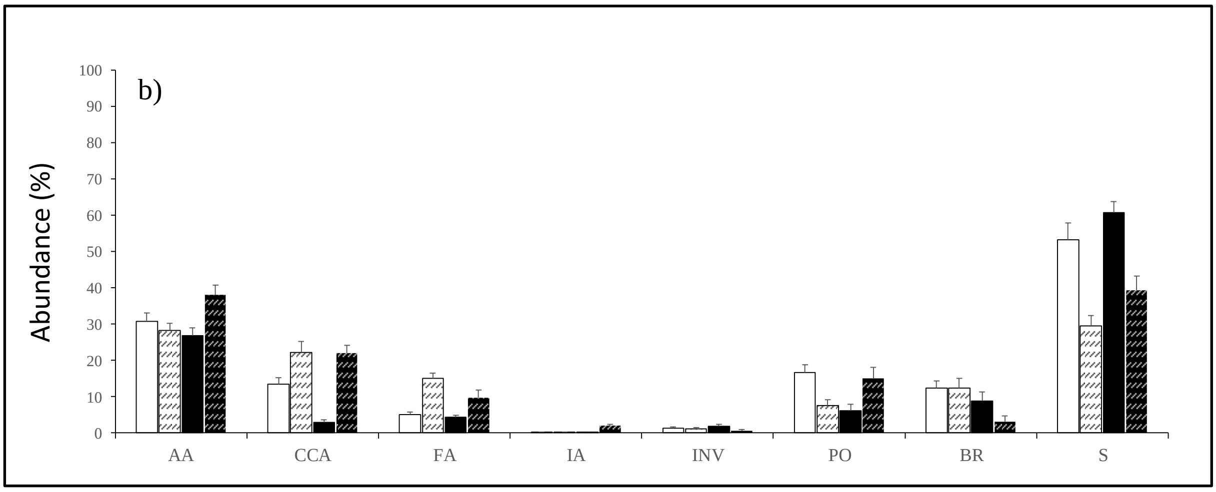


Figure 39, Average % cover (±SE) of shallow-infralittoral benthic OTUs at each sampled location at a) Torre Guaceto and b) Porto Cesareo. The different bar colour indicates the protected locations (in white) and the unprotected ones (in grey); the different textures indicate the 2 different surveyed locations for each protection condition.

Differences on the shallow-infralittoral benthic assemblage composition and structure between protected and unprotected locations were analysed by PERMANOVA. Results did not show any relevant effect of protection on the benthic assemblages at both Torre Guaceto and Porto Cesareo, although significant differences among locations within the same protection level and sites within the same location were revealed at both the study areas (Table 10).

Table 10, PERMANOVA results testing for the effect of protection on the shallow-infralittoral benthic assemblage at Torre Guaceto and Porto Cesareo. "Pr": protection; "Loc": location; "Si": site.

	Torre Guaceto			Porto Cesareo		
Source of Variation	**MS**	*Pseudo-F*	*P(perm)*	**MS**	*Pseudo-F*	*P(perm)*
Pr	4369,3	0,45	0,76	5173,3	0,53	0,718
Loc(Pr)	9581	11,18	0,0001	9708,4	6,41	0,0002
Si(Lo(Pr))	856,96	3,92	0,0001	1513,7	2,64	0,0001
Res	218,62			574,4		

- **Freus d'Eivissa i Formentera Marine Reserve**

Benthic species and taxa recorded during the survey at the Freus d'Eivissa i Formentera Marine Reserve are reported in the list below (Table 11).

Table 11, List of taxa and species found in the surveyed rocky-subtidal benthic assemblage at the Freus d'Eivissa i Formentera Marine Reserve.

TAXONOMIC LIST FOR FREUS D'EIVISSA I FORMENTERA MPA

Rhodophyceae
Articulated Corallines (AC)
Corallina elongata J.Ellis & Solander, 1786
Haliptilon virgatum (Zanardini) Garbary & H.W.Johansen, 1982
Amphiroa rigida J.V.Lamouroux, 1816
Falkenbergia rufolanosa (Harvey) F.Schmitz, 1897
Lithophyllum alternans Me.Lemoine, 1929*; L. incrustans* Philippi, 1837
Mesosophyllum alternans (Foslie) Cabioch & M.L.Mendoza, 1998
Laurencia obtusa (Hudson) J.V.Lamouroux, 1813
Peyssonnelia rosa-marina Boudouresque & Denizot, 1973; *P. rubra* (Greville) J.Agardh, 1851, *P. squamaria* (S.G.Gmelin) Decaisne ex J.Agardh, 1842
Tricleocarpa fragilis (Linnaeus) Huismans & R.A. Townsend, 1993

Phaeophyceae
Cystoseira montagnei J.Agardh, 1842*; Cystoseira brachycarpa* J.Agardh, 1896
Dictyotales (*D. dichotoma, D. dichotoma var intricata, D. mediterranea, D. implexa; Dictyopteris polypodioides)*
Padina pavonica (Linnaeus) Thivy, 1960
Stypocaulaceae (*Halopteris filicina* (Grateloup) Kützing, 1843); *Halopteris scoparia* (Linnaeus) Sauvageau, 1904
Cutleriales *(Zanardinia typus* (Nardo) P.C.Silva, 2000)

Chlorophyceae
Anadyomene stellata (Wulfen) C. Agardh, 1822
Cladostephus spongiosus (Hudson) C.Agardh, 1817
Codium effusum (Rafinesque) Delle Chiaje, 1829; *C. bursa* (Linnaeus) C. Agardh, 1822
Dasycladus vermicularis (Scopoli) Krasser, 1898
Flabellia petiolata (Turra) Nizamuddin
Green Filamentous Algae (GFA) *Cladophora* sp. Kützing, 1843
Halimeda tuna (Ellis & Solander) J.V. Lamouroux, 1816
Pseudoclorodesmis furcellata
Other algal groups
Dark Filamentous Algae (DFA); Cyanophiceae *(Symploca hydnoides* Kützing ex Gomont, 1892)
Invertebrata

For further analyses, the following operational taxonomic groups were considered: Erect Algae; Fucales; Invertebrates; Sand; Turf; Crustose Coralline Algae; *P. oceanica*; Rock; Filamentous Algae. The average abundance (±SE) for each considered OTU and the presence of bare rock and sand were also assessed and shown in the Figure 40.

All the sampled locations showed high abundances of the group Erected algae and Fucales; differences among the Fucales and other erect algae between sampling localities were evident, even within the same protection level. The protected zone showed, on average, higher abundances of invertebrates and *P. oceanica* than controls, while controls were characterised by a higher mean abundance of turf and sand than the protected locations.

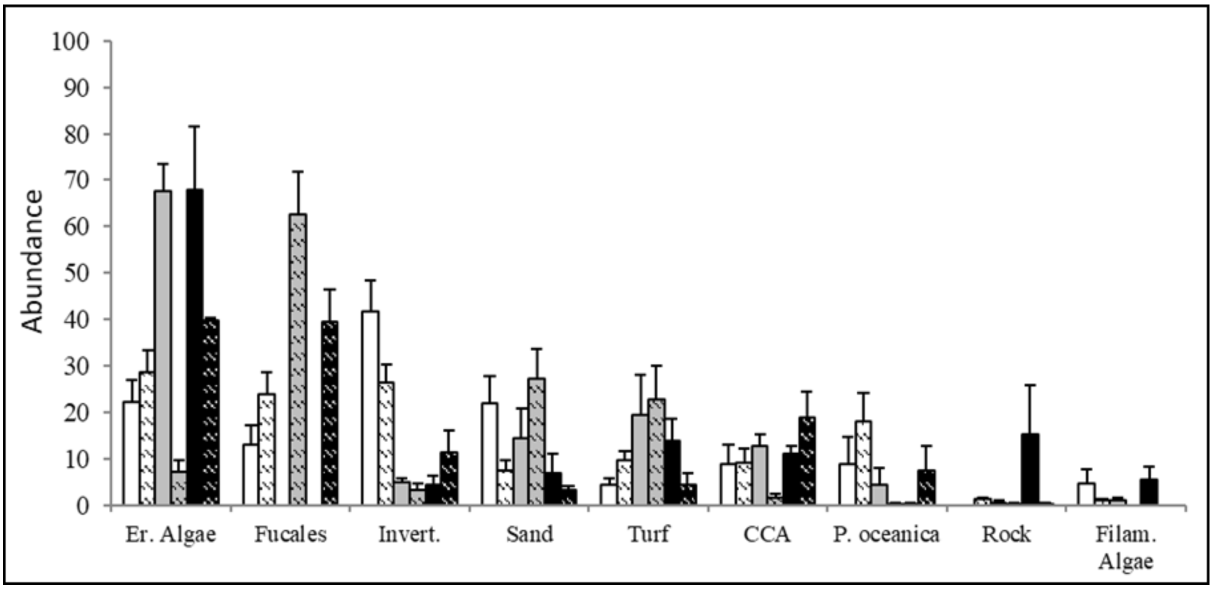


Figure 40. Average abundance (±SE) of benthic taxa revealed at both the localities within each protection level at the Freus d'Eivissa i Formentera Marine Reserve (no take zones in white; unprotected zones in grey; partially protected zones in black); the different textures indicate the 2 different locations within each protection level.

