



Life Pinna

LIFE20 NAT/IT/001122

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*Conservation and re-stocking of the Pinna nobilis
in the western Mediterranean and Adriatic sea*

DELIVERABLE

D.3.1: REPORT ON THE PINNA NOBILIS STATUS EX ANTE





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Summary

Partners involved in action D3 of Life Pinna started gathering information about the presence of fan mussels in donor and receiving sites from the beginning of the project. Unfortunately, results depicted a situation dramatically different from previous data, in particular from donor sites where *P. nobilis* density collapsed to zero.

At the end of 2021, only 8 living fan mussels were observed in the Miramare MPA, where high densities were usually measured only a couple of years before. Even more impressive, the results from Strunjan MPA in 2022, where 858 shells of *P. nobilis* were counted and measured in a total surveyed area of 6200 m² (mean density of about 14 ind/100 m²) with only 2 animals alive. Monitoring campaigns realized in 2021-2022 confirmed mass mortality event also along the northern Adriatic coasts.

Data obtained from the Tyrrhenian areas selected for the Life Pinna project (i.e. Capo Mortola MCA and Asinara MPA) confirmed the absence of fan mussels along the western coasts of Italy. In Capo Mortola, only one empty shell was observed during 2022 monitoring campaign; in other areas along the Ligurian coast, where the presence of fan mussel populations was documented in 2018, no living specimen of *P. nobilis* was observed. In the Asinara MPA, the decline of the resident population of *P. nobilis* started in 2018; in 2019, only one living specimen was observed, but its presence was not confirmed during summer 2022 monitoring. Molecular studies by the University of Sassari suggested that a multifactorial disease might be responsible for the mass mortality event (Scarpa et al, 2020; Scarpa et al, 2021).

Comforting data are arising from monitoring activities in north Adriatic small harbours, also outside MPA boundaries: in 2021/2022, more than one hundred juveniles have been observed during citizen science events. This evidence underlines the importance of promoting citizen science activities to enlarge the knowledge about the presence of *P. nobilis* along the Italian coasts and, in particular, in Life Pinna areas.



Report on the *P. nobilis* status ex ante

Miramare MPA

The numerous monitoring activities carried out in recent years in the Miramare MPA and in the waters of Friuli Venezia Giulia have made it possible to assess the distribution and density of fin specimen.

The Gulf of Trieste, in particular its coastal part, was confirmed by 2019 as a basin with high abundance for the species *Pinna nobilis*. The population of this bivalve was heterogeneous in terms of age and certainly served as a recruitment and roosting site for postlarvae, but also as a reservoir of reproducers. Overall, the stock density was very high, especially compared to that of the species in the Mediterranean.

Unfortunately, a mass mortality of *P. nobilis* populations occurred in the southwestern Mediterranean in the early fall of 2016, and since then the situation has deteriorated, gradually affecting the Spanish, French, Tunisian, Turkish, and inevitably the Italian coasts. From Sardinia to Sicily, from Puglia to Tuscany, the epidemic spread and finally reached the Gulf of Trieste at the end of 2019.

The process leading to death develops rapidly, within one to three months. The latest scientific work points to a multifactorial epidemic facilitated by the average temperature increase, which favours the pathogens involved. The mass die-off from late 2019 to 2021 hit the Upper Adriatic Sea, decimating the *Pinna nobilis* population, which is also severely compromised here.

Since then, Miramare MPA, also with the support of the competent authorities, has made significant efforts at the competent authorities, to detect the few surviving individuals. The relevance of this research, as well as for the conservation of the species at the local level, is also related to the possibility that they may be characterised by a natural resistance to the pathogens responsible for the outbreak, representing a possible crucial step for the establishment of adequate recovery plans for this species in the Mediterranean.

As part of the monitoring activities of the Miramare MPA in 2021, a series of reconnaissance actions were planned to search for survivors. The action was carried out through dives in the MPA with mapping of live specimens and subsequent checks to verify their health status.

Sampling was carried out along transects 100 m long and 6 m wide; within each transect, two divers conducted the search, identifying and pointing out live specimens as appropriate. Three transects were conducted per study area. One additional search was conducted during the remaining dive time prior to surfacing. A total of 28 live specimens were mapped during the more than 30 dives conducted (Fig. 1.1).

