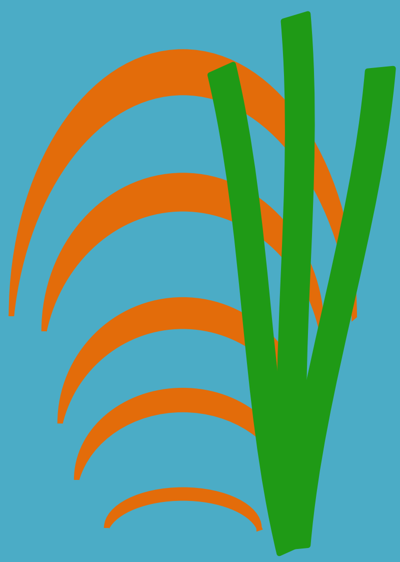
PROPOSAL OF ACTION PLAN FOR *Pinna nobilis* IN THE MEDITERRANEAN SEA IN THE FRAME OF THE MARINE STRATEGY FRAMEWORK DIRECTIVE (MSFD)



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May 2017

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PROPOSAL OF ACTION PLAN for *Pinna nobilis* in the Mediterranean Sea

1. INTRODUCTION AND BACKGROUND

The **Marine Strategy Framework Directive** (MSFD) is a European Directive aimed at achieving or maintaining **Good Environmental Status** (GES) in European seas. The aim of the EU Marine Strategy Framework Directive 2008/56/EC (MSFD) is to protect the marine environment across Europe. The MSFD was adopted in June 2008 and is the environmental component of Europe's Integrated Maritime Policy. The directive sets a target of "Good Environmental Status" which must be achieved in EU marine waters by 2020. Following the first cycle of management which ends in 2020, new programmes of measures will be set on a six yearly basis. The marine strategies to be developed by each Member State must contain a detailed assessment of the state of the environment, a definition of "good environmental status" at regional level and the establishment of clear environmental targets and monitoring programmes. Each Member State must draw up a programme of cost-effective measures to attain good environmental status. The MSFD complements the Water Framework Directive, extending environmental protection into EU marine waters beyond the coastal waters.

In the context of the European project: "Action Plans for Integrated Regional Monitoring Programmes, Coordinated Programmes of Measures and Addressing Data and Knowledge Gaps in Mediterranean Sea" (**ACTIONMED**), the main objective of this project is to support the development of regional **action plans and best practices for integrated monitoring programmes**, coordinated programmes of measures and the filing of data gaps for Mediterranean marine waters and thereby improve implementation in the next steps of the MSFD, in particular in relation to the review of the initial assessment, the GES definition and the environmental target setting in 2018, including (financial) planning as well as the related integration of data management. In particular, a) to tackle the most relevant issues at a regional level, so as to enable a systematic solution of current gaps and shortcomings in relation to Articles 8, 9 and 10 of the MSFD and building on ongoing regional and EU work; b) to develop strategies/methodologies for ongoing assessment of Biodiversity indicators; c) to set the basis for coherent design and implementation of MSFD monitoring programmes.

In marine waters, **coastal areas play an important role as biodiversity hotspots** (Coll et al. 2010), however they are vulnerable ecosystems suffering from degradation and habitat disruption that severely compromise sessile marine species. Monitoring key-species within infralittoral bottoms is essential to assess Good Environmental Status (GES) within Descriptor

1: biodiversity. The benthic filter feeder *Pinna nobilis* is an endemic, sessile vulnerable species and the largest bivalve in the Mediterranean basin which response to human derived activities (anchoring, sewage, tourisms, and coastal infrastructures, among others). Consequently, this endemic species can be used as a suitable bioindicator species to monitoring activities at different locations under a gradient of pressures. The **Mediterranean Sea** is facing increasing human pressures in coastal areas and monitoring and assessment is a high priority. Thus, short- and medium-term **actions plans** focusing on determining densities, spatial distribution and population dynamics of benthic invertebrate species such as *Pinna nobilis* at certain habitats will help to identify reliable indicators, parameters and thresholds, in order to provide GES at Mediterranean local, regional and basin scales.

The present proposal is aimed to provide guidelines in order to evaluate *Pinna nobilis* populations across the Mediterranean Sea. Specifically, we present here a case study in western Mediterranean, to use as an example, in which pen shells populations has been studied and reference values and thresholds are proposed for the studied area.

1.1. Key protected benthic species *Pinna nobilis*: a bioindicator for MSFD

The key species ***Pinna nobilis*** Linnaeus 1758 is the largest **endemic** bivalve in the Mediterranean Sea (Zavodnik et al., 1991), living up to 27 years (Galinou-Mitsoudi et al. 2006, García-March & Márquez-Aliaga, 2008) at depths ranging from 0.5 to 60 m (Butler et al., 1993). Its main habitats are soft sediments overgrown by seagrass meadows of *Posidonia oceanica* or *Cymodocea nodosa* (Zavodnik *et al*., 1991), but also in estuarine areas without vegetation (Addis *et al*., 2009) and unvegetated soft bottoms of marine lakes (Katsanevakis, 2005). In these habitats the pen shell acts as habitat forming species by adding complexity and heterogeneity to the ecosystem. Research has been conducted in investigating threats and bivalve response to anthropogenic pressures (Alomar et al., 2015; Deudero et al., 2015; Hendriks et al., 2013; Vázquez-Luis et al., 2015a). Human pressures on this species involve habitat degradation, illegal trawling, coastal construction, boat anchoring, pouching and pollution (Katsanevakis 2005; Basso et al., 2015). There is scientific evidence that this benthic filter feeder is a good indicator of changes in marine ecosystems providing information of biotic response to anthropogenic pressures (Alomar et al., 2015).

Human stressors have explained variability in density and spatial distribution of *Pinna nobilis* communities; and habitat protection affect positively *Pinna nobilis* structure (Deudero et al., 2015). In addition, physical aggression due to anchoring highly impact densities of the filter feeder in western Mediterranean Sea (Deudero et al., 2015; Vázquez-Luis et al. 2015a; Hendriks et al, 2013). On the other hand, environmental variables were seen to play a secondary role, indicating that global change processes are not as relevant in coastal benthic communities as human-derived impacts (Deudero et al., 2015). However, in MPAs without human activities environmental variables modulate densities of *P. nobilis* (Vázquez-Luis et al. 2014b). Stable isotopes analysis techniques (SIA) have been used to trace response of *Pinna nobilis* to environmental (nutrients and chlorophyll-a) and anthropogenic variables (boat traffic, anchoring allowance, waste discharges, distance to the nearest harbor and protection status) (Alomar et al., 2015). Results from this investigation demonstrated again that *Pinna nobilis* were more sensitive to human pressures and protection status of the sampling area rather than nutrients and chlorophyll-a. In addition, individuals from eutrophic environments showed

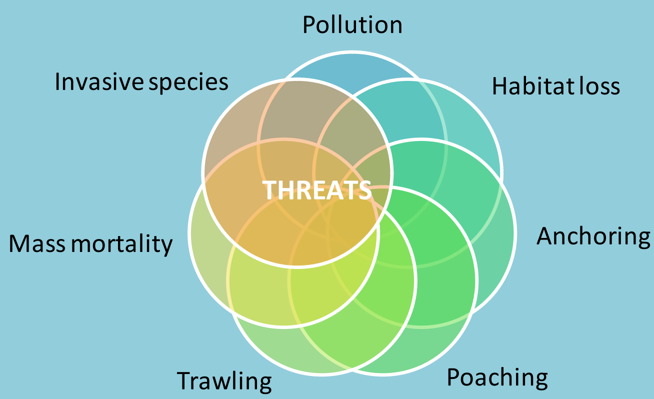
The protected bivalve Pinna nobilis, a bioindicator species acting as a good proxy to asses GES in benthic coastal ecosystems higher growth but lower survival rates than *Pinna nobilis* from oligotrophic environments. This investigation also demonstrated that pelagic food sources have a higher contribution to *Pinna nobilis'* diet than benthic sources. Waste discharges include soluble particles which dissolve in the water column and *Pinna nobilis,* as an important filter feeder contributing to water clarity, could be filtering organic and inorganic particles in impacted areas leading to physiological implications (Davenport et al. 2011, Trigos et al. 2014).

***Pinna nobilis* Linnaeus, 1758**

<u>Common name</u>: fan mussel, noble pen mussel

<u>EU protected status</u>: EU Habitats Directive 92/43/EEC, Bern Convention, Barcelona Convention Protocol

- Largest bivalve in the Mediterranean Sea
- Size up to 120 cm length
- Long life span, up to 30 years
- Sessile benthic species
- Depth range 0.5-60 m
- *Posidonia oceanica* beds preferred habitat
- Key protected species in seagrass ecosystems
- Filter feeder
- Hermaphrodite



1.2. Defining Good Environmental Status (GES) measurements for *P. nobilis*

Metrics associated to monitoring, evaluation and assessment of GES are needed within the existing controversy of methodologies. Given the scientific evidence that MPAs effectively maintain endemic *Pinna nobilis* populations and that this filter feeder can provide information on their interaction with human derived activities, this benthic invertebrate could be thought of a reliable indicator for GES within Descriptor 1: biodiversity. Common methodologies, metrics and parameters must be developed to establish reference values to determine GES. In this sense, densities and size structure of a sessile benthic invertebrate can be an optimum first approach to set up the baseline values, and population dynamics can be appropriate for long term monitoring. Additionally, other metrics including biological parameters and human variables upon the study area and species should be monitored.

1.2.1.The role of MPAs in GES

Marine Protected Areas (MPAs) are aimed to conserve and preserve biodiversity and natural habitats based on the protection of the environment from anthropogenic impacts and pressures which ensure rare and vulnerable species maintaining genetic and/or species diversity, thus preserving the structure and function of the ecosystem (Daily et al., 2000; Libralato et al., 2010). Amongst these vulnerable species *Pinna nobilis* has been widely investigated in MPAs (Vicente et al. 1980, Vázquez-Luis et al., 2014a, b; Alomar et al., 2015; Deudero et al., 2015, Prado et al. 2014, García-March et al. 2016). Part of those studies took into account environmental variables on the density of living individuals of *Pinna nobilis* including depth, slope, wave exposure habitat type and heterogeneity, as well as MPA zoning. As a rule, density and size of *P. nobilis* individuals within MPAs are higher when compared with non protected areas. MPAs which contain optimal conditions for development of *P. nobilis* populations are pristine areas appropriate to study the species without impacts and threats; and therefore can be used to provide the optimum parameters of the species = set up Good Environmental Status (GES).

Density and sizes of Pinna nobilis individuals within MPAs are higher than in non protected areas

1.2.2. The role of stressors in GES

The way environmental conditions and population processes determine the abundance and distribution of species is a central problem of ecology and biogeography (Brown 1984). Usually, variation in population density is a combination of several factors, such as spatial variables that affects distribution of organisms. Human derived stressors including globalization and international trade, climate change, aquaculture, shipping and transportation are responsible for the spread of invasive species (Streftaris et al., 2005; Occhipinti-Ambrogi, 2007) and for the modification of natural and local communities. In this regard, human stressors might guide and modify population traits in benthic species. Monitoring environmental and anthropogenic variables will help to understand differences in *Pinna nobilis* populations among sites, since it has been observed that a gradient of anthropogenic pressure influence densities around Balearic Islands (Hendricks et al. 2013, Deudero et al. 2015). Areas exposed to important tourist development, coastal human activities and sewage discharges from adjacent urban coastal areas exhibit lower densities of *Pinna nobilis* than pristine MPAs (Alomar et al. 2015). Previous studies already pointed out responses of *P. nobilis* densities to fishing mortality was much higher than natural mortality acting as important driver of the spatial distribution of this species and population viability (Katsanevakis 2007). This species is even served as seafood in restaurants (Katsanevakis et al. 2011). Regarding boating activity, anchoring in coastal waters has been shown as one of the main factors influencing *P. nobilis* population densities (Hendriks et al. 2013; Vázquez-Luis et al. 2015a), as well as boat propellers in shallow populations (Prado et al. 2014). In addition, *P. nobilis* provides a hard substrate surface susceptible to be colonized by other (flora and fauna) benthic species, including invasive species (Vázquez-Luis et al., 2014b; Banach-Esteve et al., 2015). The native epibiontic community on *Pinna nobilis* shells, has shown high variability in the number and cover of species, and richness with diversity index decreasing with the increase of invasive seaweeds, such as the red macroalgae *Lophocladia lallemandii* cover (Banach-Esteve et al., 2015).

On the other hand, environmental stressors such as hydrodynamic processes and storms also influence *P. nobilis* population. García-March et al. (2007) noted that selective pressures regulate *P. nobilis* population parameters, producing a trade-off between hydrodynamics and shell size and orientation at different shore types and water depths. In shallow populations living at 6 m depth hydrodynamic forces killed large individuals (García-March et al. 2007). Hendriks et al. (2011) demonstrated that *P. oceanica* meadows provide shelter from hydrodynamic forces to *P. nobilis*, most appreciable for seagrass meadows located in shallow waters where the smaller animals remain within the canopy. However, in the Gulf of Oristano, wave action was not a significant factor influencing the orientation of the *P. nobilis* shell due to the low hydrodynamic characteristics of the area (Coppa et al. 2013). These authors demonstrated that bottom current direction and speed have a greater influence on the spatial density patterns of the bivalve compared to exposure to waves. Sheltered bays have much lower hydrodynamics favoring recruitment of larvae and enhancing *Pinna nobilis* population which is the case of St Maria, a fully protected sheltered area (located inside Cabrera MPA) hosting the highest densities in the Mediterranean. Instead, the locality of Pollença which is also a sheltered bay exhibits similar densities as Magalluf (exposed area) probably due to the anchoring effect of boats (Hendricks et al., 2013). Deudero et al. (2015) revealed that densities of *P. nobilis* are affected by human stressors more than environmental variables. Therefore, these are an example in which a natural area with optimum characteristics to enhance densities of *Pinna nobilis* suffers a reduction due to anthropogenic effects.

Both, environmental and human stressors must be taken into account since values of GES will be obtained from pristine areas without human stressors, but the potentiality of the populations will be conditioned by environmental factors which modulate distribution, density and sizes of *P. nobilis* in a given area.