Although the bare rock reached a significant cover in one of the partially protected locations, here the patches of bare rock were small and scattered along the whole transect and no a real barren ground was observed.

Statistical analyses were carried out to test the effect of protection on each benthic taxon and the only group which showed an effect of protection measures was that represented by invertebrates (NTZ > PPZ = UPZ; $p < 0,05$), featured by the high abundance of the madreporarian *Cladocora caespitosa* (Table 11).

Table 12, Summary of univariate analysis testing for the effect of protection on the invertebrate assemblage at the FEFMR.

	FEFMR		
Source of Variation	**MS**	*F*	*P(perm)*
Pr	2145,0	11,3	0,04
Loc(Pr)	189,8	3,3	0,04
Error	57,8		

- **Coralligenous formations**

  - **Torre Guaceto and Porto Cesareo**

Taxa and species recorded during the ROV-video analysis carried out at Torre Guaceto and Porto Cesareo are reported in the Table 13, highlighting the presence of structuring taxa at both the study areas.

Table 13, List of coralligenous taxa and species at Torre Guaceto and Porto Cesareo. Structuring taxa following the MSFD framework are marked on the right column.

CORALLIGENOUS FORMATION TAXONOMIC LIST FOR TORRE GUACETO (TG) AND PORTO CESAREO (PC)					
	TG	**PC**		**TG**	**PC**
Rhodophyta			Porifera		
Articulated Corallines (AC)	x	x	*Acanthella acuta*	-	x
Amphiroa sp.	x	x	*Aplysina aerophoba* Nardo, 1843	x	x
Laurencia complex	x	x	*Agelas* sp.	-	x
Liagora viscida (Forsskål) C.Agardh, 1822	-	x	*Chlatrina* sp.	-	x
Peyssonnelia sp. Decaisne, 1841	x	x	*Chlatrina* sp.	-	x
Sphaerococcus coronopifolius Stackhouse, 1797	x	-	*Chondrilla nucula* Schmidt, 1862	x	x
Tricleocarpa fragilis (Linnaeus) Huismans & R.A. Townsend, 1993	x	x	*Chondrosia reniformis*	-	x
Wrangelia penicillata (C. Agardh) C. Agardh, 1828	x	x	*Cliona* spp.	x	x
			Encrusting Red Sponges (ERS)	x	x
Phaeophyceae			*Hemimycale columella* (Bowerbank, 1874)	x	x
Cystoseira sp. C.Agardh, 1820	x	x	*Ircinia variabilis* (Schmidt, 1862)	x	x
Dictyotales	x	x	Massive Dark Sponges (MDS)	x	x
Dumontiaceae	-	x	*Petrosia ficiformis* (Poiret, 1789)	x	x
Padina pavonica (Linnaeus) Thivy, 1960	x	x	*Phorbas fictitius* (Bowerback, 1866)	x	x
Stypocaulaceae	-	x	*Terpios fugax* Duchassaing & Michelotti, 1864	x	-
Chlorophyceae			Hydrozoa	x	-
Acetabularia (Acetabularia) acetabulum (Linnaeus) P.C.Silva, 1952	x	x			
Anadyomene stellata (Wulfen) C. Agardh, 1822	x	x	Anthozoa		
Caulerpa cylindracea Sonder 1845	x	x	*Actinia* sp.	x	x
Caulerpa prolifera (Forsskål) J.V. Lamouroux	x	-	*Balanophyllia europaea* (Risso, 1826)	x	x
Codium bursa (Linnaeus) C. Agardh, 1822	x	x	*Caryophyllia smithi* Stokes & Broderip, 1828	x	x
Colpomenia sinuosa	-	x	*Cladocora caespitosa* (Linnaeus, 1758)	x	x
Dasycladus vermicularis (Scopoli) Krasser, 1898	x	x			
Flabellia petiolata (Turra) Nizamuddin	x	x	Polychaeta		
			Calcareous Tube Worms (CTW)	x	x
Green Filamentous Algae (GFA)					
Halimeda tuna (Ellis & Solander) J.V. Lamouroux, 1816	x	x	Bivalvia		

Palmophyllum crassum (Naccari) Rabenhorst	x	x	*Gastrochaena dubia* (Pennant, 1777)	x	x
Valonia macrophysa	x	-			
			Gastropoda		
Other algal groups			Vermetidae	x	x
Dark Filamentous Algae (DFA)	x	x			
			Cirripedia		
Ascidiacea			Balanidae	x	x
Didemnidae	x	x			
Diplosoma listerianum (Milne-Edwards, 1841)	x	-	Bryozoa		
Microcosmus sp.	x	-	Encrusting Bryozoans (EB)	x	x

Further analyses were focused on the structuring taxa recorded within each video.

The structuring taxon assemblage composition and their abundance are shown in Figure 41 and Figure 42 and compared among protected and unprotected sites for each MPA. At Torre Guaceto (Figure 41), the taxon Anthozoa is the only showing a higher density at both the locations within the MPA. By contrast, a high value of abundance of the porifera *Axinella cannabina* has been recorded at one of the locations outside the MPA, the southernmost surveyed location, specifically.

Also the bryozoan *Pentapora/Smittina* complex showed on average a higher abundance at both the control locations, while the average number of the other taxa did not seem vary among protected and unprotected conditions (Figure 41).

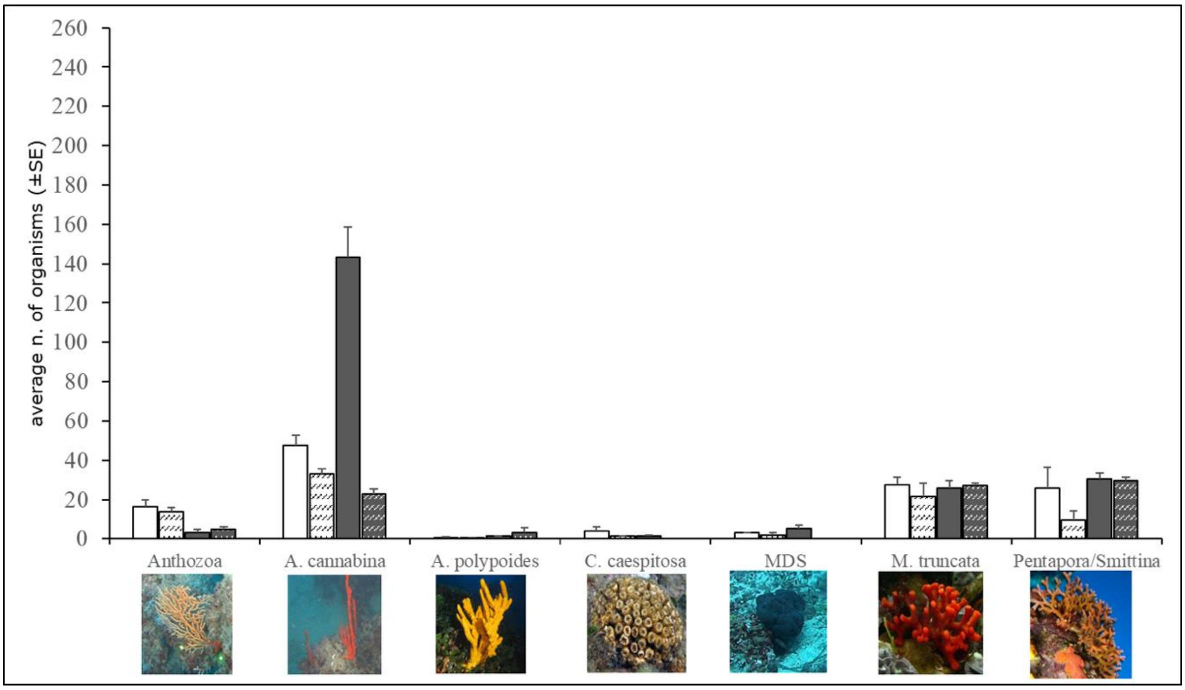


Figure 41, Average abundance (±SE) of structuring taxa among protected (in white) and unprotected (in grey) locations at Torre Guaceto. Different textures: different locations within each protection level.