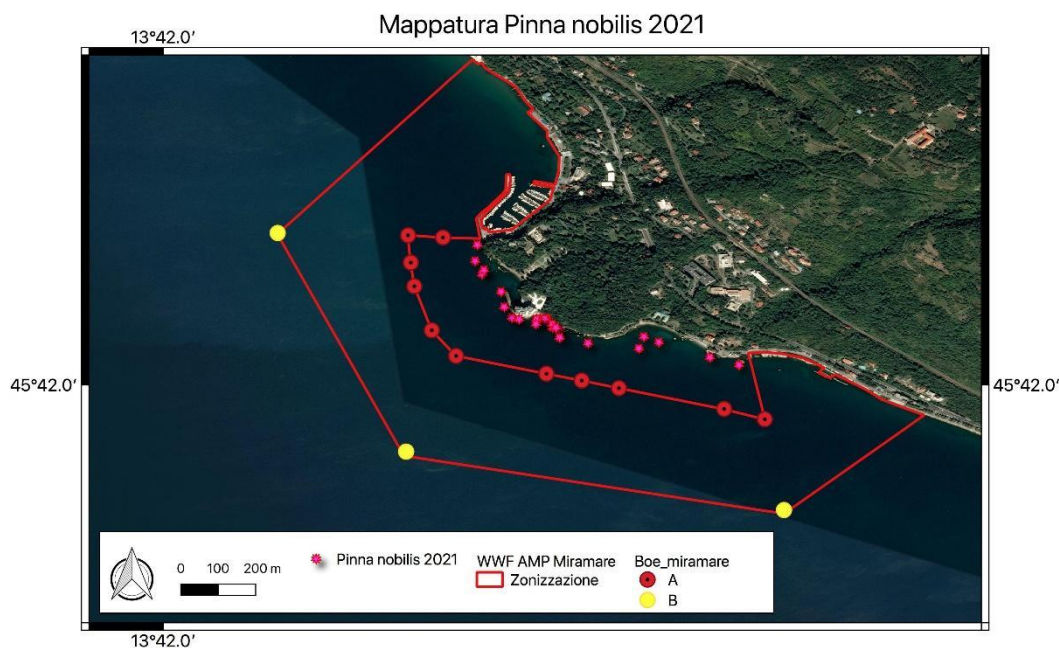


Figure 1.1. Maps of survivor in Miramare MPA in 2021.

The checks carried out showed that there were only eight live specimens as of 31/12/2021 (Fig. 1.2). It cannot be ruled out that some live specimens escaped the search operations.

During summer monitoring activities, the release of one specimen was also filmed, suggesting the possibility of additional new arrivals in future years. This hypothesis is also supported by the monitoring carried out in the areas outside the MPA, especially in the harbours, and by the collaboration with the Port Authority of Trieste, CST (Diving Club of Trieste) and Società Nautica Grignano, which led to the discovery of more *Pinna nobilis* juveniles.

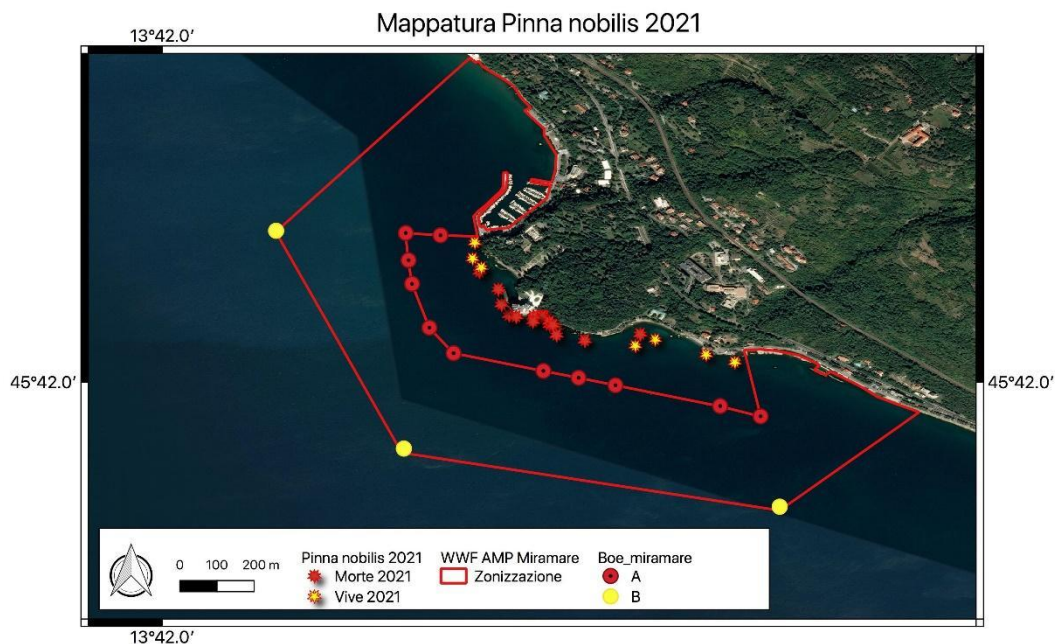


Figure 1.2. Map of dead and alive specimens in Miramare MPA at the end of 2021.

Another census, carried out on 07/11/2021 in the small port of Grignano within zone B of the Miramare MPA, revealed the presence of 18 juvenile *Pinna nobilis* individuals (max shell length outside the sediment equal to 10 cm). Twelve divers, accompanied by researchers from Miramare MPA. During this activity it was possible to map the individuals present, as shown in Figure 1.3.



Figure 1.3. Map of alive juveniles in Grignano harbour.

Other areas adjacent to the MPA were also investigated, such as the marinas and small moorings along the coast of Trieste (Sistiana, Canovella and Santa Croce). The information collected and the indications obtained also with the help of technical divers operating in the Gulf of Trieste area allowed mapping the presence of juveniles in other port areas (Pier F.lli Bandiera -Port of Trieste, Bay of Sistiana and Porto Piccolo) (Fig. 1.4).