Human and environmental stressors determine the abundance and distribution of Pinna nobilis

1.2.3. Applying metrics for GES assessment

Basic metrics including *Pinna nobilis* densities according to habitat type and depth as well as biological parameters are required to define GES. However, when developing parameters and thresholds for GES besides the basic metrics on key species *Pinna nobilis* and biological parameters, metrics including protection status and anthropogenic pressures of the study area, environmental variables, habitat characteristics and sampling strategies along with spatial and temporal information should be also considered. Each sampling on *P. nobilis* should include a subset of data, whenever is possible, for a better understanding of patterns of distribution of the pen shell. Collecting these variables will allow comparisons along the Mediterranean Sea and then will help to better define GES in a near future:

- **Data on Species:** Species name; Number of individuals sampled; Valves status (dead/alive); Valves position (vertical/lying down); Shell width (cm); Length width (cm) when possible; Number of individuals per sampling unit; Mean density (ind/100m^2); Percentage cover by invasive species (%).
- **Spatial information:** Latitude and Longitude of sampling point; Country; Area, location, site; Replicate; Sampling design; Area typology; Sampling distances among transects (m).
- **Temporal information:** Date, year, season; Sampling frequency.
- **Anthropogenic variables:** Anchoring allowance; Diving; Sewage input; Fishing activities; Fishing gear; Poaching.
- **Environmental variables of the study area:** Sediment granulometry; Nutrients; Turbidity; Chlorophyll-a; Salinity, Sea temperature; Mean wave height, Maximum wave height, Mean wave period, Direction of wave.
- **Habitat Characteristics of the study area:** Habitat type (sand, *P. oceanica* seagrass, *C. nodosa* seagrass, detritic, rocky…); Habitat composition (% seagrass, % sand); Seagrass coverage; Depth.
- **Protection status of the sampling area:** Protection level (fully protected, partially protected, not protected); Protection status (Marine Protected Area, Natural Park, Site of Community Importance…); sampling conducted Before/After establishment of protection; Years since establishment of protection.
- **Sampling methodologies:** Sampling strategy used (visual census, scuba diving, cameras towed…); Sampling method used (linear transects, quadrants…); Size of sampling unit (width x length); Sampling unit area (m^2); Scientific purpose of the sampling (Aim of study: *Pinna nobilis* study, benthic study, fish study…).

Basic metrics (i.e. density, size…) provide essential information to define Pinna nobilis distribution and determine GES

1.2.4. Enlarging the spatial extent calibration of the GES

Previous studies have focused in the Western Mediterranean and enlarging the dataset on reference values according to *Pinna nobilis* is needed to calibrate GES in other parts of the Mediterranean Sea. A wider geographically and temporal database must be analyzed in order to provide more accurate values of GES and thresholds, by taking into account possible biogeography differences. This would provide comparable and reliable scientific data available for the scientific community and stakeholders in the determination of GES required for the management and mitigation of derived human impacts upon benthic species, especially from sessile, long-lived and highly vulnerable species such as *Pinna nobilis*. For further analyses in this topic is important to remark that variability in data gathering according to all parameters exists highlighting the importance to coordinate and standardize sampling methodologies and data processing.

Need to increase sampling localities to search for global patterns

2. REFERENCE VALUES OF GES FOR *Pinna nobilis* POPULATION

In order to establish thresholds and reference values geographic variations among densities and spatial distribution on *P. nobilis* across the Mediterranean Sea should be taken into account. Here, we present a case study in the western Mediterranean Sea, specifically the *P. nobilis* metapopulation inhabiting Balearic archipelago (western Mediterranean Sea). This case study provides us with an example of how to establish reference values in a given area. We conducted the study in the five main islands: Mallorca, Menorca, Ibiza, Formentera and Cabrera; and several islets (Figure 1). Seagrass meadows (*Posidonia oceanica* (Linnaeus) Delile, 1813) are one of the most significant elements of the benthic environment of Balearic coasts, growing on carbonate sediments of biogenic origin (Marbà et al. 2005). Extensive and dense meadows up to depths of 40 m inhabit the archipelago, but many been degraded as a result of anchoring and pollution from recreational boating, which is largely unregulated around the islands (Procaccini et al. 2003). This mesoscale case study integrates variability among hundreds of kilometers covering an area of around 150,000 km^2 (Deudero et al. 2011).

A total of 661 visual censuses were conducted across 69 sampling sites in the Baleric archipelago in order to evaluate *P. nobilis* population. It is important to clarify here that several sites with different protection level were studied. An effective protection effect on *P. nobilis* populations can be seen only within the boundaries of the National Park, the highest figure of protection in the archipelago, hosting the highest densities of the species (Figure 2). In contrast, there were no differences in terms of densities between the rest of protected (Marine Reserve and Site of Community Importance) and non-protected areas, which are mainly due to many human activities are allowed (e.g. anchoring) directly affecting *P. nobilis* populations. Therefore, in order to establish thresholds for GES, the MPA of Cabrera National

Park was considered a **pristine MPA** to obtain reference values. On the other hand, the rest of sampling sites among the Balearic Islands (Figure 1) were analyzed together to integrate the diversity of the studied area and to have more analytical power; and was considered as **non-pristine MPA**.

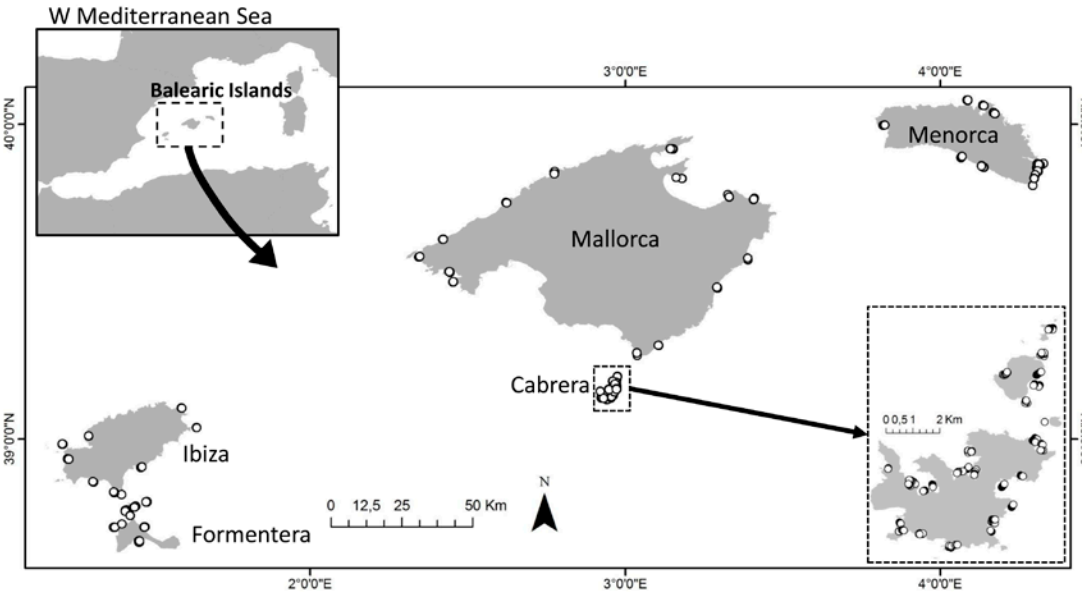


Figure 1. Study area of the case study in the Balearic Islands showing sampling localities (white dots) among islands and in the pristine MPA (Cabrera).

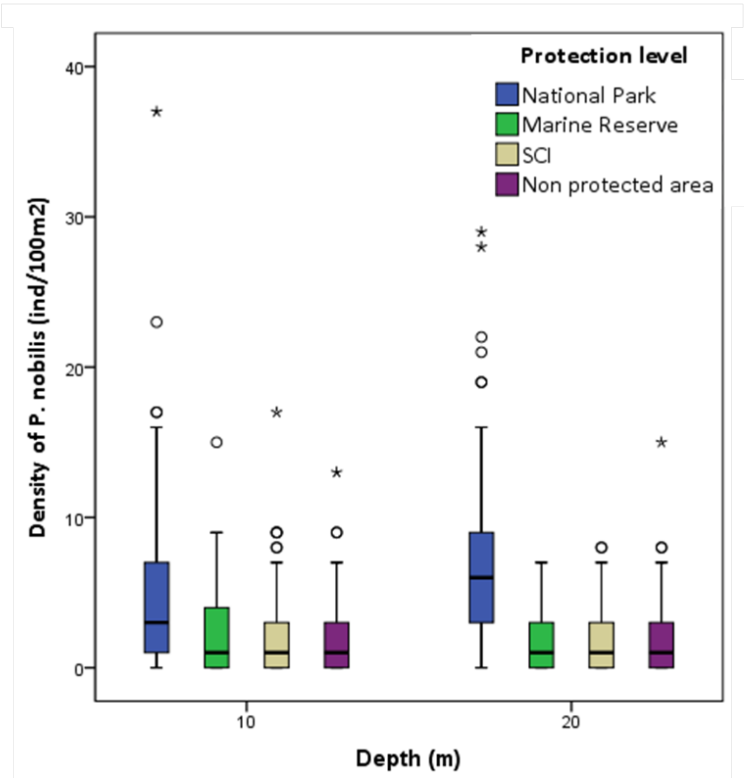


Figure 2. Mean values of density of *P. nobilis* individuals (ind./100m^2) in 10 and 20 meters depth by protection level.

2.1. *Pinna nobilis* in a pristine Marine Protected Area: Cabrera National Park case study

The Cabrera Archipelago National Maritime-Terrestrial Park (N 9º10’50”, E 2º56’0”, Balearic Islands, Western Mediterranean, hereafter “**pristine MPA**”) has been effectively, fully-protected and excluded from anthropogenic activities for more than 25 years (is located 19 km away from a port and is free of significant human pressures), and therefore it provides an optimum scenario to assess GES according to *Pinna nobilis* metrics. Scientific research in this protected area has demonstrated that populations of *Pinna nobilis* presents an optimum state of conservation (Vázquez-Luis et al., 2014a, Deudero et al. 2015) and consequently this site could be established as a reference site for *Pinna nobilis* populations in coastal areas of Balearic Archipelago. The studied population in Cabrera National Park was surveyed from 2011 to 2014. In this period, a total of 418 strip transects were conducted by scuba diving, in order to survey *P. nobilis* density, distribution and size structure at several habitats (sand, rock, coastal detritic, caves and *P. oceanica* seagrass meadows); and depths ranged from 2 to 46 meters depth. The length of each strip transects in *P. oceanica* seagrass meadow was 30 m and the width was 2.5 m, covering a total area of 75 m^2 per transect In habitats with no seagrass coverage, such as detritic, rock or sand, strip transects of different lengths and widths were also conducted according to habitat type availability. In the deepest areas, strip transects were conducted with the help of an underwater scooter. A total of 291 transects were conducted over *P. oceanica* meadows, 26 on coastal detritic bottoms, 60 on rock, 33 on sand and 8 in underwater caves (all the bottom surface of the cave). The total surveyed surface area of all habitats was 152,146.35 m^2. Specifically, 50,900 m^2 were explored on *P. oceanica* meadows, 21,604 m^2 on coastal detritic bottoms, 38,415.85 m^2 on rocky bottoms, 36,866 m^2 on sandy bottoms and 4,360.5 m^2 in underwater caves. The densities of *P. nobilis* were expressed in individuals per 100 m^2 and plotted in a geographic information system (GIS).

In order to contrast values of the pristine area, the rest of Balearic Islands were studied as a case study (hereafter “**non-pristine MPA**”). Field surveys in non-pristine MPA were carried out from July 2011 to September 2013. Underwater visual censuses along strip transects of 30 m length and 2.5 m width (Deudero et al. 2015) were conducted in order to survey the fan mussel *P. nobilis* population density and shell size (measured as maximum shell width). A total of 427 strip transects across *P. oceanica* seagrass meadows at 10 and 20 meters depth were performed. The total surveyed surface accounting for all islands was 32,025 m^2.

Data on density, size structure and demographic parameters for pristine and non-pristine MPA are provided here by setting thresholds for our case study:

- **Density**: Scientific research has shown that in the pristine MPA *Pinna nobilis* mean densities vary with depth and maximum densities are given in the first 20 m depth (Figure 3A) (Vázquez-Luis et al., 2015b). In addition, *Pinna nobilis* densities also vary according to habitat type exhibiting higher values in *Posidonia oceanica* meadows (Figure 3B) (Vázquez-Luis et al., 2015a, b). Therefore, reference values of densities in terms of ind./100m^2 must be provided according to the main habitat for *P. nobilis* by depth strata (in this case *Posidonia oceanica* seagrass meadows). It is important to remark that in the pristine MPA of this case study no human activities influencing population of pen shell exists. Reference values of densities must be established taken into account all transects done in the pristine MPA, including those transects with density values of 0. In this pristine MPA densities of *P. nobilis* in other habitat types among different depths were also studied (rocky, sand, detritic), however the replication is not enough to provide data for GES on this habitats (Table 1). Mean densities from sampled transects were of 3.79 ind/100 m^2 in all habitat types and depths, with maximum mean values in *Posidonia oceanica* seagrass meadows (5.21 ind/100 m^2). It is important to remark that for the calculation of mean values has been taking into account transect with 0 values. Therefore, among *P. oceanica* seagrass meadows in the first 20 m depth values above 4 ind./100m^2 can be considered appropriate. Comparing values of pristine and non-pristine MPA in this case study, density values were 2.3 and 3.5 times higher at 10 and 20 m depth respectively in pristine MPA (Table 1, Figure 4).