Differences in the taxon composition and abundances recorded at each transect were further analyzed and no statistically significant differences were found for the factor Protection. By contrast, significant differences were revealed among locations subjected to the same protection measures (Table 14).
Univariate analyses on each single taxon revealed a different abundance among protected and unprotected conditions relatively to the taxon Anthozoa (Table 14).

Table14, Summary of PERMANOVA and univariate PERMANOVA results testing for the effect of protection on the structuring taxa abundance at Torre Guaceto. "Pr": protection; "Lo": location.

	Torre Guaceto		
Source of variation	**MS**	*Pseudo-F*	*P(MC)*
Pr	1235,8	0,35	0,68
Loc(Pr)	3576,6	7,49	0,007
Res	477,34		
	Torre Guaceto, Anthozoa		
Source of variation	**MS**	*Pseudo-F*	*P(MC)*
Pr	8687,3	32,41	0,0029
Loc(Pr)	268,06	0,4155	0,76
Res	645,23		

At Porto Cesareo analyses on the coralligenous structuring taxon assemblage revealed significant differences of composition and abundance among protected locations and external controls (Figure 42). Overall, an evident higher average abundance of structuring taxa has been recorded at both the control locations. One of the three protected locations, also, showed a higher abundance of structuring taxa compared to the others two (Figure 42).
In detail, the controls were characterized by the exclusive presence of the group Anthozoa and by the evident high abundance of *Pentapora/Smittina* complex, *Axinella cannabina* and *Axinella polypoides.* By contrast, the bryozoa *Myriapora truncata* was mainly found at one of the protected locations. A low presence of massive dark sponges and of the madreporaria *Cladocora caespitosa* was recorded, especially outside the MPA (Figure 42).
Statistical analyses revealed a significant effect of the factor protection on the community structure, in terms of taxa composition and abundance (Table 14). A high variability among locations and sites from the same protection level was also revealed. Further univariate analyses on the abundance of each single taxon were also performed and showed significant differences for the factor protection respectively to the groups *Axinella polypoides*, *Pentapora/Smittina* and Anthozoa.

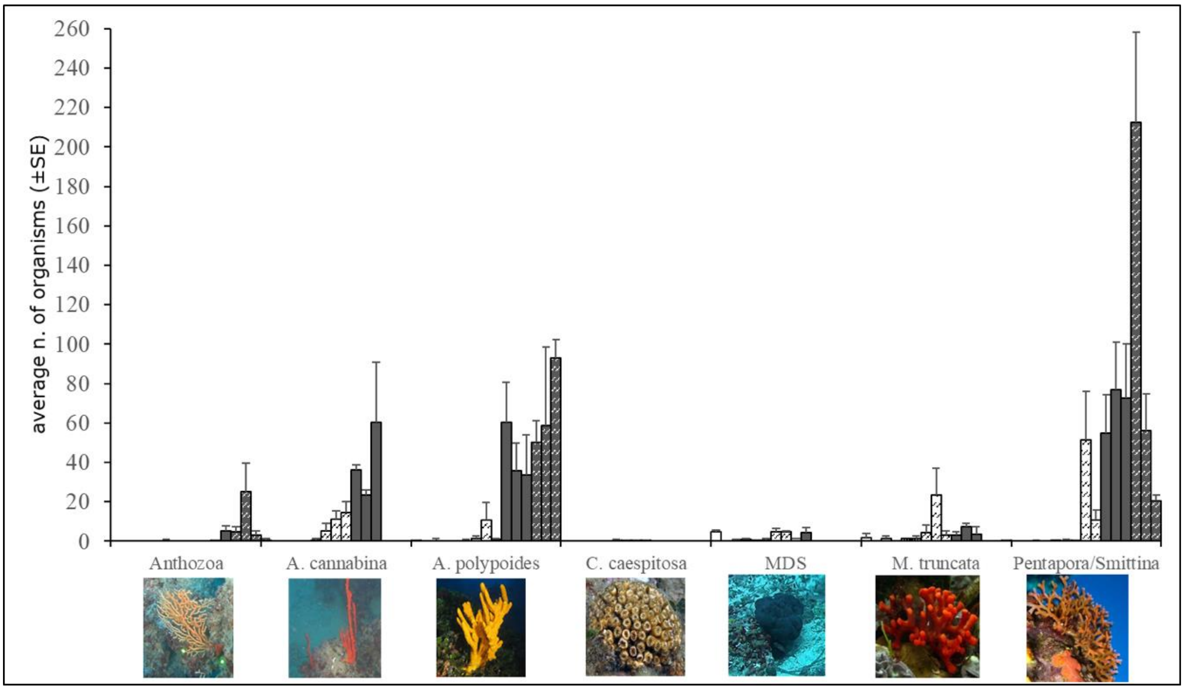


Figure 42, Average abundance (±SE) of structuring taxa among protected (in white) and unprotected (in grey) sites at Porto Cesareo. Different textures: different locations within each protection level.

Table 14, Summary of PERMANOVA and univariate PERMANOVA results testing for the effect of protection on the structuring taxa abundance at Porto Cesareo. "Pr": protection; "Lo": location; "Si": Site.

	Porto Cesareo		
Source of variation	**MS**	*Pseudo-F*	*P(MC)*
Pr	50783	6,04	0,0068
Loc(Pr)	8410,4	2,84	0,0084
Si(Lo(Pr))	2961,8	2,59	0,0004
Res	1143,3		
	Porto Cesareo, *A. polypoides*		
Source of variation	**MS**	*Pseudo-F*	*P(MC)*
Pr	60878	26,50	0,0008
Loc(Pr)	2297,1	2,86	0,05
Si(Lo(Pr))	803,03	0,82	0,65
Res	978,07		
	Porto Cesareo, *Pentapora/Smittina*		
Source of variation	**MS**	*Pseudo-F*	*P(MC)*
Pr	56575	10,73	0,01
Loc(Pr)	5273,6	2,08	0,11
Si(Lo(Pr))	2534	4,31	0,0001
Res	588,09		
	Porto Cesareo, Anthozoa		
Source of variation	**MS**	*Pseudo-F*	*P(MC)*
Pr	17895	8,97	0,02
Loc(Pr)	1995,7	1,91	0,15
Si(Lo(Pr))	1041,1	2,66	0,005
Res	391,78		

- **National Marine Park of Alonissos Northern Sporades**

Overall, compact coralligenous formations were found below 30m of depth. There, depending on the local hydrological and environmental conditions, forests of gorgonians can occur.

On each photo frame species identification was performed by visual estimation and the % cover of the structuring taxa was further analysed.

Taxa and species recorded during the coralligenous survey at the NMPANS are listed in the Table 15. Presence of structuring taxa is marked on the right.

Table 15, List of coralligenous taxa and species at the NMPANS. Structuring taxa following the MSFD framework are marked on the right column.