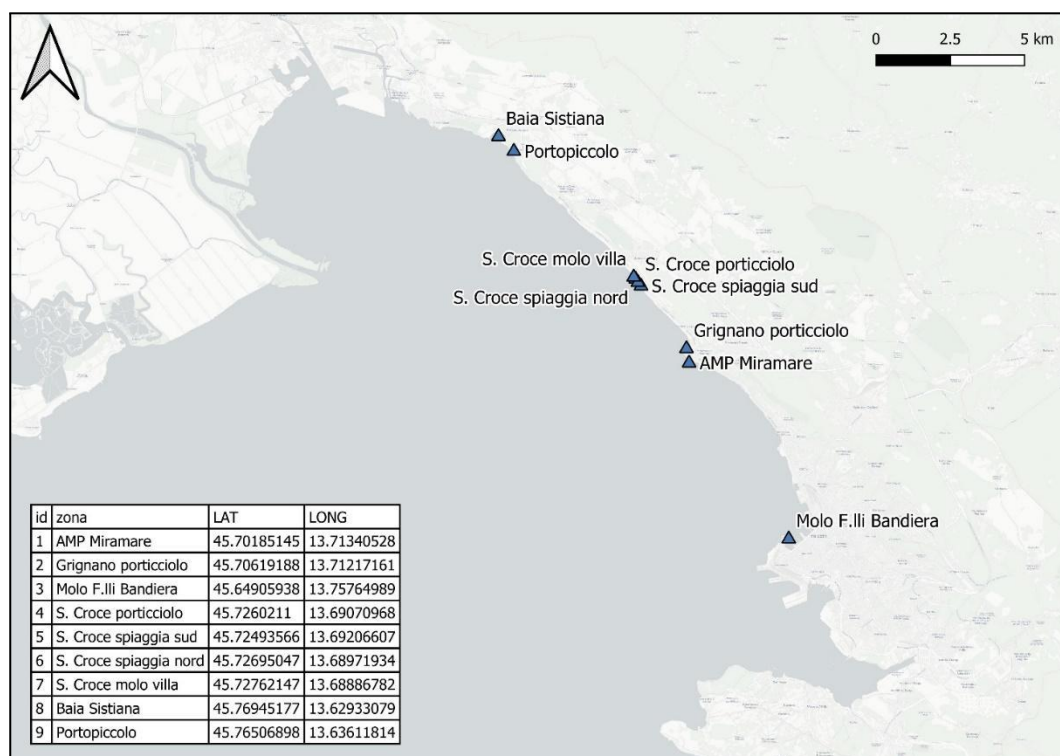


Figure 1.4. Areas in the Gulf of Trieste (blue triangles) where the presence of juvenile individuals has been reported (Bay of Sistiana, Portopiccolo, F.lli Bandiera pier) or of which there is certainty thanks to monitoring or surveys carried out (S. Croce, Grignano).

Monitoring activities conducted in the Gulf of Trieste by the Working Group (Shoreline/NIB) in 2021/2022 resulted in the identification and mapping of numerous living individuals of *Pinna nobilis*. The distribution is broad both spatially and in size, with generally more live individuals in the northernmost areas. Regarding juveniles (max. 15 cm), more than 100 specimens were found in the marine section between Barcola and Valle Cavanata in the summer of 2022 (Fig. 1.5), and were constantly monitored for viability. The number of identified live adults is about 30.



Figure 1.5. Map of monitored areas.

To this date, a very high, but not yet conclusively evaluated, percentage of juveniles have either been found dead (dead individuals with their shells still visible in the substrate but completely empty) or not found at all. This last piece of information is particularly important because the failure to find the shells suggests that the cause of death was due to predation rather than the effects of the disease that decimates *P. nobilis* populations. Many of these juveniles also settled in unsafe conditions (Fig. 1.6). The extreme rapidity with which once dead animals decompose and/or are captured makes it very difficult to collect samples useful for diagnosing the presence of pathogens partly responsible for the mass mortality. However, it was possible to examine some of the dead individuals (both captive and wild), which were variously distributed, and the presence of pathogens, especially the protozoan *Haplosporidium pinnae*, was never detected. It is also interesting to note that adults of *P. nobilis* do not appear to have suffered any significant losses.

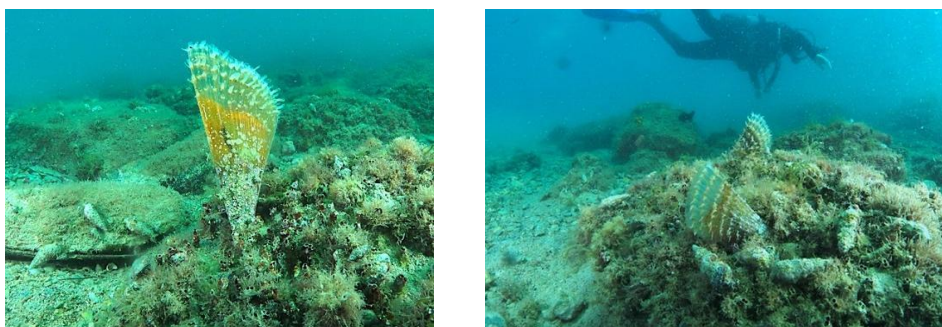


Figure 1.6. Juveniles of *Pinna nobilis* on rocks.

The combination of these activities seems to highlight the importance of harbour structures that have features conducive to rooting and subsequent growth of *Pinna nobilis* larvae, but that pose a risk to young individuals. The overhead lines, mooring lines, and bodies to which the various boats are attached can very easily injure the juveniles as these typical elements of the harbour



environment are dragged and worked. These unsafe conditions are also found in many settlements in nature (Fig. 1.6).