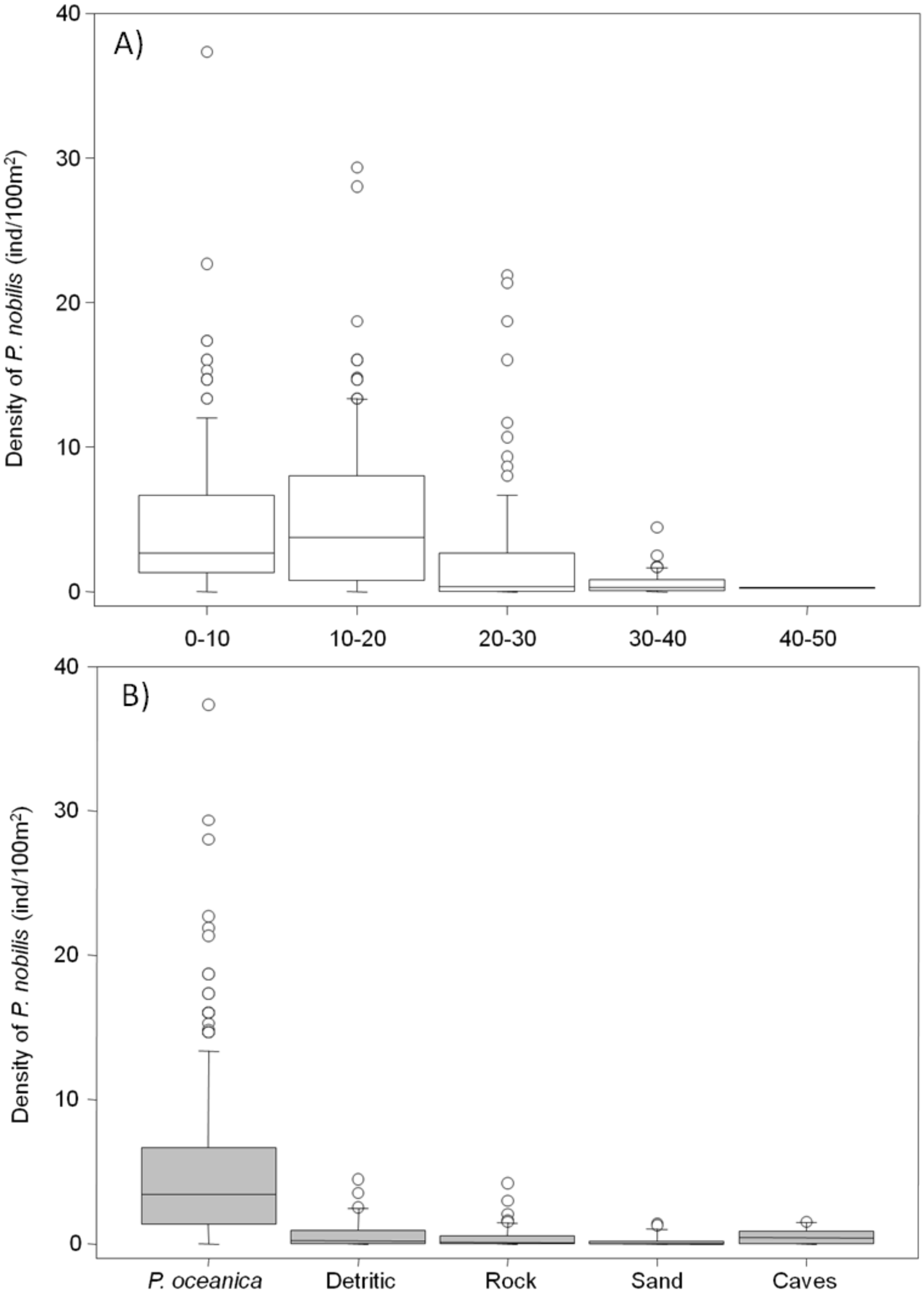


Figure 3. A) Densities of *Pinna nobilis* at different depths range in a pristine MPA reference site. Whiskers represent minimum and maximum values, the bottom and top of the box represent the first and third quartile, respectively, and the band inside the box the second quartile. B) Density box-plot of *Pinna nobilis* according to different habitat types in a pristine MPA reference site among studied habitats. Whiskers represent minimum and maximum values, the bottom and top of the box represent the first and third quartile, respectively, and the band inside the box the second quartile (from Vázquez-Luis et al. 2015a). Total number of censed living individuals: 1587 in pristine MPA in a total of 51 sampling sites.

Table 1. Mean densities (± SE) of living *Pinna nobilis* in *P. oceanica* seagrass meadows, rocky, sand and detritic bottoms according to depth stratums in pristine MPA and non pristine MPA. Depth: depth stratum, N: number of replicates, Mean density: Mean densities ± standard error (SE), Min-Max: minimum and maximum values of densities. Total number of censed living individuals: 1587 in pristine MPA and 629 in non-pristine MPA. Total of sampling sites: 51 in pristine MPA and 45 in non pristine MPA.

		PRISTINE MPA			NON PRISTINE MPA		
Habitat	**Depth**	**N**	**Mean density**	**Min-Max**	**N**	**Mean density**	**Min-Max**
P. oceanica	10	135	4.92 ± 0.48	0 - 37.33	219	2.17 ± 0.2	0 - 16.67
	20	103	6.84 ± 0.58	0 - 29.33	208	1.96 ± 0.16	0 - 15
	30	28	0.52 ± 0.12	0 - 2.5			
Total *P. oceanica*		**266**	**5,21 ± 0,35**	**0 - 37.33**	**427**	**2.07 ± 0.13**	**0 - 16.67**
Rocky	10	15	0.23 ± 0.11	0 - 1.51			
	20	6	0.71 ± 0.3	0 - 2.04			
	30	29	0.49 ±0.18	0 - 4.17			
	40	3	0.31 ± 0.06	0.25 - 0.43			
Total rocky		**53**	**0.43 ± 0.11**	**0 - 4.17**			
Sand	10	3	0.07 ± 0.07	0 - 0.21			
	20	6	0.44 ± 0.28	0 - 1.38			
	30	20	0.18 ± 0.07	0 - 1			
Total sand		**29**	**0.22 ± 0.07**	**0 - 1.38**			
Detritic	20	3	1.97 ± 0.86	0.55 - 3.51			
	30	21	0.66 ± 0.24	0 - 4.44			
	40	5	0.15 ± 0.05	0 - 0.3			
	50	1	0.21	0.21 - 0.21			
Total detritic		**30**	**0.69 ± 0.2**	**0 - 4.44**			
Total general		**378**	**3.79 ± 0.27**	**0 - 37.33**			

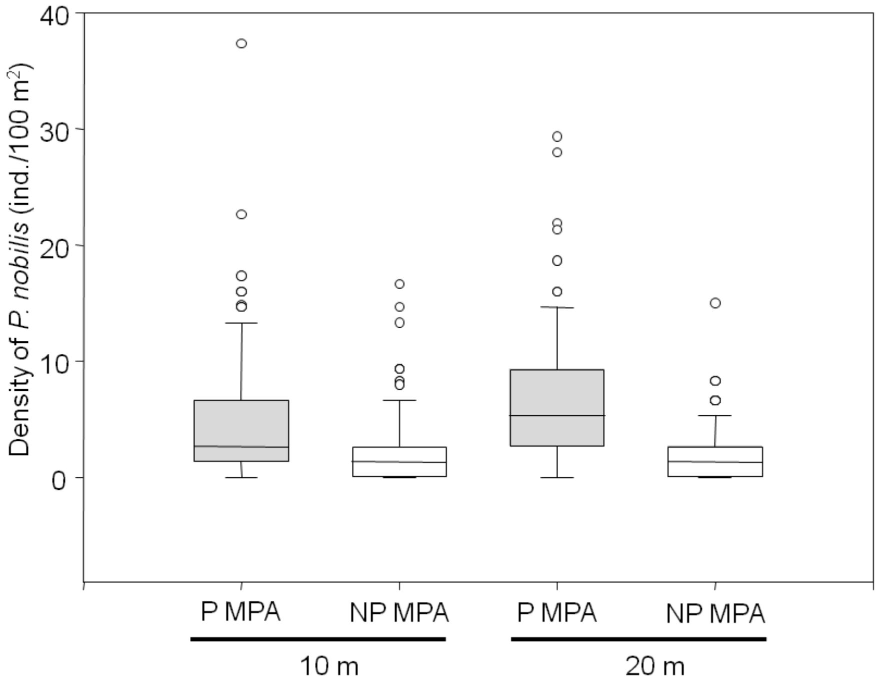


Figure 4. Mean density (ind./100m2) of Pinna nobilis at 10 and 20 meters depths (P. oceanica meadows) in a pristine MPA (P MPA) reference site and non-pristine MPA (NP MPA). Whiskers represent minimum and maximum values, the bottom and top of the box represent the first and third quartile, respectively, and the band inside the box the second quartile. Total number of censed individuals: 1081 in pristine MPA and 629 in non-pristine MPA. Total of sampling sites: 24 in pristine MPA and 45 in non-pristine MPA.

P. nobilis density is optimal in Posidonia oceanica seagrass meadows from 0 to 20 meters depth

- **Size structure**: In the case study mentioned above (densities in pristine vs non-pristine MPA) size structure of *P. nobilis* population in terms of maximum shell width was provided for size classes each 5 cm. In the pristine MPA, considering all depths and habitat types, a total of 1873 individuals were measured, which 1456 were living individuals. In general terms the most abundant size class corresponded to 15-20 cm width (Figure 5). This pattern is consisting among depth ranges, with the exception of the stratum at 30 m depth where the most common size classes was 20-25 cm (Figure 6). It is important to remark that individuals exposed to high hydrodynamics were smaller than those of the same age but living in sheltered areas (García-March, 2005). Therefore this should be taken into account for comparisons among populations. In general terms, individuals from the pristine MPA presented bigger shells at 10 and 20 m depth compared to the non-pristine MPA (Figure 7). Mean values in pristine MPA at 10 and 20 m were 16.6 ± 0.18 cm and 18.87 ± 0.15 cm respectively, while in the non-pristine MPA were 13.56 ± 0.2 cm and 17.26 ± 0.27 cm at 10 m and 20 m respectively (Table 2).

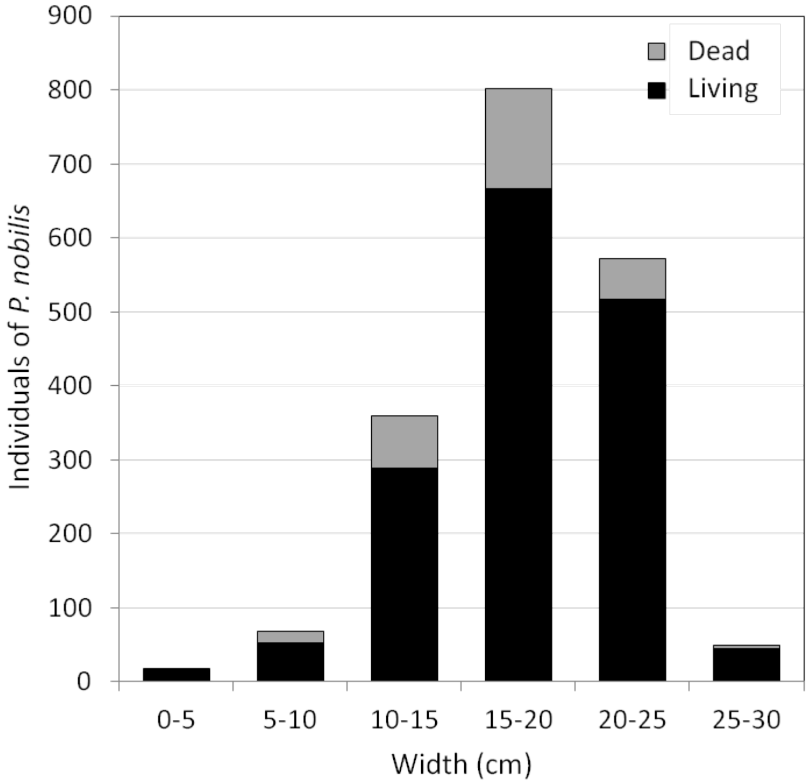


Figure 5. Size classes (maximum shell width) of the population of living and dead *Pinna nobilis* individuals in a pristine MPA reference site (N=1873 individuals).

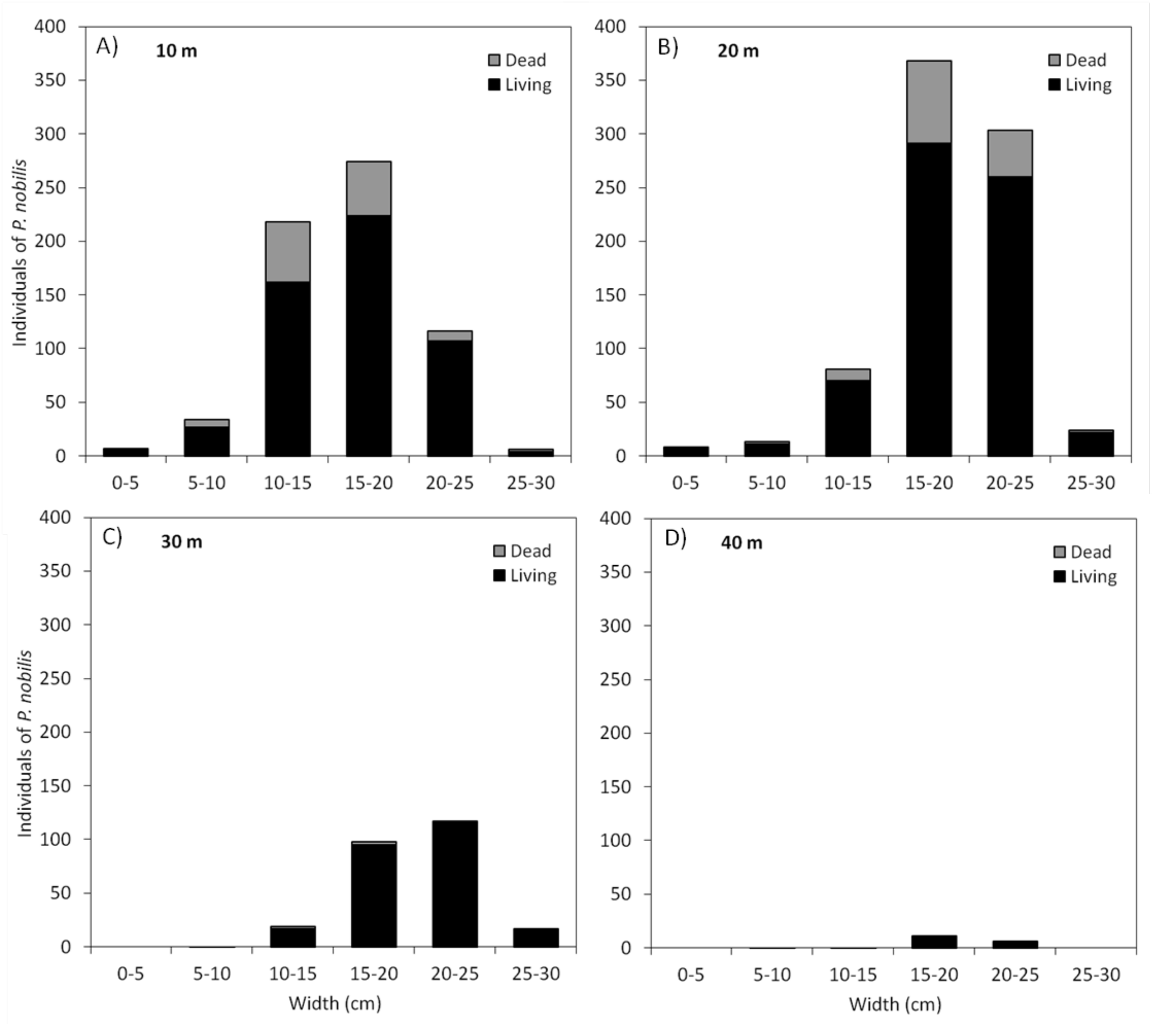


Figure 6. Size classes (maximum shell width) of the population of *Pinna nobilis* in a pristine MPA reference site at 10, 20, 30 and 40 meters depth (from Vázquez-Luis and Deudero 2013).