Taxonomic list	Structuring taxa
Rhodophyta	
Litophyllum sp./*Mesophyllum* sp.	
Peyssonnelia spp.	
Erected Corallinaceae	
Chlorophyta	
Flabellia petiolata	
Halimeda tuna	
Palmophyllum crassum	
Caulerpa sp.	
Codium bursa, C. fragile	
Dictyotales	
Padina pavonica	
Other algal groups	
Dark Filamentous Algae (DFA)	
Bryozoa	
Encrusting bryozoa	
Pentapora sp./*Smittina* sp complex	x
Chordata	
Cystodytes dellechiajei	
Halocynthia papillosa	
Cnidaria	
Eunicella cavolinii	x
Leptopsammia pruvoti	
Porifera	
Agelas oroides	
Chondrilla nucula	

Chondrosia reniformis	
ERS (Encrusting Red Sponges, Crambe/Spirastrella)	
Grey boreholing sponges (*Cliona* sp.)	
Massive Dark Sponges (MDS, *Sarcotragus foetidus*)	x
Phorbas tenacior	
Pink sponge (H. racovitzai)	
Terpios fugax	

Further analyses focused on the structuring taxa average % cover (±SE) at each partially protected surveyed site, comparing the structuring assemblage composition among different fishing pressure conditions (Figure 43).

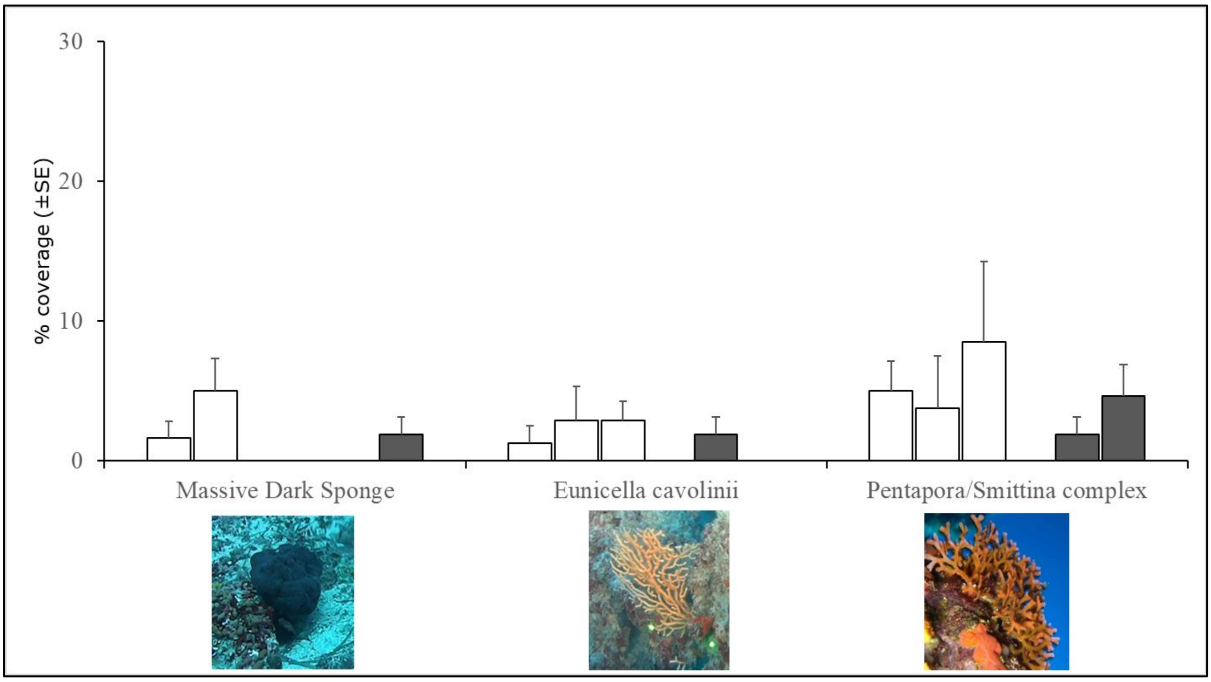


Figure 43, Average% abundance (±SE) of structuring taxa among partially protected sites subjected to a low fishing pressure (in white) and to a higher fishing pressure (in grey) at the National marine Park of Alonissos Northern Sporades.

Few structuring groups have been recorded by the photo-frame analysis. In detail, the Massive Dark Sponges, the anthozoa *Eunicella cavolinii* and the bryozoan complex *Pentapora/Smittina* were the only structuring taxa found.

Despite the low diversity of structuring taxa, their average % cover increased at the sites subjected to a lower fishing pressure, where a higher frequency of their presence was also recorded (Figure 43). Furthermore, one of the three sites exposed to a higher fishing pressure did not show the presence of any of the identified structuring taxa, highlighting a different distribution of structuring organisms at the site-scale.

These high variability among the sites was not confirmed by statistical analyses and the structure of the considered assemblages did not significant differ neither among levels of fishing pressure.

- **Marine litter survey**

  - **Torre Guaceto and Porto Cesareo**

During ROV- video analyses, information on fishing tools and debris presence at Torre Guaceto and Porto Cesareo were also recorded. Collected data are shown in the Figure 44 and Figure 45.

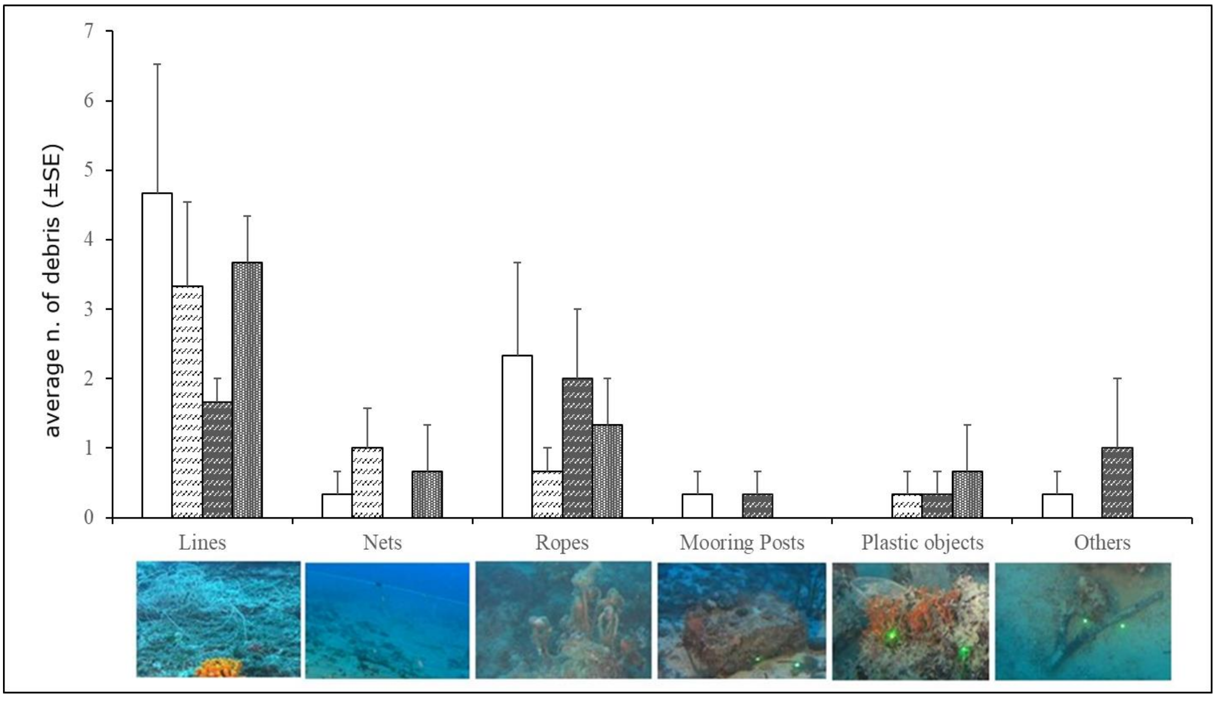


Figure 44, Average number of debris (±SE) found within the protected zone (in white) and at the external controls (in grey) at Torre Guaceto. Different textures represent different locations sampled within each protection level.

At Torre Guaceto the higher presence of lines within the protected locations was unexpected (Figure 44), since the use of this fishing tool is totally forbidden within the MPA. Furthermore, also the presence of other fishing tools at both the protected locations, nets and ropes specifically, was recorded, while plastic objects and other typology of litters are more abundant, on average, outside the MPA. However, further analyses performed did not revealed statistically significant differences in the debris composition and abundance among protected and unprotected locations.