Strunjan MPA

The Strunjan Landscape Park was founded on February 2, 1990. The main goal of the institution was the protection of natural values and the preservation of biodiversity and landscape diversity. The park has three close protected areas (Fig. 2.1): Strunjan Nature Reserve (Naravni rezervat Strunjan), Strunjan-Stjuža Nature Reserve (Naravni rezervat Strunjan-Stjuža) and Natural Monument Pine Trees Avenue (Naravni spomenik Pinijev drevored). The park area preserves the landscape with its mosaic distribution of landscape structures, the ecological characteristics of the salt pans, the lagoon and the seashore, as well as the natural processes and connections between the supralittoral, the midlittoral and the infralittoral zones (KPS, 2019).

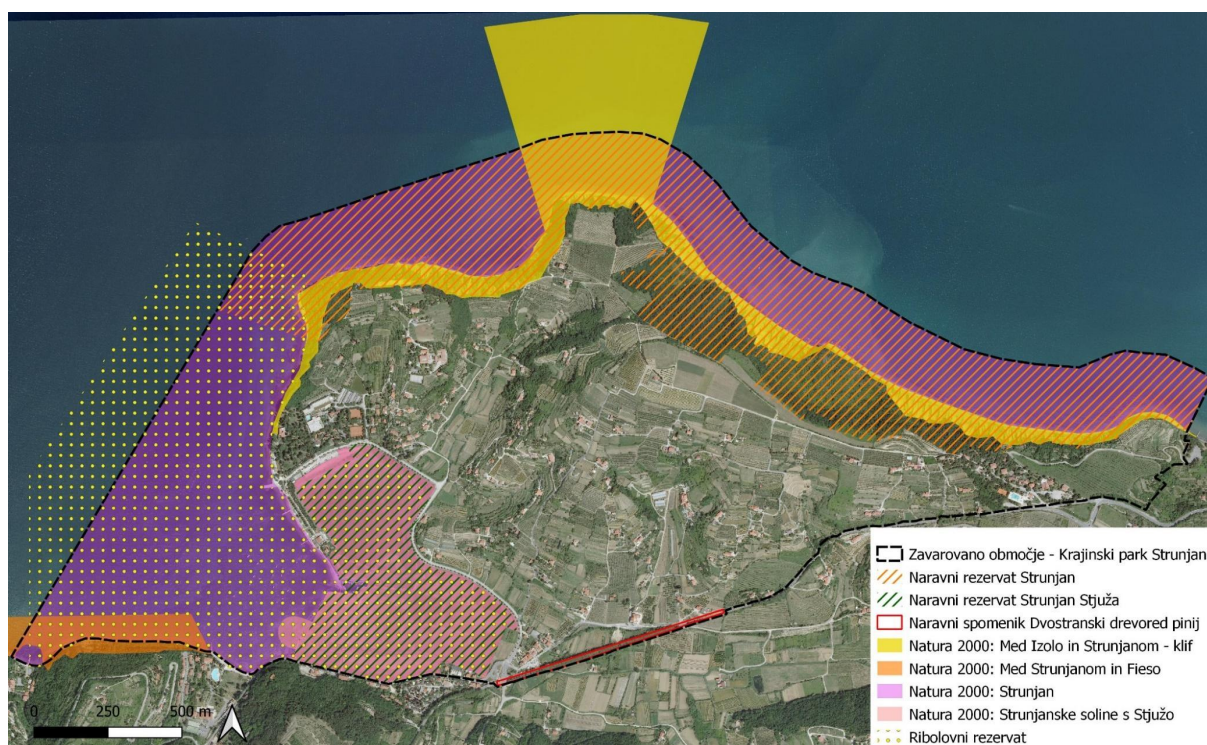


Figure 2.1. Protected areas of the Strunjan Landscape Park.

The selected donor and receiving areas are located in the Strunjan Landscape Park, within the Natura 2000 and fishing reserve area (Fig. 2.2; 45°31'56.22 "N; 13°35'58.42 "E), and include the area of the mussel farm where the collection of *Pinna nobilis* is planned. They were selected and described within the Actions A1.1 (deliverable D_A1.2) and A1.2 (deliverable D_A1.2).



Figure 2.2. Donor and receiving site area in the Landscape Park Strunjan (white polygon, 45°31'41.55" N; 13°35'55.76" E).

Within this zone the monitoring of the status of *P. nobilis* ex-ante was performed during spring and summer 2022 by conducting visual censuses under scuba diving. Divers were equipped with video camera, GPS, and gauge. Each transect was also recorded with a GoPro. Survey areas were selected based on cartographies and expert judgement. They were located within 12 m of depth, since deeper the conditions of sea bottom are no more suitable for *P. nobilis*. An additional transect performed at 18 m, in the area where the mussel farms are located confirmed the absence of *P. nobilis* shells. During monitoring activities data on the health, abundance, and size composition of individuals was carried out. Additionally, a citizen science activities (CS) was conducted on the 22/05/2022 with the collaboration of Strunjan MPA, Shoreline and diving center Ocean Divers. With those activities additional information on *P. nobilis* in the Strunjan MPA were collected.

Altogether 858 shells of *P. nobilis* were counted and measured in a total surveyed area of 6200 m². Only two of them were alive and have been observed within the Strunjan MPA, outside donor and receiving area, during the citizen science activities. Those two specimens were adults and measured 32 x 15 x 10 cm, and 38 x 24 x 16 cm, respectively.