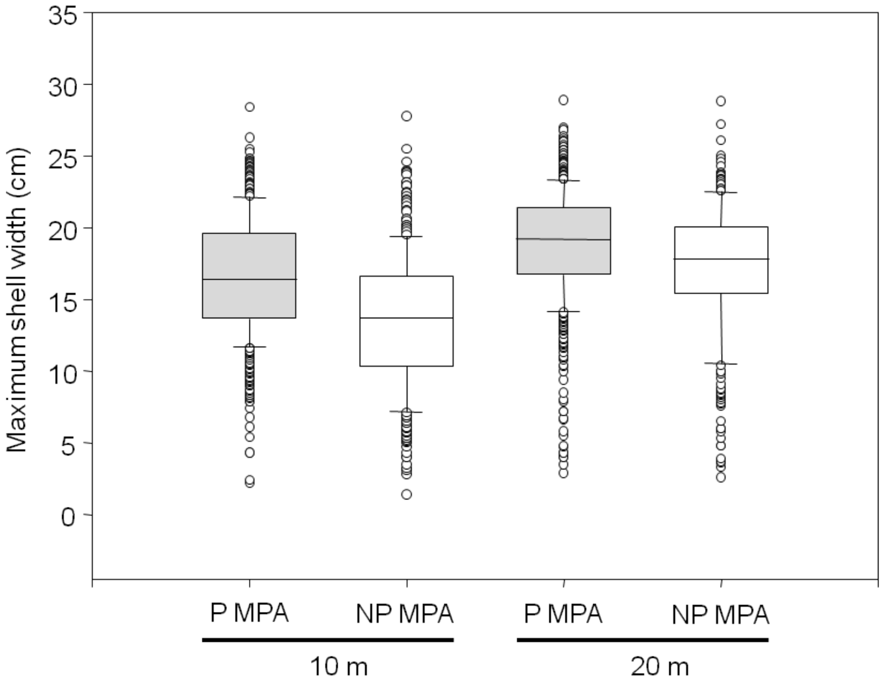


Figure 7. Maximum shell width (cm) of *Pinna nobilis* at 10 and 20 meters depths in a pristine MPA (P MPA) and non-pristine MPA (NP MPA). Whiskers represent minimum and maximum values, the bottom and top of the box represent the first and third quartile, respectively, and the band inside the box the second quartile. Total number of censed individuals: 1081 in pristine MPA and 629 in non-pristine MPA. Total of sampling sites: 24 in pristine MPA and 45 in non-pristine MPA.

Table 2. Mean shell width (± SE) in cm of living *Pinna nobilis* according to depth stratums in pristine MPA and non-pristine MPA. Depth: depth stratum, N: number of replicates, Mean shell width: Mean shell width in cm ± standard error (SE), Min-Max: minimum and maximum values of densities.

	PRISTINE MPA			NON-PRISTINE MPA		
Depth	**N**	**Mean shell width**	**Min-Max**	**N**	**Mean shell width**	**Min-Max**
10	530	16.6 ± 0.18	2.2 - 28.4	344	13.56 ± 0.2	1.4 - 27.8
20	662	18.87 ± 0.15	2.9 - 28.9	285	17.26 ± 0.27	2.6 - 28.8
30	246	20.51 ± 0.24	9 - 30			
40	17	19.39 ± 0.46	15 - 22			
50	1	19	19			
Total general	**1456**	**18.19 ± 0.11**	**2.2 - 30**	**629**	**15.24 ± 0.2**	**1.4 - 28.8**

P. nobilis in pristine area showed an unimodal distribution of size classes with mode values between 15-20 cm of maximum width. At deeper depth individuals were larger.

- **Demographic parameters**: Demographic studies require long-term monitoring to obtain estimates of population evolution by measuring mortality, recruitment and net growth rates. We monitored the population of pen shell in permanent plots installed in 2011 within the pristine MPA at three depths (10, 20 and 30 m) and outside the MPA at 10 m depth (non-pristine MPA in San Telmo, Mallorca). As mentioned above the highest density of *P. nobilis* population within the pristine MPA was located in the area of maximum protection, sheltered, with low hydrodynamics, and conditions that favors the high development. In contrast, the pen shell population of non-pristine MPA was located in an area without legal protection and highly exposed to hydrodynamics since it is located near a channel between two islands (Mallorca and Dragonera). The population values of net growth rates integrate mortality and recruitment rates. In pristine MPA the *P. nobilis* population tends to remain stable over time, regardless of depth strata, with net growth rates close to zero during the 4 years of monitoring (Figure 8). The populations of non-pristine MPA presented a similar pattern in terms of net growth (Figure 8A) and the general trend is also stable over time in spite of the slight differences detected among plots (Figure 8B). However, the population dynamics were very different between the pristine and non-pristine MPA plots. Both annual mortality and recruitment rates are higher in non-pristine MPA (Figure 9, Figure 10, Table 3), which supposes a higher population renewal rate than in pristine MPA populations. The survival rate of tagged individuals in the pristine MPA at time 0 exceeds 80% in all studied depths, while in the non-pristine MPA it did not reach 40% in 4 years of monitoring (Figure 11). The population of non-pristine MPA remained stable since recruitment is high and sufficient to counteract the high mortality rates of adults (Figure 12). However non-pristine MPA populations were expected to be more vulnerable to disturbances because of their lower resilience. Moreover, individuals are larger (in size) in the pristine MPA compared to non pristine MPA (Figure 13).

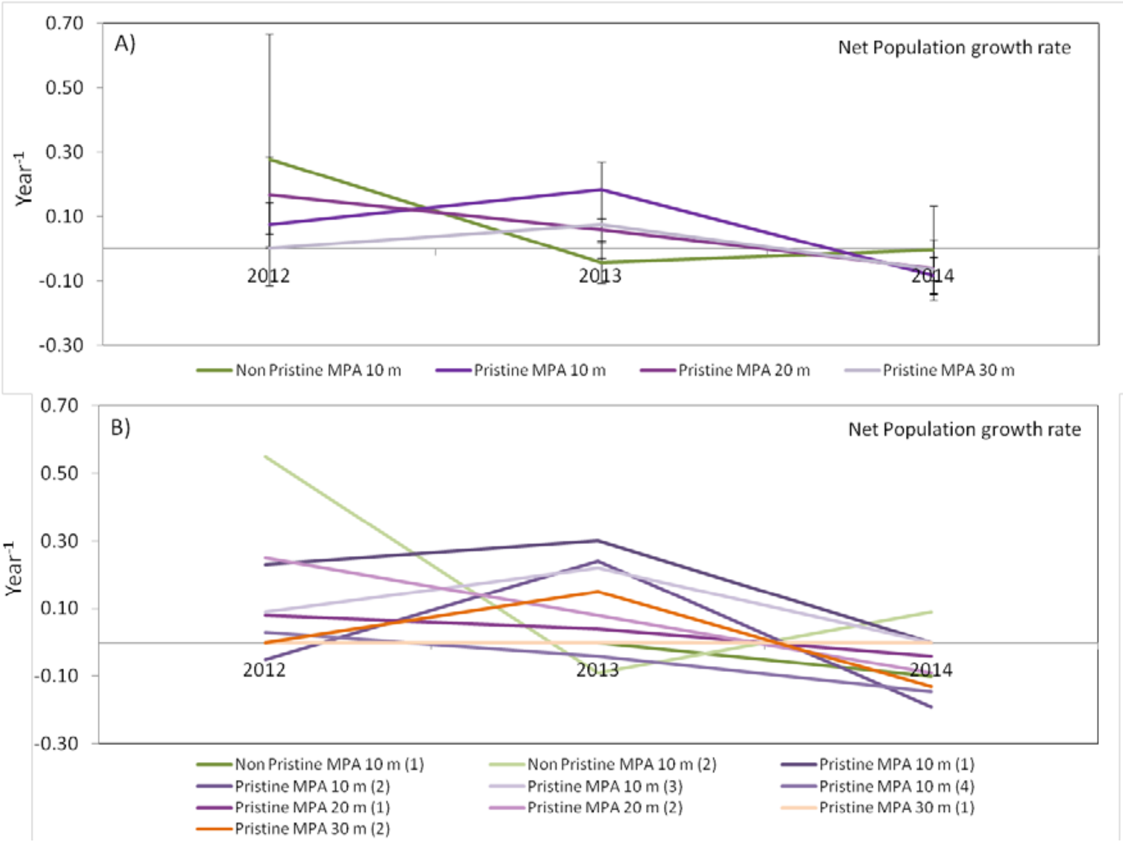


Figure 8. A) Average of net population growth rate (yr^{-1}) (± SE) of tagged *Pinna nobilis* population between 2011-2014 in pristine MPA (10, 20 and 30 m), and non-pristine MPA (10 m); and B) Net population growth rate (yr^{-1}) of *Pinna nobilis* among plots between 2011-2014 in pristine MPA (10 m: 4 plots, 20m: 2 plots and 30 m: 2 plots), and non-pristine MPA (10 m: 2 plots).

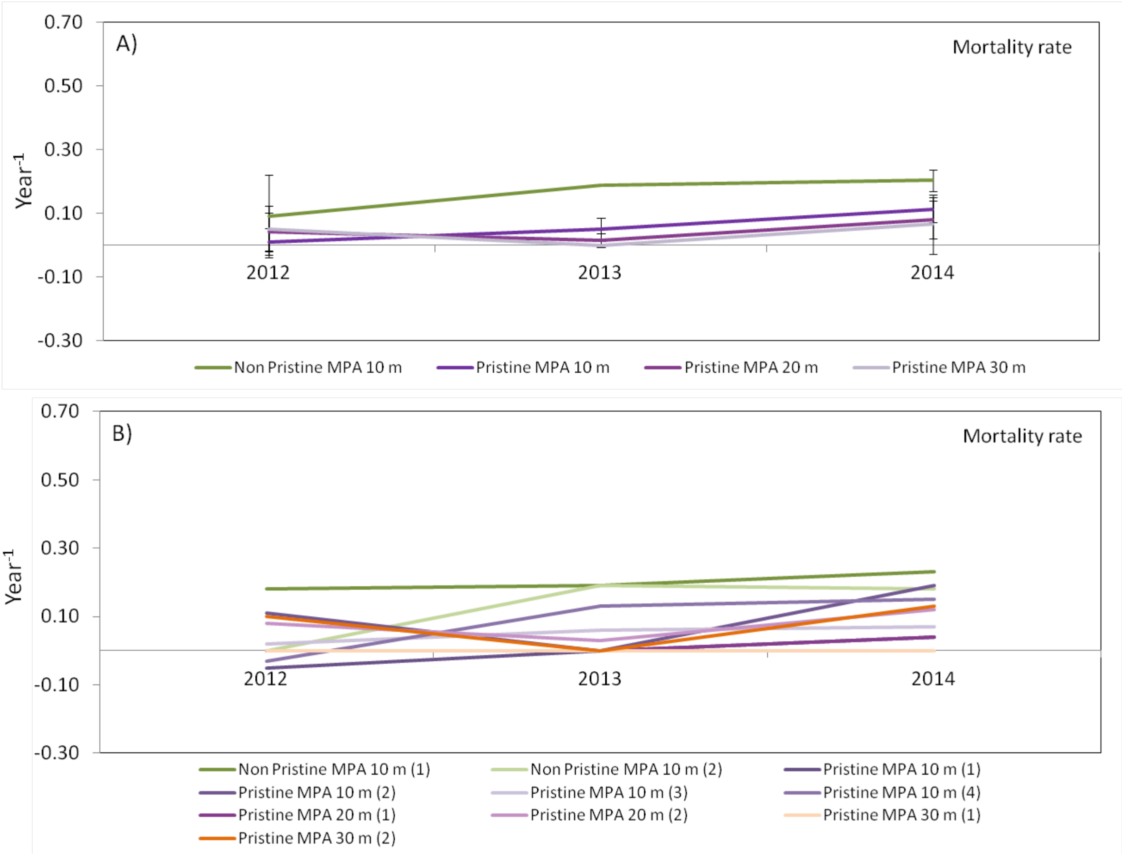


Figure 9. A) Average of mortality rate (yr^{-1}) (± SE) of tagged *Pinna nobilis* population between 2011-2014 in pristine MPA (10, 20 and 30 m), and non-pristine MPA (10 m). B) Mortality rate (yr^{-1}) of *Pinna nobilis* population between 2011-2014 in pristine MPA (10 m: 4 plots, 20m: 2 plots and 30 m: 2 plots), and non-pristine MPA (10 m: 2 plots).