At Porto Cesareo, a higher number of litters was found, on average, at the locations within the MPA (Figure 45). Also at Porto Cesareo fishing tools were the most representative typology of debris. Pot presence and mooring posts were recorded exclusively within the MPA, which in general showed a higher average abundance of ropes and nets. By contrast, lines were rather scarce within the MPA and most abundant at the external controls and plastic objects were common at all the surveyed areas (Figure 45).

Multivariate analysis did not reveal significant differences in the abundance of marine litter among protected and control condition, although differences among sites within the same location were found.

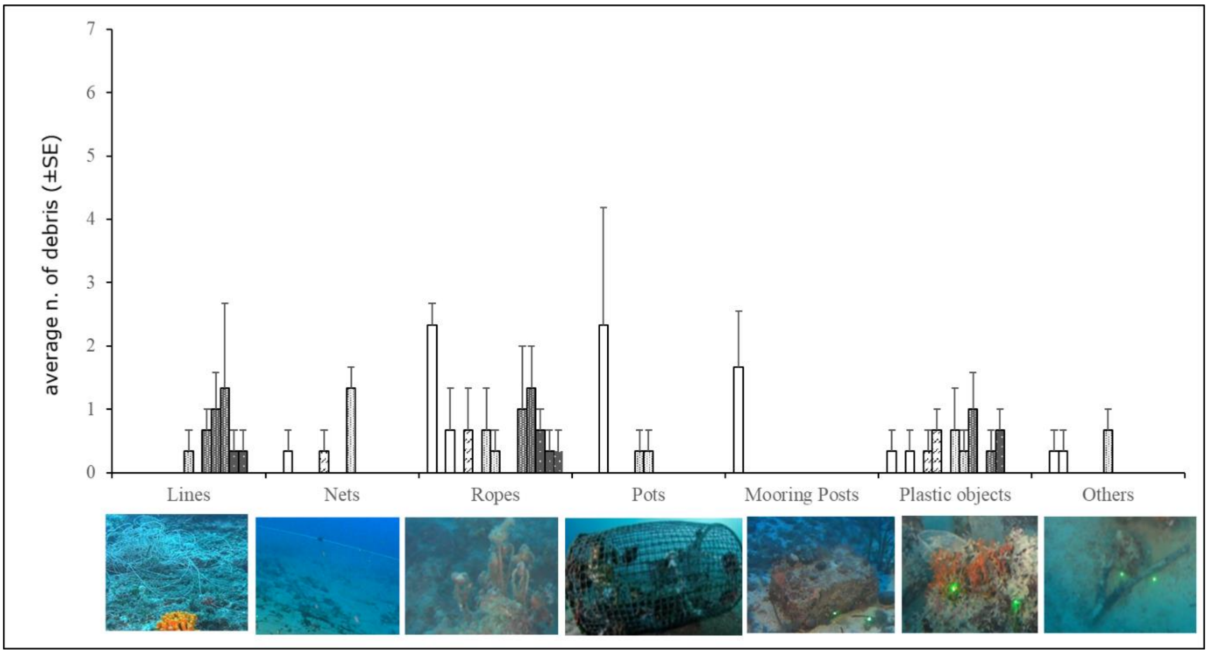


Figure 45, Average number of debris (±SE) found within the protected sites (in white) and at the external controls (in grey) of Porto Cesareo. Different textures represent different sites sampled within each protection level.

- **National Marine Park of Alonissos Northern Sporades**

Marine litter has been observed at several protected and partially protected locations at the NMPANS, during the *P. oceanica* meadow and the Coralligenous formation surveys.

At the sites where the seagrass meadow was monitored, most of the observed litter was represented by plastic bottles, soft-drink cans and other type of single use material, while at the sites with coralligenous reefs, almost all of the observed litter was abandoned fishing gear, either nets or longlines.

A description of the total abundance and typology of marine litter recorded at the NMPANS is provided by Figure 46.

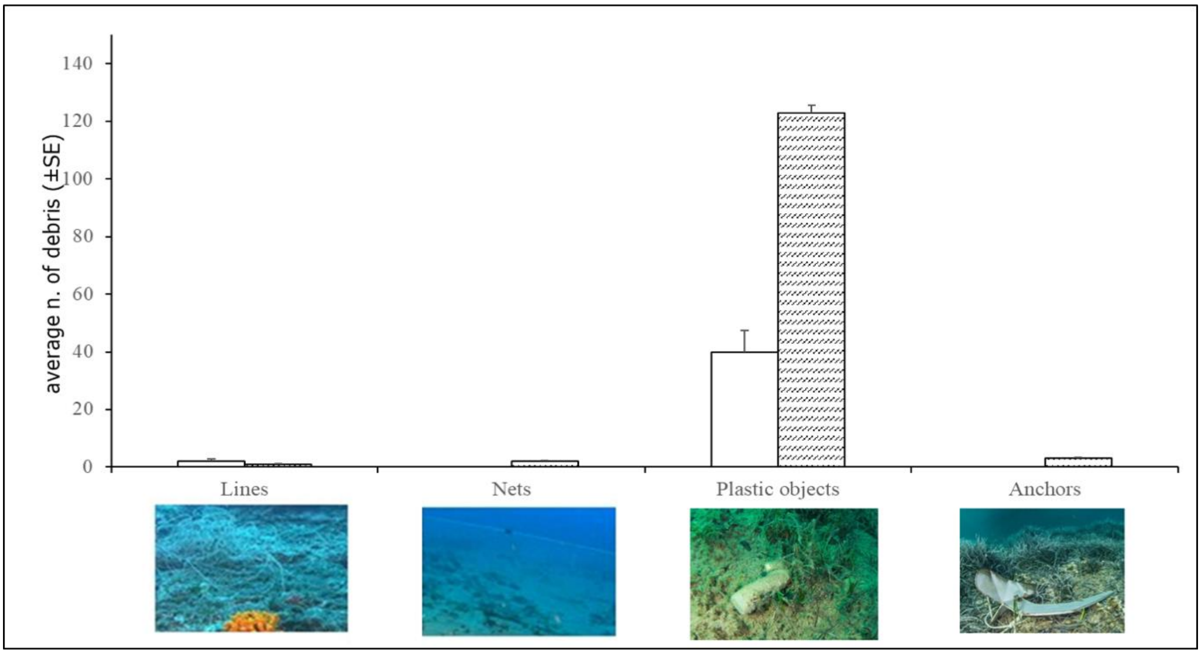


Figure 46, Average number of debris (±SE) and typology found within the protected zone (in white) and the partially protected zone (textured white) of the National Marine Park of Alonissos Northern Sporades.

A low abundance of fishing tools was found at the NMPANS, which consisted of longlines and nets. These last, moreover, were only recorded at the partially protected zone (textured white bars, Figure 46). By contrast, a high density of plastic debris, bottles and soft-drink cans in detail, was recorded, with the highest densities at the partially protected zone of the marine park. Here, anchors were also present.

Despite these differences, further statistical analyses did not reveal a significant variation of litter abundance and typology among totally protected and partially protected conditions.

- **Stakeholder engagement**

- **Torre Guaceto**

A first stakeholder workshop was organised in September 2019 by the Management Consortium of Torre Guaceto Marine Protected Area (MPA), with the support of the AMAre Lead Partner CoNISMa. The workshop was titled "Modelli di Governance e misure di conservazione per la SAC di Torre Guaceto" (Models of Governance and Conservation Measures for the Torre Guaceto SAC). A wide variety of stakeholders from the world of fishery, tourism, territorial governance, MPA personnel and from research institutions has been invited to participate.

The target of this event was to discuss the recent commitment of the SAC "Torre Guaceto & Macchia San Giovanni", instituted the 28th of December 2018, to the Management Consortium of Torre Guaceto MPA (DGR 1267, 08/07/2019).

The meeting was an important opportunity to discuss which measures suggest to the Apulian Region regarding the SAC management, incorporating the responses received from locals, concerning the following topics: the regulation, the management, the monitoring plans, the economic incentives and the educational programs.