Considering the empty shells of *P. nobilis*, 659 were still upright, whereas 199 were lying down to the floor, and 67 of them were broken.



Only 11 shells belonged to juveniles (less than 15 cm of height) and they were all empty. They were found mainly between 5 and 7 m depth, with only 2 juvenile shells found between 3 and 4 m depth. The average height of shells was 34 (± 9 SD) cm, the average maximum length was 17 (± 3 SD) cm, minimum length was 12 (± 3 SD) cm.

The average density was 14 (± 9 SD) empty shells for 100 m², with the lowest density at 11 m depth (0.5/100 m²) and the highest density found at 4-5m depth (39/100 m²). Those data are in accordance with the survey conducted in 2012 (Lipej et al., 2012), when average densities of *P. nobilis* of 14 to 33 specimens/100 m² was recorded in Strunjan MPA. The highest density (38 specimens/100 m²) was observed at 4 m and 8 m depth (Lipej et al., 2012). Therefore, our data confirmed the depth range of 5-8 m as the most suitable for transplanting *P. nobilis* as planned within the Action A.1.2.

In the receiving area no evident signs of anthropogenic disturbance, such as signs of anchoring, fish nets or marine litter, have been observed during the monitoring activities, confirming it as a proper site for *P. nobilis* transplantation activities.

Most of *P. nobilis* shells were observed in sandy area. In the past the area was inhabited by a seagrass meadow of *C. nodosa*, that has drastically declined between 2017 and 2018, likely related to sediment deposition and increased water turbidity that occurred during the construction of the fishing pier and the restoration of the western lagoon shore (Lipej et al., 2018; Ivajnsiĉ et al., 2022). Later on it completely disappeared (more details in deliverable A.1.2). Seagrass meadow will likely recover and sediment deposition should not be a problem for the recruited specimens of *P. nobilis* in the future, since no construction or other encroachment is planned in this area.

To have a clearer view on the status of *P. nobilis* along the Slovenian coast, 10 additional sites were monitored, for a total surveyed area of 2700 m². Altogether 648 dead shells were counted and measured, 13 of them were juvenile (less than 15 cm). Only 2 alive specimens were found and georeferenced at two sites (Debeli rtiĉ and Ankaran), and they were both juvenile, measuring 10 x 4.5 cm and 11 x 4 cm respectively.

Considering the empty shells 170 were lying on the seafloor, while 394 were still upright. The highest density of dead shells was found at Seĉovlje between 2 and 3 m depth (142 empty shells for 100m²). The depth with the highest density varied according to the site, ranging from 2-3m at Seĉovlje to 10m at Piran Valobran (32 empty shells per 100m²). In deeper waters only few single individuals were found. Shells of juvenile were found only in the depth range between 2 and 7 m.

Moreover, with citizen science activities a database of *P. nobilis* sightings was created. Thanks to the collaboration of colleagues and other divers a total of 24 records were collected, for a total of 28 living specimens of *P. nobilis*. Most of them were juveniles, but few specimens of 20 cm were also reported.

Capo Mortola MPA

Capo Mortola is a rocky promontory located in the westernmost part of the Ligurian coast. Since 2020, Regione Liguria established the Capo Mortola Marine Conservation Area (MCA), as part of the Hanbury Botanical Gardens Regional Protected Area, included in the Nature 2000 site "Fondali Capo Mortola – San Gaetano" (IT 1316175).

The seabed is characterised by the presence of a vast *P. oceanica* meadow that develops to a maximum depth of 30 m, and rocky outcrops and coralligenous formations which provides shelter to a great number of animal and plant species, including vertebrates and invertebrates protected by European regulations. The highly diversified coralligenous population settles within the meadow and beyond its lower limit, at depths greater than 30 m (Fig. 3.1).

The MCA is also a valuable conservation area due to its geological and geomorphological features, including a karstic freshwater spring, known as the "Polla di Rovereto", which gushes out 1 km from the coast at a depth of about 40 m (Calvino and Stefanon, 1963). The presence of *Pinna nobilis* in this area has been documented by several scientific and recreational divers before mass mortality event.

Monitoring activities were carried out on November 2022 by UBICA srl with Underwater Scientific Divers on behalf of ARPAL, following the national safety standards for environmental monitoring (ISPRA, 2013).