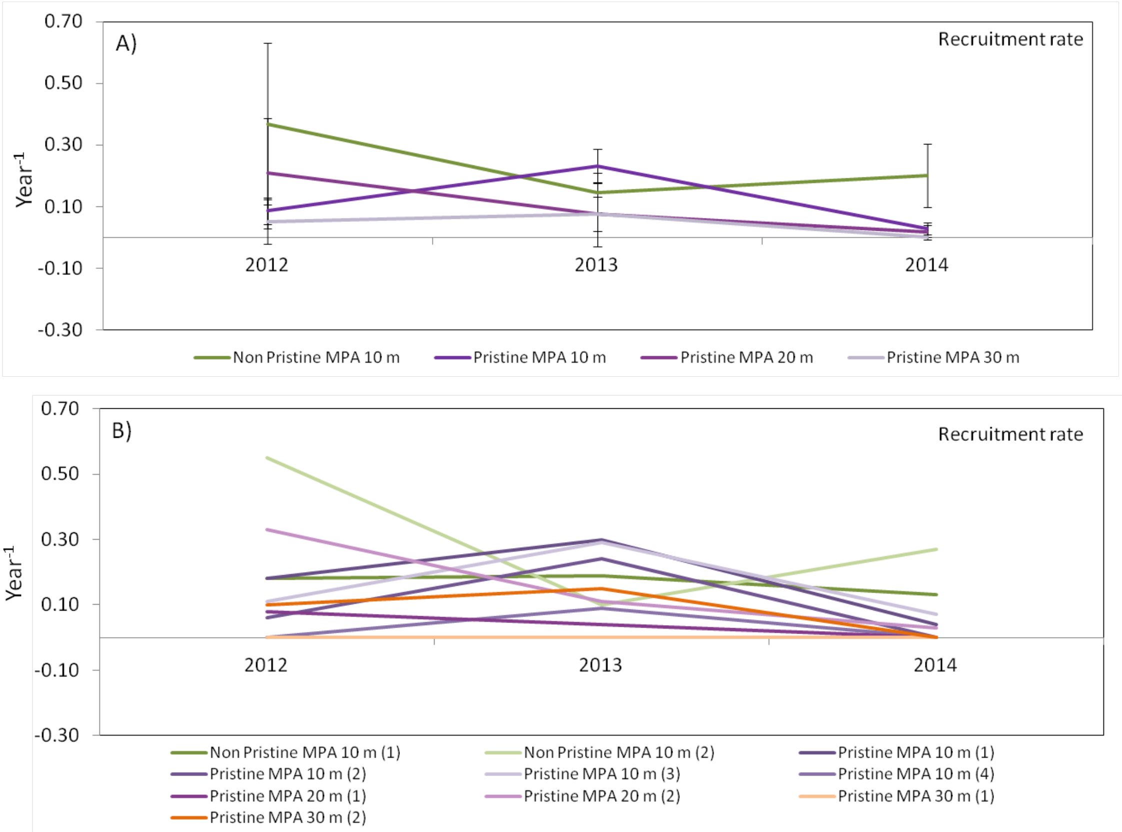


Figure 10. A) Average of recruitment rate (yr^{-1}) (± SE) of tagged *Pinna nobilis* population between 2011-2014 in pristine MPA (10, 20 and 30 m), and non-pristine MPA (10 m). B) Recruitment rate (yr^{-1}) of *Pinna nobilis* population between 2011-2014 in pristine MPA (10 m: 4 plots, 20m: 2 plots and 30 m: 2 plots), and non-pristine MPA (10 m: 2 plots).

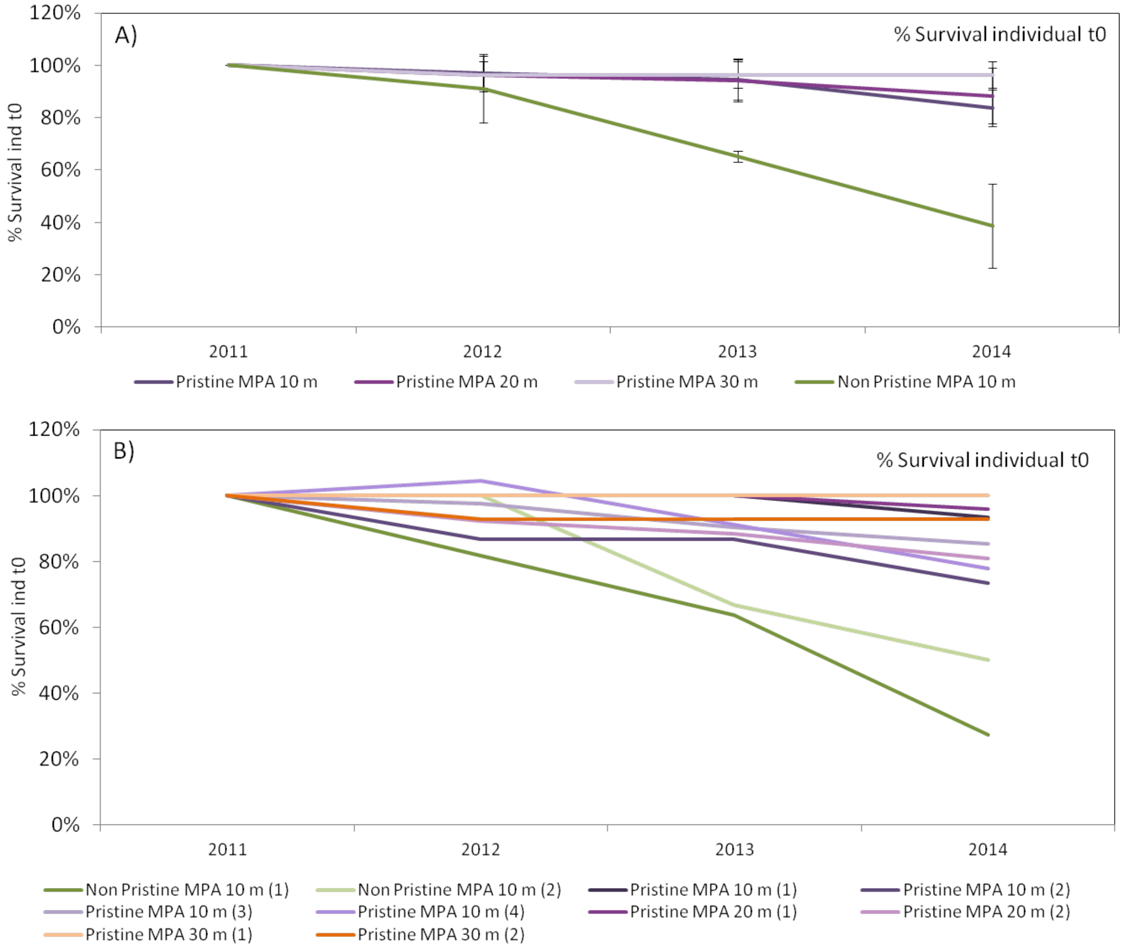


Figure 11. A) Average of percentage (± SE) of survival of tagged *Pinna nobilis* individuals between 2011-2014 in pristine MPA (10, 20 and 30 m), and non-pristine MPA (10 m). B) Percentage of survival of tagged *Pinna nobilis* individuals between 2011-2014 in pristine MPA (10 m: 4 plots, 20m: 2 plots and 30 m: 2 plots), and non-pristine MPA (10 m: 2 plots).

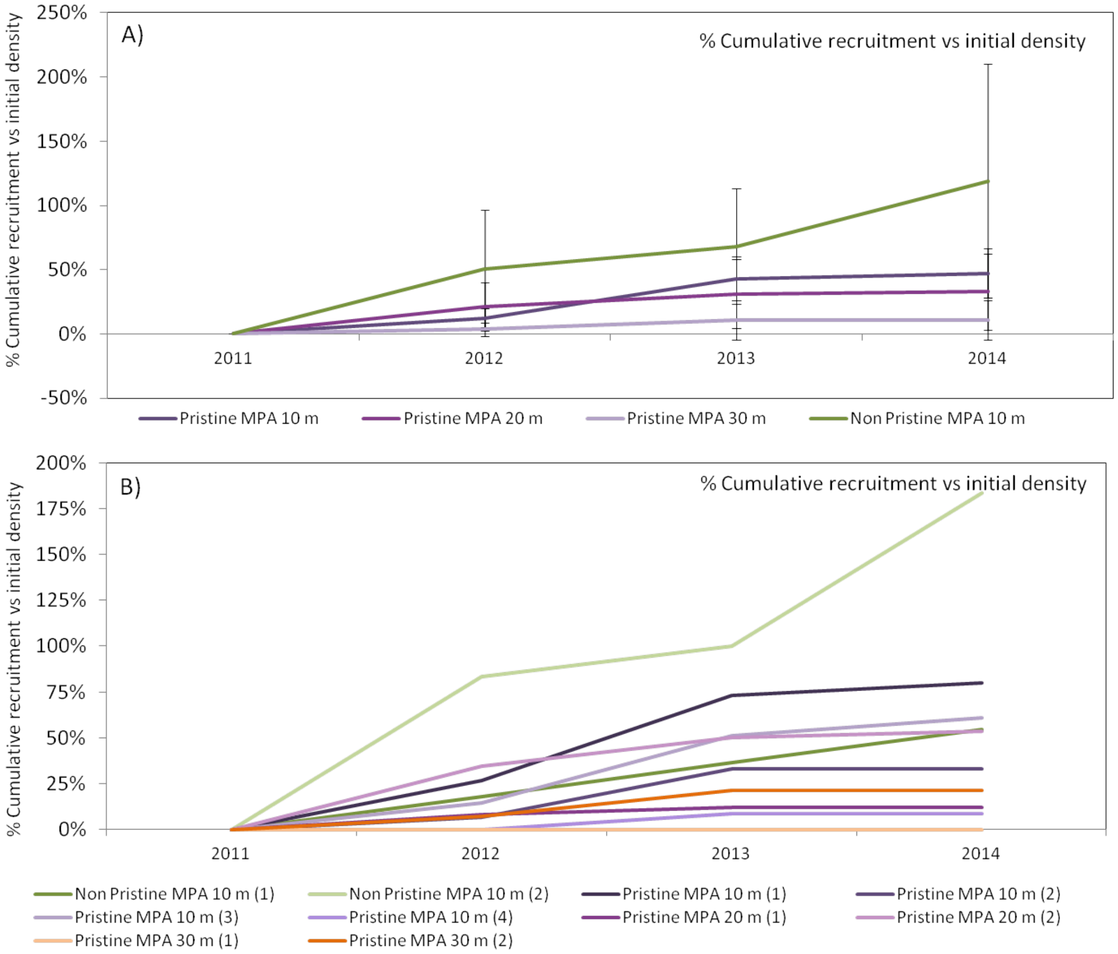


Figure 12. A) Average of percentage (± SE) of cumulative recruitment versus initial density of tagged *Pinna nobilis* individuals since 2011 to 2014 in pristine MPA (10, 20 and 30 m), and non-pristine MPA (10 m). B) Percentage of cumulative recruitment versus initial density of tagged *Pinna nobilis* individuals between 2011-2014 in pristine MPA (10 m: 4 plots, 20m: 2 plots and 30 m: 2 plots), and non-pristine MPA (10 m: 2 plots).

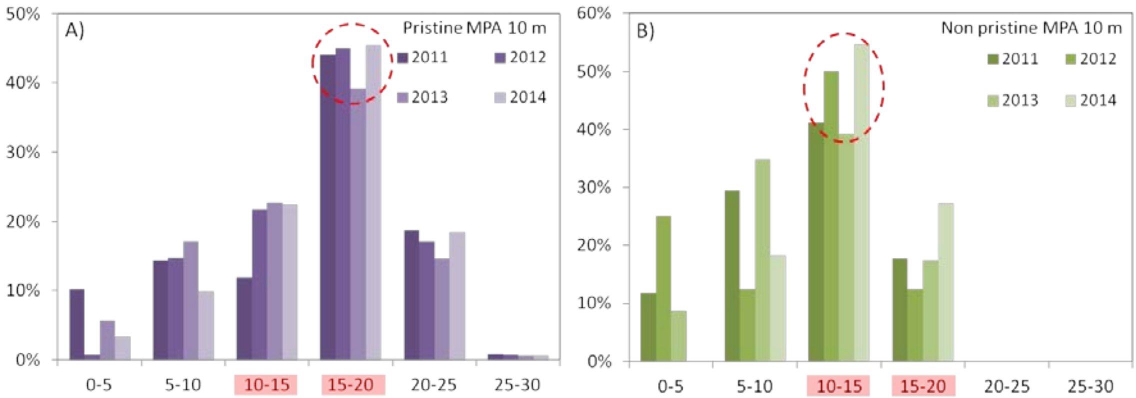


Figure 13. Size classes (maximum shell width) of the population of living and tagged *Pinna nobilis* individuals in a pristine and non-pristine MPA (N in pristine MPA 10 m: 2011=118, 2012=129, 2013=158 and 2014=152; N in non-pristine MPA 10 m: 2011=17, 2012=24, 2013=23 and 2014=22).

Table 3. Demographic parameters of *Pinna nobilis* in permanent plots in pristine MPA and non-pristine MPA, from 2011 to 2014. Mean values (± SE) of rates are also provided. Depth: depth stratum, Plot: number of plot, Year: year of measurement, Mortality rate, Recruitment rate and Net population growth rate in y-1; N initial: number of tagged individuals at time 0 (2011) and N final: number of tagged individuals in the last visit.