Another relevant point addressed during the workshop concerned the status of conservation of the marine habitats and biological resources in relation to the Torre Guaceto MPA presence and to the occurrence of regulated human activities within and outside the MPA (such as: fishing, underwater tourism, boating). With this respect, the result from the monitoring activities and the field surveys carried out within the framework of the InterregMed project AMAre (Actions for Marine Protected Areas) have been shown, providing a critical contribution to reach conclusions regarding the conservation and the management models in place within the MPA Torre Guaceto.

A participatory approach among the presents was sought during the discussions, as one of the main issues to actively support effective conservation beside to a sustainable development.

Furthermore, the workshop was also an opportunity for a practical exercise focused on the formulation of priority conservation measures for the SAC management which involved all the participants.

The meeting "Marine Protected Areas" was another relevant workshop held in Bari in November 2019 and was an important opportunity for discussing with the Apulian Protected Areas (PA) Managers about the PA current status, administrative issues and criticisms. The recent research outcomes gathered from the AMAre field surveys were presented and discussed at the event showing the impact of current management plan and discussing the implementation of alternative conservation strategies and models.

A detailed description of the two stakeholder workshops is available in the deliverable 4.9.2.

- **National Marine Park of Alonissos Northern Sporades**

A stakeholder workshop was organised in Volos in April 2019, where three main topics were presented: the AMAre objectives and outcomes from the pilot surveys at the National Marine Park Alonnisos Northern Sporades (NMPANS), good practices, the stakeholder perceptions related to conservation issues and the future development of the NMPANS. A session dedicated to the ecosystem services provided by the priority habitats under study was also organised, to raise the awareness of the participants on this subject.

For boosting a participatory approach and in order to develop recommendations on spatial allocation of activities/uses based on the project outcomes, the Web-GIS technology "PPGIS" was used during the event, being an efficient tool to support a public participatory process. Indeed, the PPGIS is a smart tool for sharing of information between stakeholders and decision makers. It has been used widely in policy making and planning processes in order to disseminate the outcomes of research and projects to stakeholders, allowing them to express comments or concerns and work towards integrated outcomes. Using PPGIS techniques important information related to for example small scale fishery fishing grounds has been gathered to better understand the areas that are important for fishers' operations and at the same time evaluate fishing footprint on the habitats under study. Then, also the SeaSketch platform (www.seasketch.org), a web-based spatial data platform that addresses the technical needs arising from collaborative area-based planning workflows, has been used in the framework of the pilot activities carried out at the NMPANS in order to be accessible by local stakeholders and enable further interactions and collection of their feedbacks.

Following the event, a three-days stakeholder workshop was organized in Alonnissos between the 26 th and 28th of August 2019, in order to discuss spatial outcomes in the Greek study area of AMAre, conduct a planning exercise and make recommendations for actions that may contribute to a more efficient management of the NMPANS.

Each day a different focus group of stakeholders was invited, except for the NMPANS officials who were present during all three days. More precisely, the small-scale fishery associations, local diving centers and the marine tourism sector joined the discussions along with the NMPANS officials.

Further details on the stakeholder workshop are discussed in the deliverable 4.10.2.

Furthermore, another relevant stakeholder workshop has been organised in Malta the 4th of June as part of the of the European Maritime Day 2019 (annual two-day event during which Europe's maritime community meet to network, discuss and forge joint action). The event topic was "Safeguarding the Marine Environment Together - Bridging Conservation and Stakeholder Uses in the NE Marine Protected Area" and was organised by the Physical Oceanography Research Group (Dept. of Geosciences, University of Malta) in collaboration with Malta Marittima Agency, Malta Council for Science & Technology and AquaBioTech Group. More than 80 people attended the workshop, involved in a practical marine spatial planning exercise to investigate possible solutions to meet conservation objectives without disrupting essential economic activities. The analyses of conflicts and the possible solutions were focused on the North- East Marine Protected Area of Malta. A report detailing the main topics addressed during the workshop has also been produced and uploaded to the AMAre website.

6. Conclusions

Monitoring activities are crucial in providing fine scale information that accurately assess the status of the protected ecological components and contribute to effective decision-making in meeting conservation objectives. The provision of feedbacks to the current management strategies of the MPAs involved in the project through fine-scale field surveys was one on the main focus of the AMAre project.

This activity was motivated by the observation that the collection of fine scale data actually describing the ecological status of the marine biodiversity is often missing. Despite being recognised a crucial issue of the management plans, indeed, a direct, quantitative assessment of the conservation status of ecologically relevant ecosystems and habitats is seldom achieved by the current MPAs. Moreover, consideration of spatial information on biodiversity and human uses are needed to feed the current management plans and for the choice of adaptive solutions, as this study underlines.

The selection of the surveyed habitats was critical for the application of the ISEA Framework and for a better understanding of the potential effects such as tourism frequentation and fishery on different ecological components. The core of the pilot activities discussed in this document was on *Posidonia oceanica* meadows, the rocky subtidal-shallow infralittoral benthic assemblages and the coralligenous formations, selected as priority habitats of conservation concern largely distributed at all the surveyed MPAs. Also, the assessment of marine litter was included in the pilot activities. In addition, in order to increase the stakeholder perception on the conservation of marine habitats and their current ecological status, an additional effort focused on the organization of a number of communication events in the project study areas, aimed at involving the local community, disseminating the outcomes from the pilot activities and supplying appropriate recommendations stemming from the AMAre project achievements. These meetings represented an important opportunity for the discussion among MPA managers, scientific staff and stakeholders directly contributing to the MPA prosperity and benefit from them (e.g. from fishing and tourism sectors), that is a focal point for the implementation of an adaptive management, in order to fulfil both the ecological and the socio-economic MPA objectives.

The main conclusions about the management outcomes for each monitored target of conservation are reported below.

- *Posidonia oceanica* meadow:

At Torre Guaceto, *P. oceanica* meadow positively responds to current protection measures and this is confirmed by the significantly higher shoot density/m^2 recorded at the protected and partially protected locations compared to unprotected ones. Despite an overall trend of seagrass regression at local (Fraschetti et al., 2013) and regional scale (De los Santos et al. 2019; Telesca et al. 2015), local management of fishery and anchoring inside the MPA provides insurance against human pressures. Moreover, the analysis of the ecological status of the meadow carried out taking into account threshold values reported by Pergent et al. (1995) reveals good seagrass conditions within the MPA while a very disturbed status outside the MPA.

At Malta, the ecological status of *P. oceanica* meadow is good at all the surveyed locations, with values of shoot density recorded at all the surveyed sites corresponding to not disturbed meadows. Protection from anchoring and urbanization shows an effective enhancement of the seagrass conservation status at the surveyed protected locations with a significant increase of the shoot density.

P. oceanica meadow at Porto Cesareo does not differ among protected and unprotected locations, for both the surveyed depth conditions. Management measures currently in force within the MPA do not seem to enhance the seagrass density compared to unprotected conditions. However, it has to be stressed that no site (within or outside the MPA) shows very disturbed conditions. The analysis of the meadow ecological status following Pergent indications shows a prevalence of sites in which *Posidonia* is not disturbed at shallow depth while disturbed conditions were observed in the meadows at deeper sites. Furthermore, the northernmost part of the surveyed area is characterized by a seagrass density identified as “not disturbed”, in spite of the southern sites which exhibit, on average, lower densities. Despite spatial information about fishery activities are available, no clear relation with fishery intensity was possibly found.

At the Freus d’Eivissa i Formentera Marine Reserve *P. oceanica* conservation status appears good everywhere and no sign of regression, such as extensions of the dead matte or occurrence of clearing areas within the meadow, has been reported by the surveys. Overall, the seagrass meadow is in undisturbed conditions. The seagrass shoot density at the no-take zone of the MR and outside its boundaries is higher than at the partially protected zone. Current regulation of activities at the partially protected zone should consider this result, although no specific pressure has been recorded here (e.g. water quality, anchoring).