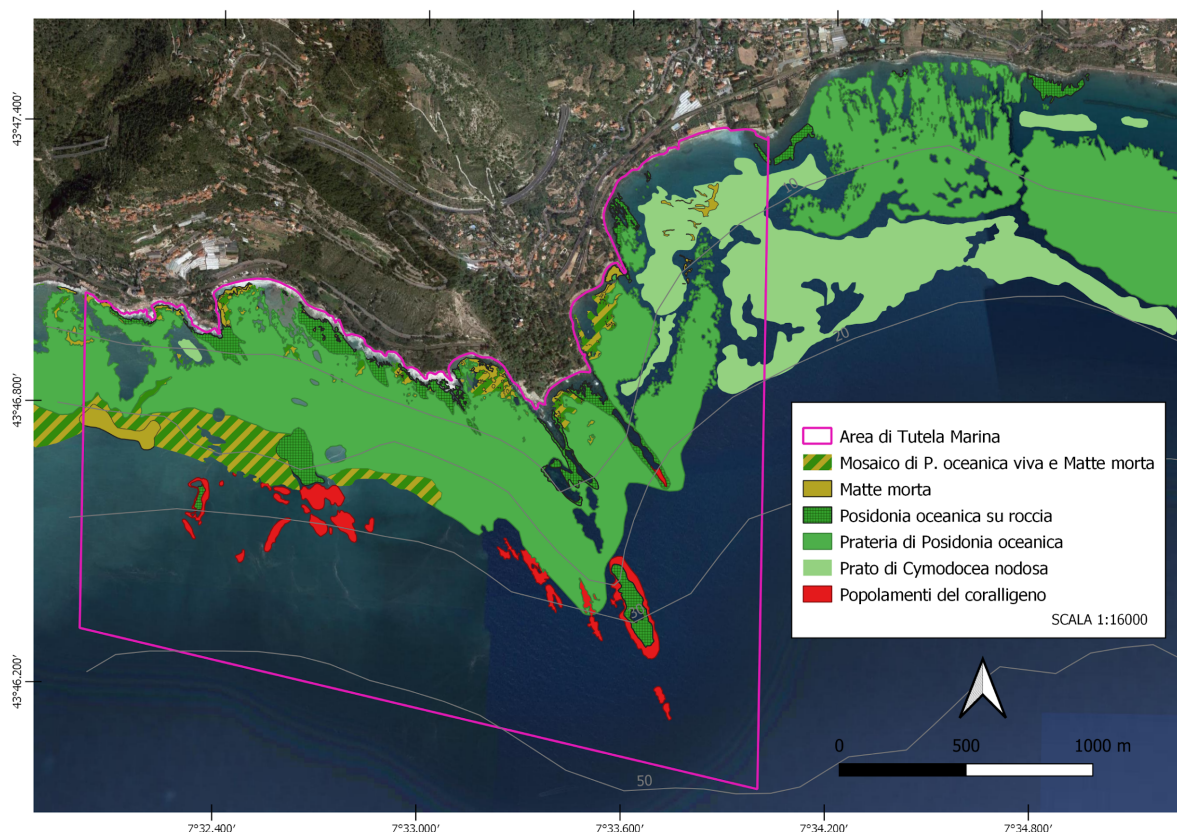


Figure 3.1. Bionomic map of habitats in the Capo Mortola MCA (Coppo et al., 2020).

In order to monitor presence and health status of *P. nobilis* in the Capo Mortola area ex-ante and ex-post transplantation of the organisms for restocking, 3 sites were identified (Tab. 3.1 and Fig. 3.2).

The sites were chosen on the basis of:

- the opportunity to investigate different habitats at different bathymetries;
- the need to focus the survey effort on the Capo Mortola area, considered the most suitable for the possible transplantation of organisms;
- evidence of *P. nobilis* presence in the area before the mass mortality event.

Site	Lat.	Lon.	Depth (m)	Note
MOR-A	43.780600	7.538360	28	Report of <i>Pinna nobilis</i> (2017) by Reef Check (id 59473634)
MOR-B	43.776105	7.558325	15	Shallow point, near coast
MOR-C	43.77252	7.560065	20	Deep Point, far from the coast

Table 3.1. Description of the sites with name, coordinates (latitude and longitude) and depths of the starting point of the first transect and notes on site characteristics.