Locality	Depth	Plot	Year (t0-tf)	Mortality rate (y^{-1})	Recruitment rate (y^{-1})	Net population growth rate (y^{-1})	N initial	N final
PRISTINE MPA	**10**	1	2011-2012	-0.05	0.18	0.23	14	19
		2		0.11	0.06	-0.05	15	14
		3		0.02	0.11	0.09	41	46
		4		-0.03	0.00	0.03	45	47
		Mean 10 m 2011-12		0.01 ± 0.04	0.09 ± 0.04	0.07 ± 0.07		
		1	2012-2013	0.00	0.30	0.30	19	26
		2		0.00	0.24	0.24	14	18
		3		0.06	0.29	0.22	46	58
		4		0.13	0.09	-0.04	47	45
		Mean 10 m 2012-13		0.05 ± 0.04	**0.23** ± 0.06	**0.18** ± 0.09		
		1	2013-2014	0.04	0.04	0.00	26	26
		2		0.19	0.00	-0.19	18	15
		3		0.07	0.07	0.00	58	58
		4		0.15	0.00	-0.15	45	39
		Mean 10 m 2013-14		**0.11** ± 0.04	0.03 ± 0.02	-0.08 ± 0.06		
	20	1	2011-2012	0.00	0.08	0.08	25	27
		2		0.08	0.33	0.25	26	33
		Mean 20 m 2011-12		0.04 ± 0.06	**0.21** ± 0.18	**0.17** ± 0.12		
		1	2012-2013	0.00	0.04	0.04	27	28
		2		0.03	0.11	0.08	33	36
		Mean 20 m 2012-13		0.01 ± 0.02	0.07 ± 0.06	0.06 ± 0.03		
		1	2013-2014	0.04	0.00	-0.04	28	27
		2		0.12	0.03	-0.09	36	33
		Mean 20 m 2013-14		0.08 ± 0.06	0.02 ± 0.02	-0.06 ± 0.04		
	30	1	2011-2012	0.00	0.00	0.00	8	8
		2		0.10	0.10	0.00	14	14
		Mean 30 m 2011-12		0.05 ± 0.07	0.05 ± 0.07	0.00 ± 0.00		
		1	2012-2013	0.00	0.00	0.00	8	8
		2		0.00	0.15	0.15	14	16
		Mean 30 m 2012-13		0.00 ± 0.00	0.07 ± 0.11	0.07 ± 0.11		
		1	2013-2014	0.00	0.00	0.00	8	8
		2		0.13	0.00	-0.13	16	14
		Mean 30 m 2013-14		0.09 ± 0.00	0.00 ± 0.00	-0.07 ± 0.09		
NON-PRISTINE MPA	**10**	1	2011-2012	0.18	0.18	0.00	11	11
		2		0.00	0.55	0.55	6	11
		Mean 10 m 2011-12		0.09 ± 0.13	**0.37** ± 0.26	**0.28** ± 0.39		
		1	2012-2013	0.19	0.19	0.00	11	11
		2		0.19	0.10	-0.09	11	10
		Mean 10 m 2012-13		**0.19** ± 0.00	0.14 ± 0.06	-0.04 ± 0.06		
		1	2013-2014	0.23	0.13	-0.10	11	9
		2		0.18	0.27	0.09	10	12
		Mean 10 m 2013-14		**0.20** ± 0.03	0.20 ± 0.10	-0.005 ± 0.14		

Pristine MPA reduce Pinna nobilis mortalities and recruitment rises up to 30% after 4 years

2.2. Reference values for *P. nobilis* populations

Densities of *P. nobilis* populations several decades ago before the decline of its population are unknown. However, historical records point out the existence of large aggregations of "*giant mussels"* in several areas along the Mediterranean Sea (Berard, 1789). Here, we can only provide a first proposal of thresholds in our study area to evaluate conservation status of *P. nobilis* in *P. oceanica* seagrass meadows based on current knowledge of *P. nobilis* population in pristine MPA areas not influenced by human impacts. However, it is very likely that we will start from fragmented populations in most cases.

Based on the present study, values of mean density and sizes of *P. nobilis* population are different among depths and according to pristine and non-pristine MPAs (Figure 14A, B). According to the model and field observations, a first proposal of thresholds to establish GES are provided in Table 4 for each studied descriptor. Thus, intervals of values for density, sizes and net population growth rate (demography) for good, moderate or bad, among 10 and 20 meters depth are proposed. In the present study predicted density and sizes for pristine and non-pristine MPA are clearly different and depends on depth (Figure 14A, B). It is important to consider that a given values of an area may not be representative of what happens in other parts of the Mediterranean Sea. Therefore, this is a first proposal of values that should be expanded and revised with other geographical areas. In any case, instead of a given value (of density, size, net population growth rate) it would be appropriate talk about trends in indicators or variables to evaluate if we are on the way to reach good environmental status. Moreover, to assess *P. nobilis* population from a multimetric point of view or to include more variables by combining data on densities, sizes and demographic parameters are highly recommended. Thus, developing a multimetric/multicriteria index will be of great help and further possibilities of including other variables must be explored.

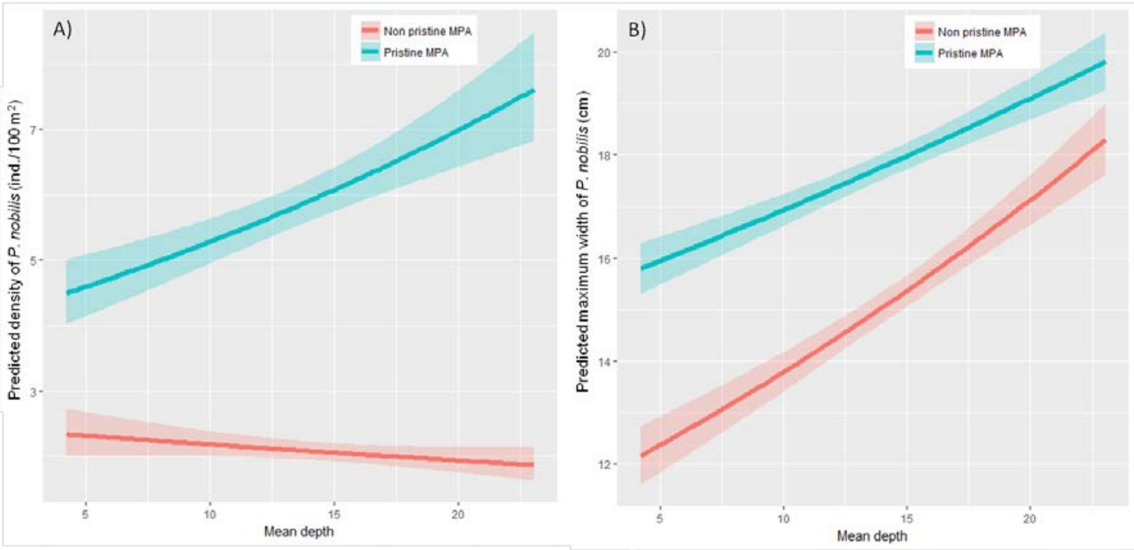


Figure 14. General lineal model for: A) Predicted density of P. nobilis (ind./100m2) among the interaction of Depth and protection level (pristine and non-pristine MPA); and B) predicted maximum shell width for P. nobilis (cm) among the interaction of Depth and protection level (pristine and non-pristine MPA.

Table 4. Environmental status of *P. nobilis* population based on thresholds for: density (individuals of *P. nobilis*/100 m^2), size (maximum shell width in cm) and demography (net population growth rate in terms of average yearly). Three groups of environmental status can be distinguished: **green → good**, **orange → medium** and **red → bad**.

GES for *Pinna nobilis* in *Posidonia oceanica* meadows and thresholds			
Depth (m)	**Density** (Individuals/100m^2)	**Size** Maximum shell width (cm)	**Demography** (Net population growth rate yr^{-1})
10	>4	Unimodal, mean width>16 cm	Positive, 0 or > -0.1 yr^{-1}
	2-4	Unimodal, mean width 14-16 cm	-0.1 to -0.2 yr^{-1}
	<2	Unimodal, mean width<14 cm	< -0.2 yr^{-1}
20	>6	Unimodal, mean width>18 cm	Positive, 0 or > -0.05 yr^{-1}
	2-6	Unimodal, mean width 16-18 cm	-0.05 to -0.1 yr^{-1}
	<2	Unimodal, mean width <18 cm	< -0.1 yr^{-1}

3. ACTION PLAN FOR *Pinna nobilis* IN THE FRAME OF THE MSFD

Several actions are proposed in this action plan in order to evaluate the environmental status of *P. nobilis* populations in the Mediterranean Sea with standardized methodologies, and to identify stressors in order to propose a program of measures to achieve or maintain GESs. The **Marine Strategy Framework Directive** (MSFD, European Directive) aimed at achieving or maintaining Good Environmental Status (GES) in European seas. For the above mentioned purpose, a definition of "**good environmental status**" at regional level, detailed **assessment of the state of the environment** and the establishment of clear **environmental targets and monitoring programmes** must be developed by each Member State. The benthic filter feeder *Pinna nobilis* is an endemic species of the Mediterranean Sea, so its distribution is exclusive of its Sea. Therefore common protocols across Mediterranean Sea to evaluate conservation status of *P. nobilis* must be shared and applied. Here, assessments of density, size and demographic parameters of the species are proposed. In order to evaluate the conservation status of *P. nobilis* in a region in the frame of MSFD several actions are planned:

Definition of "good environmental status" at regional level,
detailed assessment of the state of the environment,
establishment of clear environmental targets and monitoring programmes
must be developed by each Member State within MSFD

Action 1: Steps to a monitoring programme: baseline surveys

To evaluate GES status in *Pinna nobilis* population is desirable to carry out a review of the previous information on the species in a given area. In order to establish a monitoring programme several steps must be followed taking into account previous information of the species (if exists):

- Each member state must establish a monitoring programme to evaluate *P. nobilis* population in its waters. For this purpose selection of several monitoring points (sampling stations) has to be established across the region.
- Baseline surveys across regional area in order to find optimal points to fix for monitoring (sampling stations); and to establish baseline values have to be carried out.
- At each locality/site, the methodology proposed in the Annex 1 must be applied.

Action 2: To propose a definition of GES and thresholds for *P. nobilis* in the region

After the first year of baseline surveys a proposal of GES for *P. nobilis* in the region must be provided. Moreover, proposal of thresholds at regional scale is highly recommended. It is important to remark that taking into account that data is only provided in one year sampling, the thresholds proposed are temporary and should be reviewed and updated later. However, if previous data are available in the region it should be also considered.

Action 3: Establishing monitoring stations of *Pinna nobilis* populations

Sampling stations for monitoring have to be established after baseline surveys (Action 1). Once a year sampling station have to be sampled and monitored to accomplish with the assessment of the state of the environment.

Action 4: Assessment of the state of *Pinna nobilis* populations

Assigning a value of conservation status of sampling stations based on GES and thresholds proposed in Action 2 (see also section 2). In this way we will classify stations into **good**, **moderate** or **bad**.

Action 5: Apply measurements to achieve or maintain GES

In areas where *Pinna nobilis* population is under good environmental status (GES), it is highly important to identify disturbing sources in order to propose measurements to be applied at each case. At this point, clear environmental targets and engagement of managers and administrations is essential for cost-effective measures to attain good environmental status.

Action 6: Long term monitoring

The annual monitoring of proposed variables (Annex 1) among sampling stations is fundamental in order to evaluate evolution of populations. Fast detection of several kinds of disturbances, but also evaluation of the effectiveness of measurements applied against an impact through the recovery or not of the species will be allowed.

Action 7: Review thresholds

It is advisable to revise the thresholds annually until a steady trend. After several years of monitoring, the available information and data on *P. nobilis* population will be increased. Therefore, it will be necessary to analyze wider data in order to review thresholds of GES for *P. nobilis* on a larger scale namely across the Mediterranean basin, by taking into account biogeography differences and anthropogenic and environmental variables. This would provide reliable data for GES establishment at basin level.

Extreme events

A **mass mortality event** (MME) has been recently detected in *Pinna nobilis* population in the Spanish Mediterranean population. This event has caused mortalities exceeding 80% in most cases reaching 100% in many areas (Vázquez-Luis et al. 2017). If this MME spreads to the rest of pen shell populations in the Mediterranean Sea, a rapid assessment is highly valuable. If evidence of anomalous mortality is detected several tasks are recommended to apply:

- ✓ Monitoring of variables described in Annex 1. At the time of conducting the censuses it is important to distinguish in the specimens, the livings that are in good condition, of those who are alive but ill. Living specimens in good condition generally close upon detection of the diver when approaching. Sick/diseases pen shells take longer to close and need a contact stimulus to close permanently, moreover the mantle is retracted. For the dead specimens, we must distinguish (at least during this first period) the recently dead *P. nobilis* from the ancient dead ones. In the freshly killed specimens the clean and bright shell is observed in the interior, where the characteristic pearly area is also seen in the front part of the shell (the most pointed part). As for the orientation with respect to the substrate, in all cases they may be vertical, which is their most common position, or lying down, such as after a storm. Dead specimens of long time are easily identified because they are those in which the inner part of the shell is covered by epiphytes and fouling organisms.
- ✓ If it is not possible to conduct the methodology proposed in Annex 1, at least the number of living, sick, recently dead and ancient dead individuals per area should be noted.
- ✓ In the worst of scenarios where almost all individuals have died, it is important to: (i) monitor remaining living individuals in the area, (ii) rake large areas searching new living individuals, and (iii) search for new recruits.

Extreme events require rapid response and assessment to implement management measures

ANNEX 1

4. ANNEX 1

Descriptors for the characterization of *Pinna nobilis* populations and for the evaluation of their conservation status are presented in this annex. These descriptors include: density of individuals, size structure and population dynamics (demography). For specific sampling and monitoring programs of the species, a complete sampling including all descriptors is recommended.

4.1. Descriptor 1: Density of individuals

Proposed methodology: to study the abundance of individuals of *Pinna nobilis* population, density of living and dead individuals will be recorded. Due to spatial distribution of the species, density will be expressed as ind./100 m^2. As for the orientation with respect to the substrate individuals can be found vertical or lying down. Two non destructive methodologies are proposed to apply in optimal habitats (mainly seagrasses), although other methodologies can be applied to determine abundances (for further details see: García-March & Vicente, 2006).

Methodologies:

A – Strip transects will be conducted in areas with similar depth and by two trained divers (Figure A1. 1). Visual censuses were conducted along transects of 30 m length; the width were variable depending on the habitat type and choosing an optimum width (so as not to lose efficiency in the detectability of individuals). For instance, in the case of *Posidonia oceanica* seagrass meadows the total width might be 2.5 m obtaining a total prospected surface per transect of 75 m^2 (Vázquez-Luis et al. 2014b). Therefore, prospected surface will be calculated in order to express density in ind./100 m^2. At least 5 transects per station must be conducted. The GPS point at one of the extremes of each transect will be registered through a surface buoy; as well as initial and final depth. Each diver record the direction of the census (from 0 to 30 m, or from 30 to 0 m). When detecting an individual of *P. nobilis*, maximum shell width is recorded to provide valuable information on population size structure (see descriptor 2). The tape meter where is situated the individual is also recorded. Thus, from each individual of *P. nobilis* it must be recorded: position according to the tape measure in which it is found, state (alive, dead), shell position (vertical, lying down), and maximum width of the valve (cm).