At the National Marine Park of Alonissos Northern Sporades, the conservation status of *P. oceanica* is highly influenced by touristic frequentation. Overall, most of the surveyed sites which showed a bad ecological status of the seagrass meadow host anchoring, while locations with limited natural access or within the totally protected zone of the park show a good or excellent ecological status. The only site within the protected area of the marine park where *P. oceanica* meadow was found very disturbed, is actually used as temporary anchorage location by visiting boats during summer and, also during the field work, more than 10 boats were anchored there. Moreover, additional surveys reported in Table 7 revealed that the shallower meadows (until 15 m of depth) are the mostly affected by human pressures, such as anchoring.

- Rocky subtidal- shallow infralittoral benthic assemblages

As widely reported, benthic assemblages may exhibit different ecological responses to protection, since a wide range of factors are largely responsible for their structure. In the present study, no direct responses of benthic assemblages to protection measures have been revealed at the surveyed MPAs, accordingly to other authors (Sala et al., 2012). Both at Torre Guaceto and Porto Cesareo no effect of protection on benthic assemblages was shown and a high variability of abundance of specific benthic taxa across scales was found at both MPAs. Invasive macroalgae do not represent a major pressure since a negligible abundance of *Caulerpa cylindracea* and *C. prolifera* were observed at all surveyed locations, despite shallow water assemblages in the Mediterranean are largely affected by their presence (Klein et al., 2005). Previous studies in Porto Cesareo showed significant differences in the structure of assemblages according to different combinations of threats, indicating distinct responses of marine habitats to different sets of human pressures (Guarnieri et al. 2016). A more complex three-dimensional structure, higher taxon richness and β-diversity characterized assemblages subject to low versus high levels of human pressure, consistently across habitats. The main drivers of change were: closeness to the harbour, water quality, and the relative extension of beaches. Overall, the results we obtained are in line with recent analyses showing that subtidal rocky reefs among the most impacted habitats across the Mediterranean Sea, stressing the need for prioritizing conservation initiatives on these productive and diverse environments since actual measurements are apparently not efficient enough.

At the Freus d'Eivissa i Formentera Marine Reserve the no take zone of the MR is characterized by a higher abundance of invertebrates. In addition, canopy-forming algae of the genus *Cystoseira* sp. and *Sargassum* sp. were found abundant at one of the partially protected locations and at one of the external controls. This is an important result considering present indications of canopy loss across the Mediterranean Sea (Fabbrizzi et al. 2020). The high abundance of *Cystoseira* sp. is considered an indicator of good environmental status and is described at sites with good water quality and without overgrazing (Ballesteros et al., 2007; Thibaut et al., 2005; Pinedo et al., 2007). This is conducive to suppose a good environmental condition both at the surveyed locations within the partially protected area of the FEFMR and at the control locations.

The presence of canopy forming algae at the two Italian MPAs is negligible for different reasons. On temperate rocky reefs one of the most frequent and persistent regime shift is represented by a transition from macroalgal-dominated habitats to barren grounds as a result of sea urchin overgrazing. Anthropogenic stressors have been demonstrated having a crucial role in trigger and maintain the shift from one state to the other because of the onset of feedback processes able to erode resilience of desirable macroalgal beds while strengthening resilience of urchin barrens. The depletion of populations of sea urchin predators by overfishing has been invoked as the main driver leading to kelp beds collapse in different regions of the world. Overgrazing was discussed as a critical factor in Porto Cesareo, impairing the recovery of the system and requiring focused actions to restore canopies that were originally abundant in the MPA (Guarnieri et al., submitted). In Torre Guaceto, high sedimentation rates have been identified as a relevant factor to drive the system from canopies to turf. Also in this case specific interventions should be implemented to achieve a higher effectiveness of protection, in line with an integrated approach to the management of marine resources (EBM, ecosystem-based management, Katsanevakis et al., 2011).

- Coralligenous formations

At Torre Guaceto, a complex pattern was observed with Anthozoa showed higher densities at both the locations within the MPA, *Axinella cannabina* with higher density at one of the locations outside the MPA and the bryozoan *Pentapora/Smittina* complex with higher abundances at both the control locations. Most of the other taxa did not seem vary among protected and unprotected conditions.

The surveyed locations, indeed, are exposed to a gradient of fishing activities as the fishing effort map reports (Figure 46). Thus, the obtained results might be driven by the different intensity in the fishing effort, that has been reported to be lower in the southern control location and in the northern protected location.

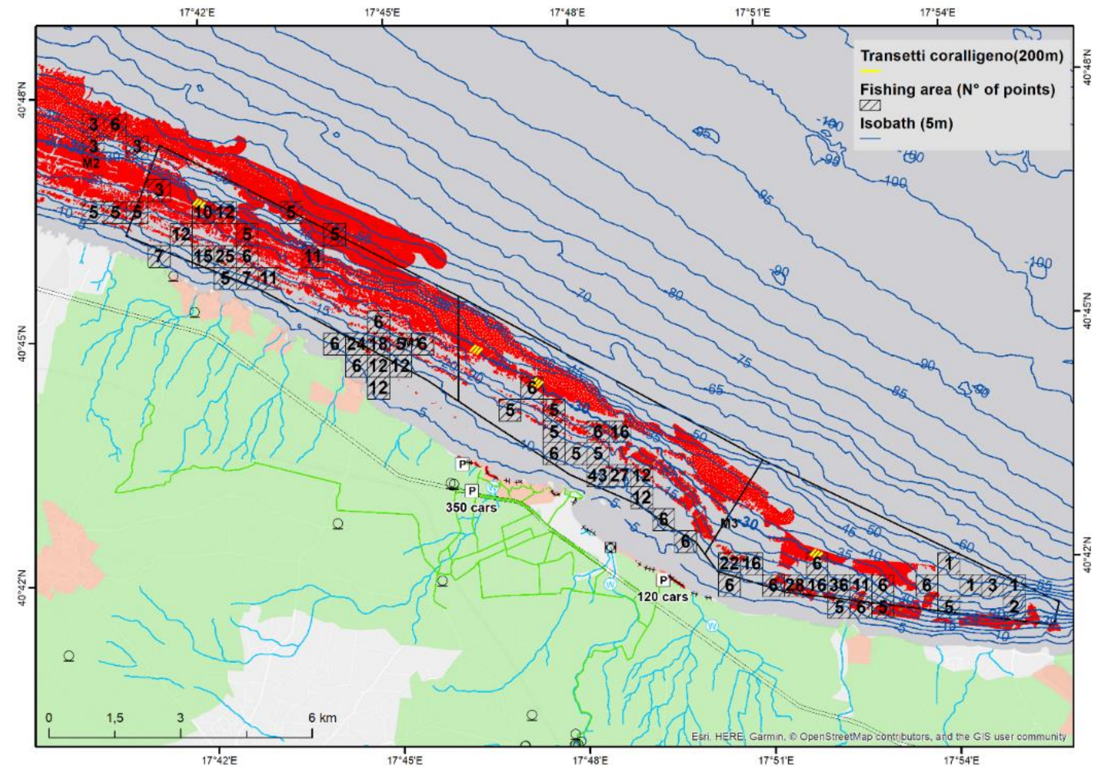


Figure 46, Fishing effort map for the area of Torre Guaceto. The numbers in the cells show the fishing effort intensity. The yellow lines represent the video transects run by ROV.

An interesting result considering the mass mortalities events recorded across the Mediterranean Sea (Garrabou et al. 2019), is that no bleaching or necrosis signs on the structuring organisms have been reported in the whole surveyed area.

Survey at Porto Cesareo revealed an unexpected picture of the coralligenous formations. Both the control locations host an evident higher abundance of structuring taxa. Benefit from current protection measures seems to be limited on this habitat and an effort should be carried out to obtain a fine scale mapping of human pressures inside and outside of the MPAs to design measures, priorities, and decisions with respect to local needs.

Illegal fishing activities also occur within the MPA at a wider scale than that officially recognised and allowed, representing a real threat for the conservation of the coralligenous formations. More enforcement would be necessary to control this activity, beside of supporting management strategy through a participatory approach of local fisherman cooperatives.

Also at Porto Cesareo no bleaching or necrosis signs on the structuring organisms have been reported.