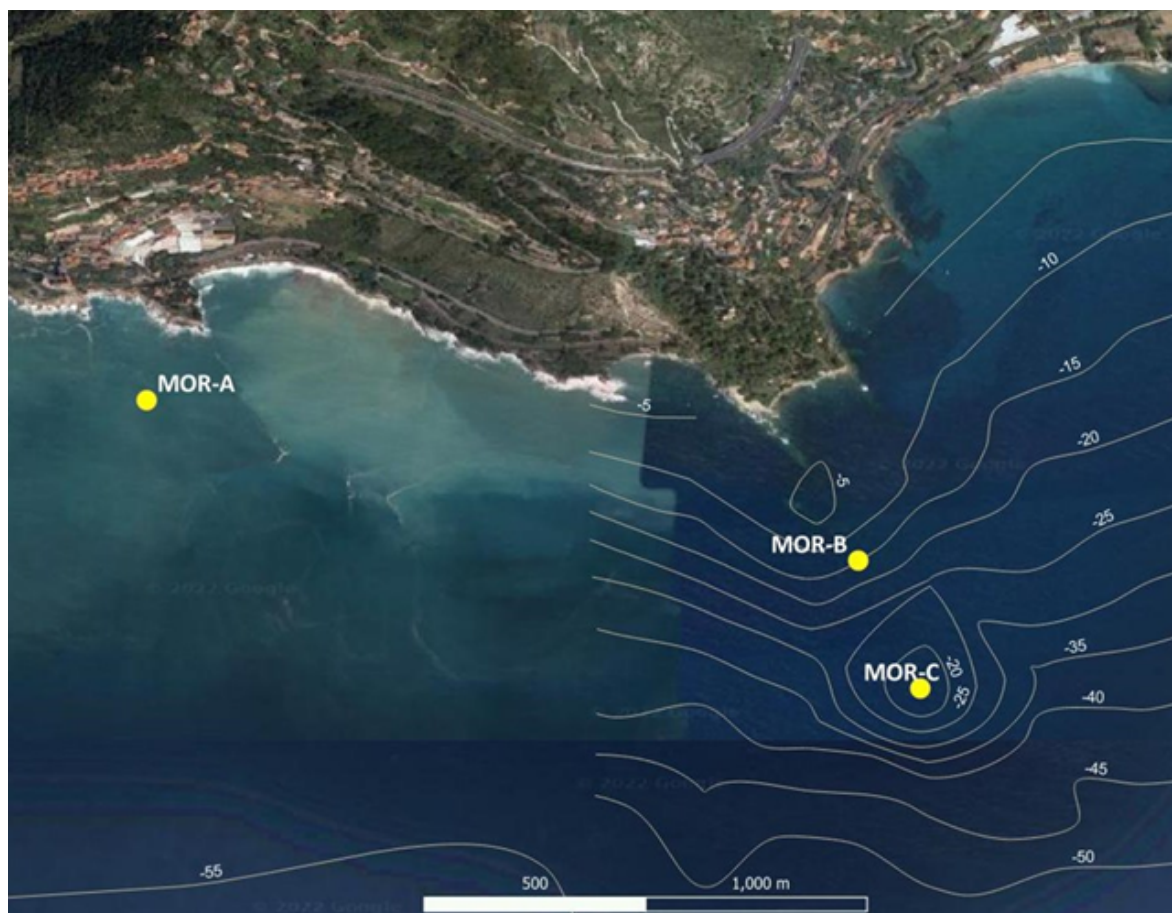




Figure 3.2. Map of the 3 monitoring sites MOR-A, MOR-B, MOR-C.

At each study site (corresponding to cells of 100 m x 100 m), 3 linear transects of 100 m length have been defined. Data are acquired by visual census considering an area of 600 m² per transect (100 m length and 6 m width) (La Mesa et al, 2019). All specimens of *P. nobilis* are counted and measured for collecting information on:

- health status (alive, dead or injured);
- coordinates (latitude, longitude and depth);
- substrate (e.g. Posidonia meadow, sandy bottom);
- impacts from human activities.

Measured biometric variables are (Fig. 3.3):

- unburied length (h);
- maximum shell width (W);
- shell width at the base (w).

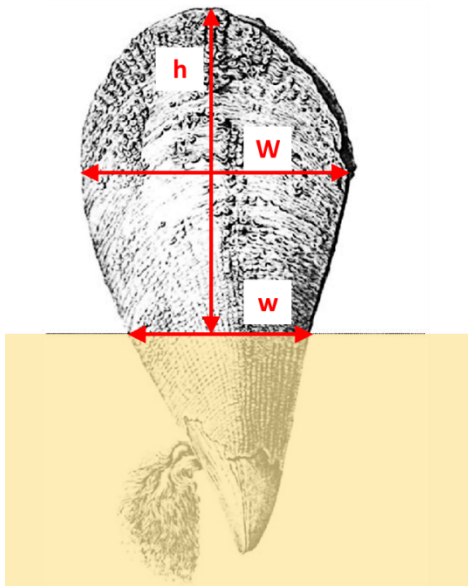


Figure 3.3. Biometry of *P. nobilis*.

Monitoring was carried out at 3 sites at different depths (- 28 m, - 20 m and - 15 m depth, respectively) by surveying, at each site, 3 transects of 100 m each, about 30 m apart (Fig. 3.4).



Figure 3.4. Map of the 3 transects of 100 m length (TA, TB and TC, respectively) at the 3 sites (MOR-A, MOR-B and MOR-C).

The MOR-C site is located about 20 m deep and characterized mainly by pre-coralligenous formations with calcareous bio-concretions, sponges, gorgonians (e.g. *Eunicella cavolinii*) (Fig. 3.5A) and bryozoans. A still intact and upright shell of *P. nobilis* was found at the 50th meter of the 3rd transect, at a depth of 21 m. The two valves were photographed and measured (Fig. 3.5B).



A



B

Figure 3.5. (A) MOR-C site characterized mainly by pre-coralligenous formations with calcareous bio-concretions, sponges, gorgonians (e.g. *Eunicella cavolinii*) and bryozoans; (B) dead but still intact and upright shell of *P. nobilis*.

The MOR-B site, located 15 m deep, is characterized by *Posidonia oceanica* meadows interrupted, in the deepest part, by rocks covered with coralline algae and, in the shallowest part, by leafy algae. No specimens of *P. nobilis*, either alive or dead, were found at this site (Fig. 3.6).



A



B

Figure 3.6. (A) Site MOR_B characterized by *P. oceanica* meadow interrupted by rocky outcrops in the superficial areas; (B) Deep areas of site MOR-B.

MOR-A, the deepest site (- 28 m depth) and the westernmost of the 3 sites, is characterized by a dense *P. oceanica* meadow with boulders of different dimensions and sandy corridors, probably due to the disturbance of anchorages (Fig. 3.7A). In this site a fragment of a *P. nobilis* shell was found, measured and photographed (Fig. 3.7B).



A



B



Figure 3.7. (A) MOR-A site characterized by dense *P. oceanica* meadow with boulders and sandy corridors;
(B) fragments of *P. nobilis* shell found at the site.

Additional efforts to gain information on the presence of *P. nobilis* specimen have been carried out in the area of Capo Mortola in 2022.

In the framework of action A.1 of the Life Pinna project, eight HD video transects (about 500 m of length) have been realized in the Capo Mortola area; other six analogous videos have been recorded in the eastern nearby zone (Fig. 3.8). These fourteen videos have been analysed to find insights on the presence of fan mussels: unfortunately, no evidence has been found.