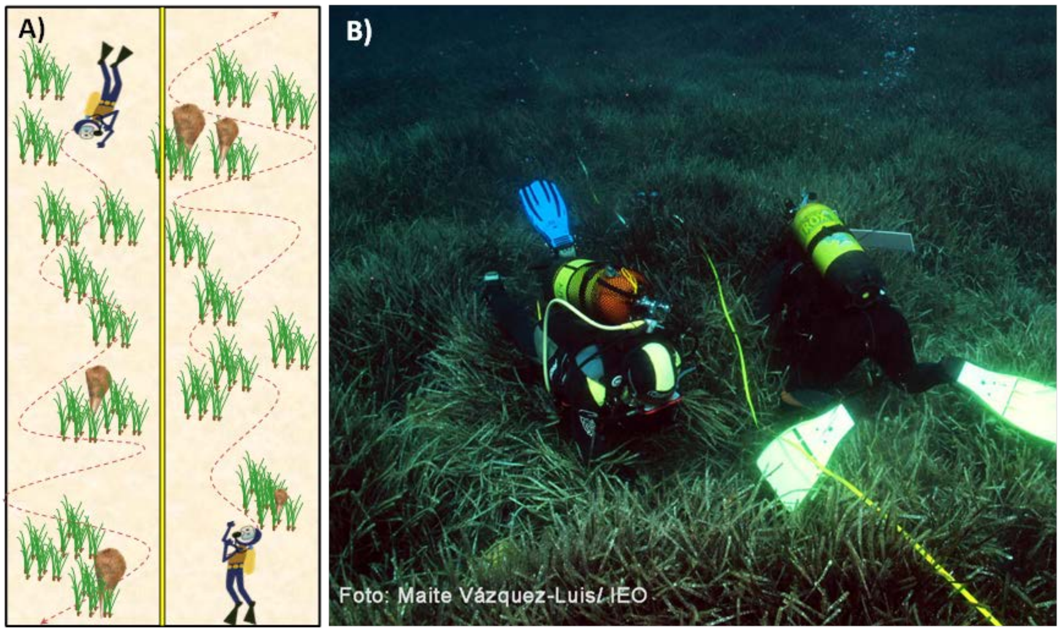


Figure A1. 1. Scheme of strip transects (left), and scuba divers making a strip transect (right).

B – Circle sampling will be conducted in areas with similar depth and by two trained divers (Figure A1. 2). In this methodology the survey of individuals is done in a circle area of at least 6 m of radius (prospected surface of 113 m^2). The center of the circle will be marked with a metal bar attached to a buoy at the surface in order to register the GPS point. At least 3 replicates per station must be conducted. The prospection of this surface can be done in two ways: 1) from the center of the circle two metric tapes or plumbed ropes will be extended from N-S and E-W direction (e.g. for a circle of 6 m radius the rope will have a length of 12 m). Therefore each diver will carry out the prospection of 2 quadrants. Or: 2) from the center of the circle a metric tape is extended with the chosen radius where the two divers will be placed dividing the surface to be explored proportionally (e.g. one diver explores the area between 0-4 m and the other 4-6), (for more details see: García-March & Nardo 2006). For both types of search, the depth of the center of the circle will be GPS positioned. Thus, from each individual of *P. nobilis* it must be recorded: orientation in degrees (0-360º), state (alive, dead), maximum valve width (cm), and shell position (vertical, lying down).

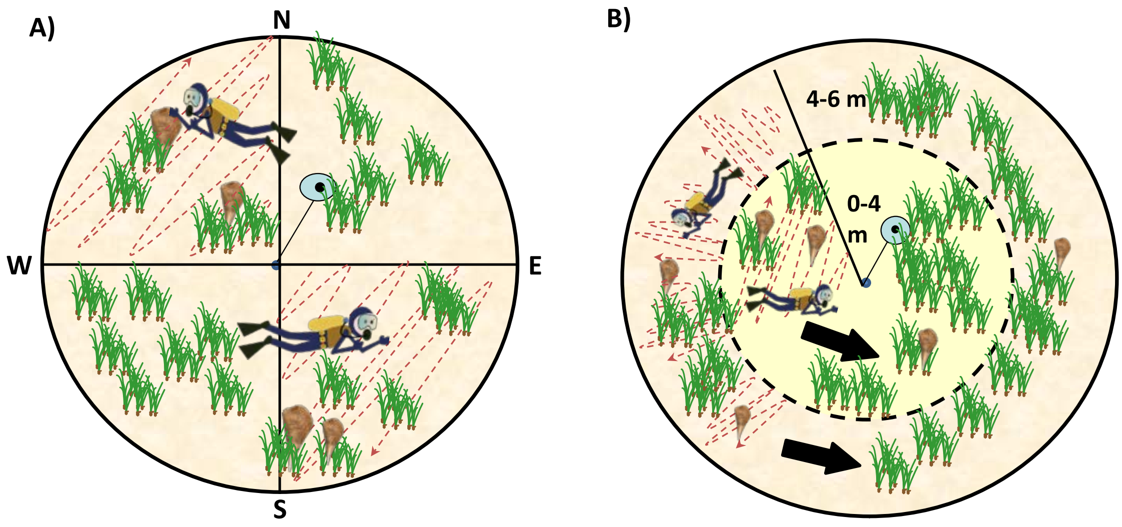


Figure A1. 2. Scheme of the circular search by the two methods. A) Search of individuals dividing the circle into 4 sectors; and B) Search of individuals by dividing the circle into two sectors based on the radius of the circle.

Results Descriptor 1: Mean density of *P. nobilis* (ind./100m^2). The calculation of the mean density of individuals in a given area will be made taking into account all the replicates (transects/circles) made in the area, even if the density value is zero.

Frequency: It is recommended that the assessment of the abundance of pen shell populations be carried out annually or at least every two years. Regarding the time of year in which the work should be done, it is advisable to take into account the periodicity and the different morphology of the meadows of *P. oceanica*, its main habitat. The leaf length will condition the detection power of the specimens. During summer pen shell individuals are hidden by the foliar canopy of the meadow, while in winter the leaf length is smaller and facilitates the encountering of specimens. Therefore, the most favorable time for sampling is between October and December when the length of the leaf canopy is lower. In any case, in this type of work, it is advisable that annual or biennial follow-ups are always carried out at the same time of the year.

4.2. Descriptor 2: Size structure of the population

Proposed methodology: sampling of this variable is done simultaneously with variable 1. To determine size structure of *P. nobilis* population it is proposed to use only the biometric measure of maximum shell width. This measurement has already demonstrated to be correlated with total length of the individual. In young specimens the maximum width can be found in the top of the valve, however in adults can be found in the inflection point (Figure A1. 3). It is important to remark that in old and big specimens maximum shell width is found over the inflection point, in these cases is recommended to register both widths (at inflection point and the maximum). The maximum width values of all measured individuals will be used to estimate the size structure of *P. nobilis* in size classes at each station. In order to measure the maximum shell width of each *P. nobilis* individual, L-shaped device designed for this purpose is proposed to use (Figure A1. 4a), which serves also to record related data. The device is easy to construct and can be made in various materials of plastic type, in the example presented is of the same material of the tablets, a white PVC in which can be written with pencil. To measure the width of the shell, once the pen shell is found, the device is placed horizontally, as perpendicular as possible to the valve. Its L-shape makes it easy to have one side supported on the leaflet (the short side of the L), while on the long side it is where the maximum width is marked with the pencil (Figure A1. 4b). For each width mark, related information about each individual must be recorded: transect number (T1, T2, ...), the tape meter in which is found (1 m, 3.5 m, 24 m ...), the state (alive, dead) and the position in which the specimen is (lying down, vertical).

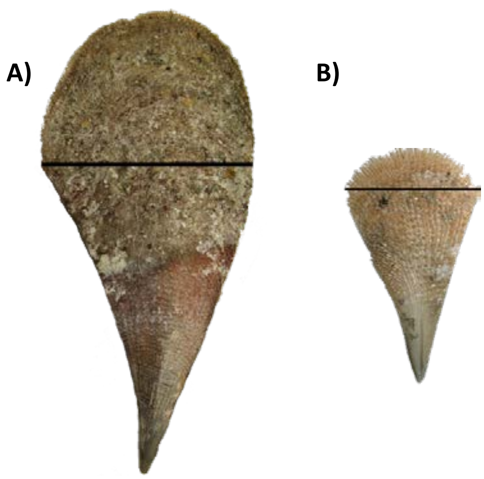


Figure A1. 3. Maximum shell width of two individuals of *Pinna nobilis*: A) maximum width measured in inflection point of an adult; and B) maximum width measured in a juvenile.

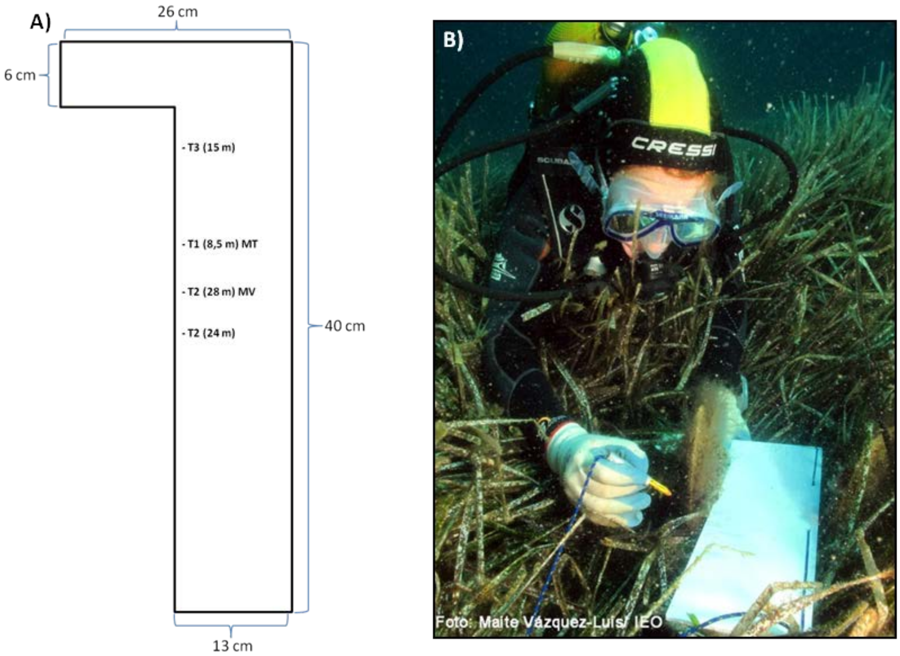


Figure A1. 4. Scheme of L-shape board designed to record maximum shell width of each valve and additional information (transect number, tape meter, position and state) (left); and a scuba diver using the device (right).

When the specimens die they are usually found lying down, for example after a storm, especially in shallow areas. In these cases it is possible to measure also the total length of the shell. To take the measure of the total length of the shell the device can be used for its longest part, or use the tape meter. It should be noted that because of the curvature of the shell the measurement of the total length is that of the projection of the valve on the tape or the device (Figure A1. 5).

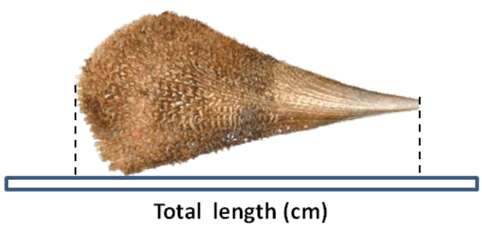


Figure A1. 5. Projection of the total length of the leaflet on the tablet for the measurement of the total length.

Results Descriptor 2: The census population will be classified according to the maximum width of the valve in size classes of 5 cm interval. In this way a number of individuals for each size class will be obtained, which can also be expressed as a percentage.

Frequency: It is proposed that both the periodicity and the time of the year in which this variable is to be evaluated, are associated with the ABUNDANCE variable.

4.3. Descriptor 3: Population dynamics of *Pinna nobilis* (demography)

Long-term studies are necessary to know the dynamics and evolution of *Pinna nobilis* populations, and demographic studies are very useful in evaluating the stability of populations and their evolution over time (Garcia-March et al., 2007). Distribution of pen shell in a meadow is usually contagious and population density is the most commonly less than 10 individuals per 100 m^2 (García-March 2005; Basso et al. 2015). The study of the demography of *Pinna nobilis* presents difficulties; one of them is related with the distribution of the species, often fragmented, that makes difficult the location of specimens. For demographic monitoring it is necessary to have a minimum number of specimens per plot, since it involves monitoring the same specimens for several years. This reason will determine the type of plot to be designed to contain a sufficient number of tagged specimens.

Proposed methodology: it is necessary a previous exploratory phase to know densities of pen shell on a given area since it is recommended to install demographic plots in areas with high density. For the study of the population demography it is therefore proposed to define limited areas with marked individuals who will be monitored annually. These areas or plots, which may have variable size, are proposed to be installed in areas of high density of individuals or in areas of aggregation of several individuals. These permanent plots should have a minimum surface area of 100 m^2 to accommodate a sufficient number of specimens. The shape of the plot may vary; in the present protocol we propose two types: the square plot, 10 x 10 m side; and the circular plot, about 5.6 m radius.

<u>Plot installation</u>

The shape of the plot may vary, in this protocol two different approaches are proposed (circular of 5.6 m of radius and square of 10 x 10 m).

A- <u>Square plot</u>: In the case of the square plot, the precision in the sampling of the demographic monitoring is increased, but the installation of the plot is time-consuming. It is recommended to install in protected areas or with low hydrodynamics. The corners of the plot are delimited by metallic bar or other resistant material. To determine the position of the bars and make a regular square it is recommended to use two metric tapes and follow step-by-step instruction of Figure A1. 6. It is important that the bars are marked by a buoy floating above the foliage canopy for easy location. Later, the bars will be joint with each other by a rope forming the external line of the plot. Once the contour is installed, the plot can be subdivided into several parts to facilitate the location of the specimens (Figure A1. 7). It can be chosen to subdivide the plot into 16 squares of 2.5 m long if the density of specimens is high, as in the case of Cabrera.