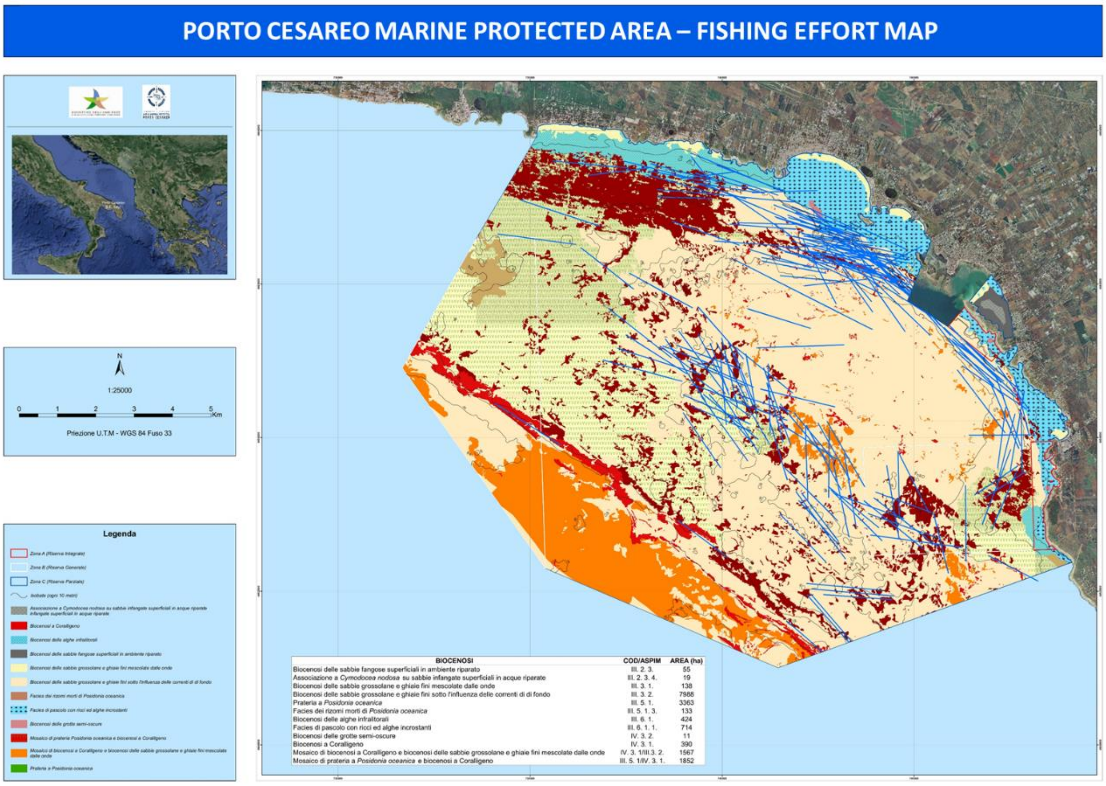


Figure 47, Fishing effort map at Porto Cesareo MPA. The blue lines represent the distribution of the fishing effort.

At the National Marine Park of Alonissos Northern Sporades the average % abundance of structuring taxa was higher at the sites under a lower fishing pressure. Furthermore, one of the three sites exposed to the higher fishing pressure does not show the presence of any of the identified structuring taxa, highlighting the effect of fishery on this habitat.

- Marine Litter

Marine Litter survey at Torre Guaceto revealed a consistent presence of fishing tools within the MPA, specifically nets, ropes and lines. The presence of lines within the MPA was unexpected, since their use is totally forbidden within the boundaries of the MPA. This data supports the occurrence of illegal fishing activity within the protected zone and the need to increase the enforcement measures.

Furthermore, although plastic objects are more abundant outside the MPA, entanglement by a plastic bag was recorded also within the MPA, even though for just one individual belonging to the taxon *Pentapora*/*Smittina*.

At Porto Cesareo, plastic objects were common at all the surveyed locations. Fishing tools were higher within the MPA compared to the external controls. Also, pot presence was recorded exclusively within the MPA and entanglement by a line was reported limitedly to one individual of the species *Axinella polypoides* in one location within the MPA. The higher presence and frequency of marine litter recorded within the two MPAs compared to external controls highlight the necessity to include this threat within the management plans and implement measures to monitor its influence on the ecological status of the marine resources.

At the National Marine Park of Alonissos Northern Sporades litter presence was recorded at several locations during the field surveys and the plastic represented the majority of the litter found. More specifically, at sites where *P. oceanica* meadow surveys were conducted, plastic objects were among the most frequently observed litter. At sites where coralligenous formations were monitored, almost all of the observed litter was abandoned fishing gear, either nets or longlines.

Considering the marine litter data collected at the three MPAs, other than the accidental loss of fishing tools, another important contribution derives from the not sustainable practice to discard damaged gears at the sea, that continue to fish (such as in the case of ghost fishing), representing a risk of entangle for marine organisms, especially for sessile species such as those belonging to the coralligenous formations.

Raising awareness of fishermen on this very important issue, in order to influence behavioral change and prevent such practices, should be included in the activities of knowledge transfer/raising awareness conducted by the MPA Management authorities. In addition, promotion of activities related to the removal of discarded gears and other kinds of marine litter to produce products from recycled fishing gears is currently considered as a promising option in the frame of the circular economy agenda strongly endorsed by the recently adopted EU Plastics Strategy, in line with the EU Circular Economy Action Plan.

7. Recommendations

From the survey carried out across the AMAre surveys the following recommendations are provided:

1- **Fine scale data on biodiversity within and outside MPAs are needed to assess their performance to reach biodiversity targets**.

Little is known on whether management measures are implemented in a large proportion of MPAs and if they are, whether they are effective to reach stated conservation targets: good ecological and environmental data collected at appropriate spatial and temporal scales are simply missing. More investments are needed on mapping the distribution and status of ecosystems, habitats and species and setting observation platforms to improve our knowledge of biodiversity, abiotic variables and ecosystem functioning. Monitoring only inside MPAs is not enough. AMAre introduced new knowledge about the status of several habitats under protected versus non-protected conditions with a full recognition of the importance of the context in which each MPA is implemented.

2- **Systematic reporting about human uses and refinement of threat assessments within and outside MPAs will enhance management and conservation capacity**.

A fine scale mapping of human pressures inside and outside of the MPAs is also critically needed to design measures, priorities, and decisions with respect to local needs and GES of protected biodiversity. AMAre is showing that the achievement and maintenance of good ecological conditions requires fine scale data of human activities and their ensuing pressures to marine ecosystems. Cumulative effects assessment models could be of crucial help, only if based on sound and representative data linking the status of ecosystems to the cumulative level of human pressure.

3- **Implementation of integrated land-sea conservation and management.**

An integrative approach should be adopted when managing MPAs. Integrated conservation planning allows to meet conservation targets more efficiently, to account for human uses occurring on land affecting marine habitats. AMAre showed the importance of assessing land-sea interactions for the implementation of effective management plans.

4- MPA managers must work closely with other actors that use space in the vicinity of their MPAs, with effects on the status of biodiversity within their MPA.

This calls for effective implementation of all environmental legislations, such as the Birds and Habitat Directives, the MSFD, and the MSP, at all relevant scales. AMAre showed the importance of starting a dialog with different stakeholders for extending governance models and conservation measures also to Sites of Conservation Interest / Special Areas of Conservation often totally lacking management and external to the MPAs.

5- **MPA managers should better coordinate their activities across MPAs.**

At present, a real coordination among MPAs is rarely occurring while synergies in management, monitoring and conservation tools across MPAs represent an opportunity to better manage human pressures in all marine spaces simply using current legislation. Despite success stories are surely ongoing, AMAre showed the difficulties in the interactions with and among Management Bodies. More interactions are needed to put in place more robust collaborative framework.

6- **Improving data availability and accessibility.**

Coordinated monitoring and management require coordinated data collection and a common data infrastructure to ensure that effectively accessible and comparable information is shared across managers and policy makers but also with the public, supporting the production of consistent evidence-based messages valuable for different stakeholders. The Spatial Geoportal developed in AMAre goes in this direction. The AMAre approach consists of building a common and standardized relational spatial database. The AMAre Geodatabase collects and manages in a coordinated manner all the spatial data and the related information. Using this tool, all MPA managers spoke the same standardized language and for the first time, they can access the available information in the area of interest and in the other MPAs.

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