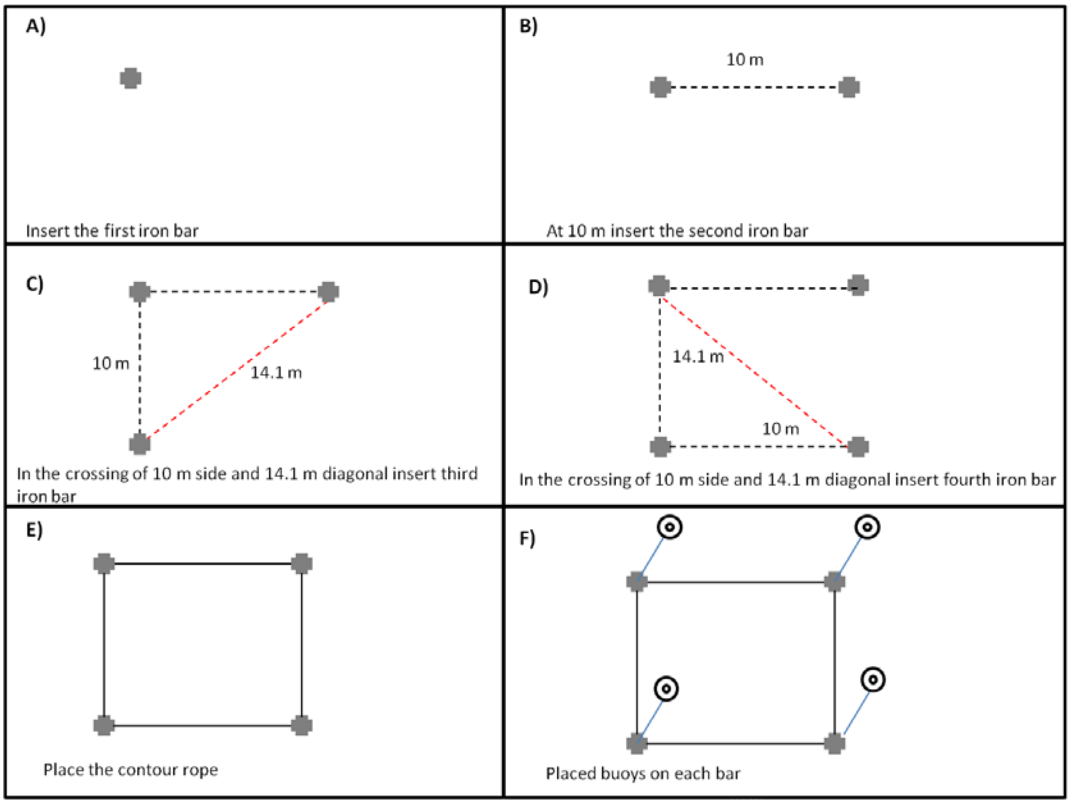


Figure A1. 6. Scheme of installation of bars of the square plot of 10 m side. The gray crosses represent the bars, the black lines the sides of the square and the red lines the measurement of the diagonals (necessary to place the third and fourth bar).

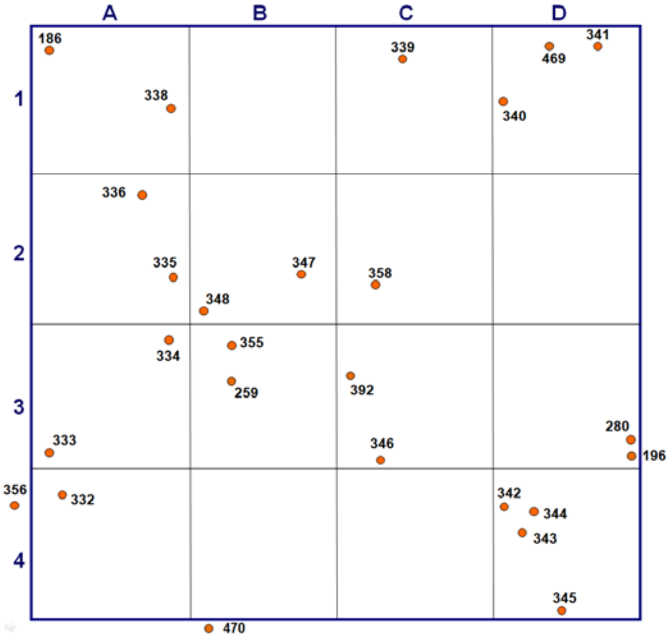


Figure A1. 7. Permanent plot situated in pristine MPA (Cabrera National Park) at 20 meters depth.

- Circle plot: the installation of the circle plot is simplest than the square, since only will be marked the center of the circle with a metal bar or an anchor structure marked with a buoy to be easily located. The center of the circle is a single structure that will remain permanently at the bottom. Similar to abundance censuses with circle, the circle must be subdivided into 4 sectors with a signal line. In the case of a circle of radius 6 m, there would be two 12 m ropes, oriented according to the geographical coordinates (N-S and E-W, Figure A1. 8). The diver that samples each sector will carry a tape measure from the center, with a length equal to the radius of the circle (6 m according to the previous example), to know where the end of the circle is and to correctly sample the entire surface of the sector).

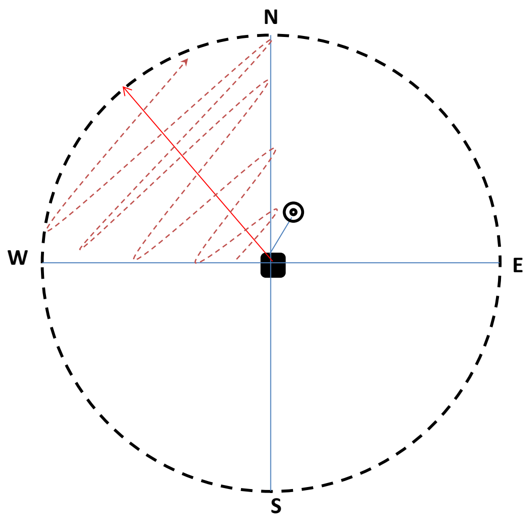


Figure A1. 8. Diagram of a circular plot, with the anchor that indicates the center of the circle and the lines that divide it into 4 sectors. The red arrow represents the tape meter.

Search and tagging of individuals

In both types of plots (square plot or circle) the position of each specimen will be mapped and marked with an identifying number. Maximum shell width will be also registered in order to study size structure of the population. To tag the pen shells 3 different non invasive methods and sufficient durability are proposed according to the size of the animal. Two types can be used for adults and one for juveniles:

- Two-component putty: attaching a numbered plastic tag to one of the valves of each individual with the aid of polyvalent two-component putty (IVEGOR, Figure A1. 9A).
- Zip tie: attaching the same plastic tag with a cable tie at the base of the individual (only with large individuals, Figure A1. 9B).
- Tent peg: attaching the same plastic tag with a cable tie at the base and near of the individual (only with small individuals, Figure A1. 9C).

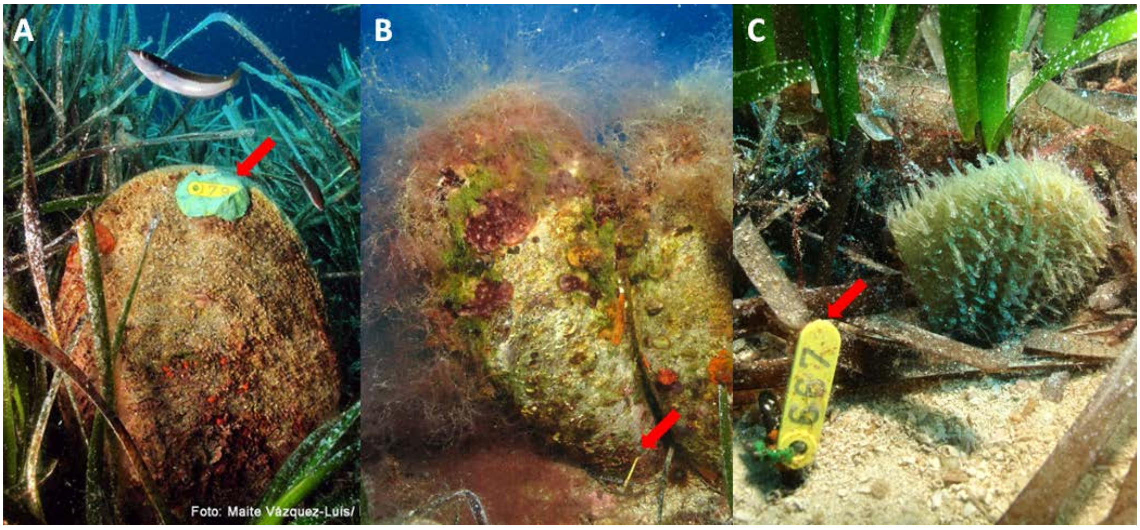


Figure A1. 9. (A) Adult individual of *P. nobilis* marked in the valve with two-component putty, (B) adult individual marked in the base with a cable tie, and (C) juvenile marked with a tent peg.

Demographic parameters require long-term monitoring to obtain recruitment and mortality rates. The first year the zero state of marked and mapped individuals is obtained and an annual monitoring of these plots will be carried out. In the annual visits maintenance tasks of the plots should be made (revision of ropes, iron bars, buoys, tent pegs, marks...), as well as demographic sampling. For demographic sampling, the mapping of the individuals present in each plot is counted, so that the individuals marked initially are reviewed to confirm their survival or mortality. Unmarked individuals (recruits) must be searched, and if found mapped and marked for tracking. To each of the specimens of the plots, in addition to record the status (alive, dead), measures of the maximum width are taken in order to assess the size structure of population.

Results: annual demographic rates of population: mortality rate, recruitment rate and population growth rate. Size structure of the population in number or percentage.

Calculation of demographic rates:

To calculate demographic rates (mortality, recruitment and growth) the formulas proposed for this species must be applied (García-March and Nardo 2006).

Specific mortality rate M (year^{-1}) is calculated based on the formula:

$$N_2/N_1 = e^{-Mt}$$

Where N_1 is the number of tagged individuals counted in the first visit, N_2 is the total number of individuals counted in the second visit, and t (days) is time elapsed between censuses.

Specific recruitment rate R (year^{-1}) calculated as:

$$\mathbf{N_2=N_1 \cdot e^{Rt}}$$

Where N_1 is the initial number of tagged individuals in the plot, N_2 total number of censed individuals during the second visit, and t (days) is time elapsed between censuses.

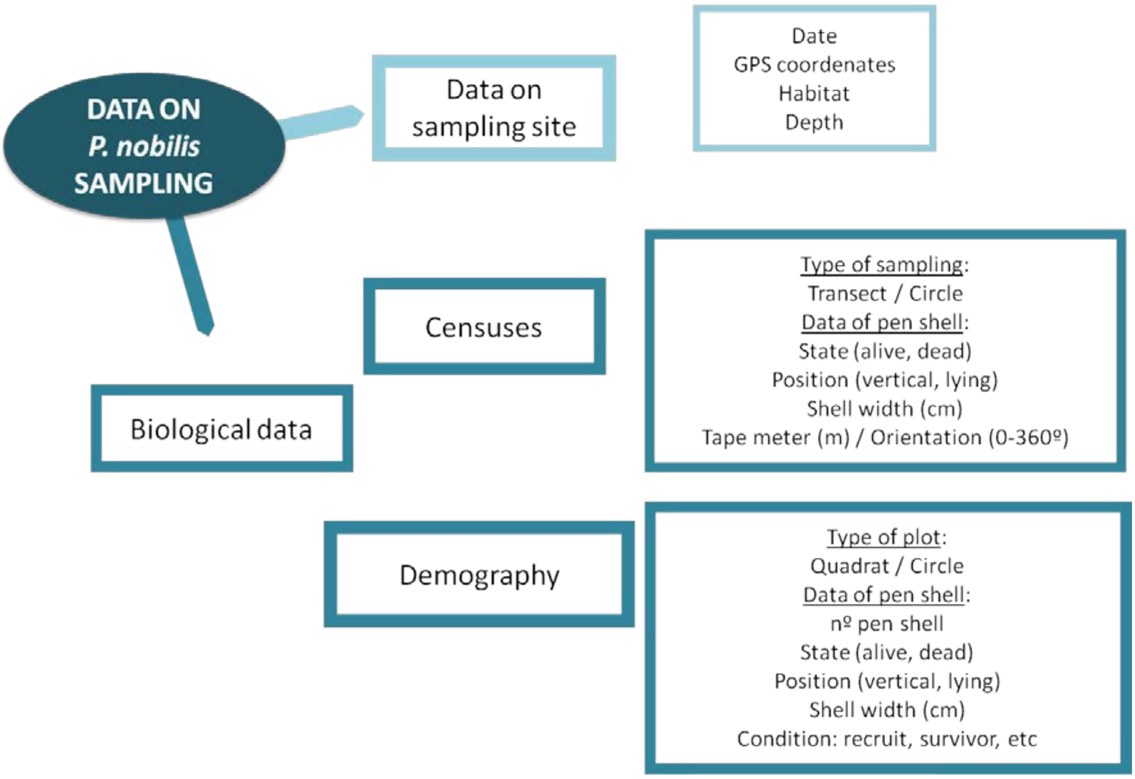
Net growth rate μ (year^{-1}) of *P.nobilis* population calculated as the difference between the mortality rate and the recruitment rate:

$$\mathbf{\mu= M-R}$$

The net population growth rate (μ) is indicative of the evolution of the population. To determine demographic trends it is necessary to carry out long term monitoring. The evolution tends to zero if population density remains stable ($\mu = 0$), is expanding ($\mu > 0$) or regressing ($\mu < 0$).

Frequency: The monitoring of plots has to be done once by year. It is recommended to do it in autumn-winter coinciding with the shorter leaves length of *Posidonia oceanica*, since allows better detection of recruits and juveniles of *P. nobilis*.

Here a diagram summarizing the descriptors proposed and data to collect on *Pinna nobilis* assessment can be found:



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