Furthermore, a rapid monitoring protocol, based on the circle monitoring approach (García-March & Vicente, 2006), has been developed and applied in two sites (Fig. 3.8). For each site, a visual census has been performed in a circle of 10 m radius (surface of about 300 m²): no evidence of fan mussel has been observed.

The drastic reduction of *P. nobilis* population along the Ligurian coast was confirmed during a monitoring campaign realized in 2022 in three areas where the presence of fan mussel was documented in 2018.



Figure 3.8. Lines represent HD video transects. Stars indicate circle monitoring areas.



Asinara MPA

The Asinara National Park - Marine Protected Area "Isola dell'Asinara", established in 1997, is an institutional body emanating from the Ministry of the Environment and Energy Security, responsible for the protection of biodiversity (habitats and species), the landscape and of cultural and historical values, as well as promoter of sustainability education actions, scientific dissemination and sustainable use of environmental assets.

As far as the marine environment is concerned, the island is characterized by two main sides which determine a high variety of macro and micro environments which is reflected in the associated biological component.

In particular, the limited depth and slope of the eastern seabed and the weather-marine characteristics of this side favor the development of extensive and well-structured *Posidonia oceanica* meadows, which represent the typical elements of this part of the island.

Overall, the prairie appears in excellent condition, and in general there are no degraded points, even if there are small areas in regression and areas in which traces of trawling activity can be found, despite this being a strictly prohibited activity within the Marine Protected Area.

The state of general well-being of the *Posidonia* meadows of Asinara is also demonstrated by the analysis of the structure, by the periodic presence of flowers and fruits and by the high values of leaf density.

The prairies together with the sandy and muddy bottoms of the rias of the eastern and southern slopes represent the ideal habitat for the distribution of *Pinna nobilis*.

As regards the population of *Pinna nobilis* within the Marine Protected Area of the Asinara Island, until 2018, a good overall number and density of individuals was reported, with important points of aggregation, as well as a unimodal distribution of the population, which indicate a good recruitment of the youth classes and a general situation of balance.

However, starting from the first months of 2018, important mortality events were reported, which can be estimated at a variable percentage between 20 and 50% of individuals and a general state of reduced reactivity of many live specimens, with valve closing reaction times even of 8 seconds, which highlighted an infection in progress.

An agreement was thus activated between the Park and the Department of Veterinary Medicine of the University of Sassari, as part of the Marine Strategy project coordinated by the Ministry of the Environment, to statistically ascertain the phenomenon and study, also with samples of biological tissue with non-lethal techniques, the possible causes and to investigate the population also from a genetic point of view. The scientific activity was coordinated by Prof. Marco Casu. In fact, from the 2018 "Marine Strategy" study, in which 55,800 m² were accurately surveyed within 31 cells (310,000 m²), it emerged that mortality deteriorated very rapidly, going from 50% at the beginning of August, with just over 350 individuals, at 80-90% at the end of October 2018 with less than 50 individuals present.



In 2019, the Asinara MPA, within a regional plan ("Regional Action Plan for the conservation of *Pinna nobilis*" Resolution G.R. n. 61/39 of 12/18/2018), promoted a specific monitoring of 6 sample areas, already studied in the past (in the areas of Fornelli, La Reale and Cala d'Olive and an external area on Island Piana) supported by genetic analyzes aimed at identifying the aetiological agents that caused their mortality mass.

The results confirmed the seriousness of the situation for the *Pinna nobilis* species in the study area and at the end of the censuses only one surviving individual was found at the Island Piana site. This individual, on the basis of the parameters defined by the protocol, was found to be asymptomatic since following external stimuli it responded with a rapid and complete closure of the valves. These characteristics made it an extremely important specimen as it could have been the representative of a resistant lineage. Unfortunately, its presence was confirmed only until 2021. In fact, further inspections carried out by the research group of Prof. Marco Casu in conjunction with the censuses/sampling of the *Paracentrotus lividus* species in the summer of 2022 did not confirm its presence and given a negative result throughout the island, so much so that at the end of 2022 there were no surviving individuals of *Pinna nobilis* within the Isola dell'Asinara MPA.

The various molecular surveys carried out from 2018 to today have confirmed that the disease that causes mass mortality is not carried by a single species-specific pathogen, rather by an ecologically plastic environmental pathogen adapted to infect multiple filter-feeding organisms. Furthermore, the alternation of pathogens in the different specimens analyzed suggests that the mortality events of *Pinna nobilis* individuals are caused by a multifactorial pathology never described before for these species. In this context, it is conceivable that the pathogens found are always present in the water column, going through phases of different virulence influenced by the oscillations of the environmental parameters and which influence each other.

In this context, since within the Life Pinna project the Asinara Island is a receiving specimens area of *Pinna nobilis*, considered the current context and given the fluctuating presence of the pathogens found so far, during the preparatory actions it is of fundamental importance to understand the level of presence of pathogens through the use of sentinel species in order to identify the "pathogen-free" sites in which to reinstall individuals recruited elsewhere or reproduced in the laboratory.

In particular, the sites located in Cala di Sombro di Dentro, in an area of maximum respect, and Cala Marcutza have been identified at the moment. However, the latest analyzes are underway to confirm the suitability of these areas (Fig. 4.1).

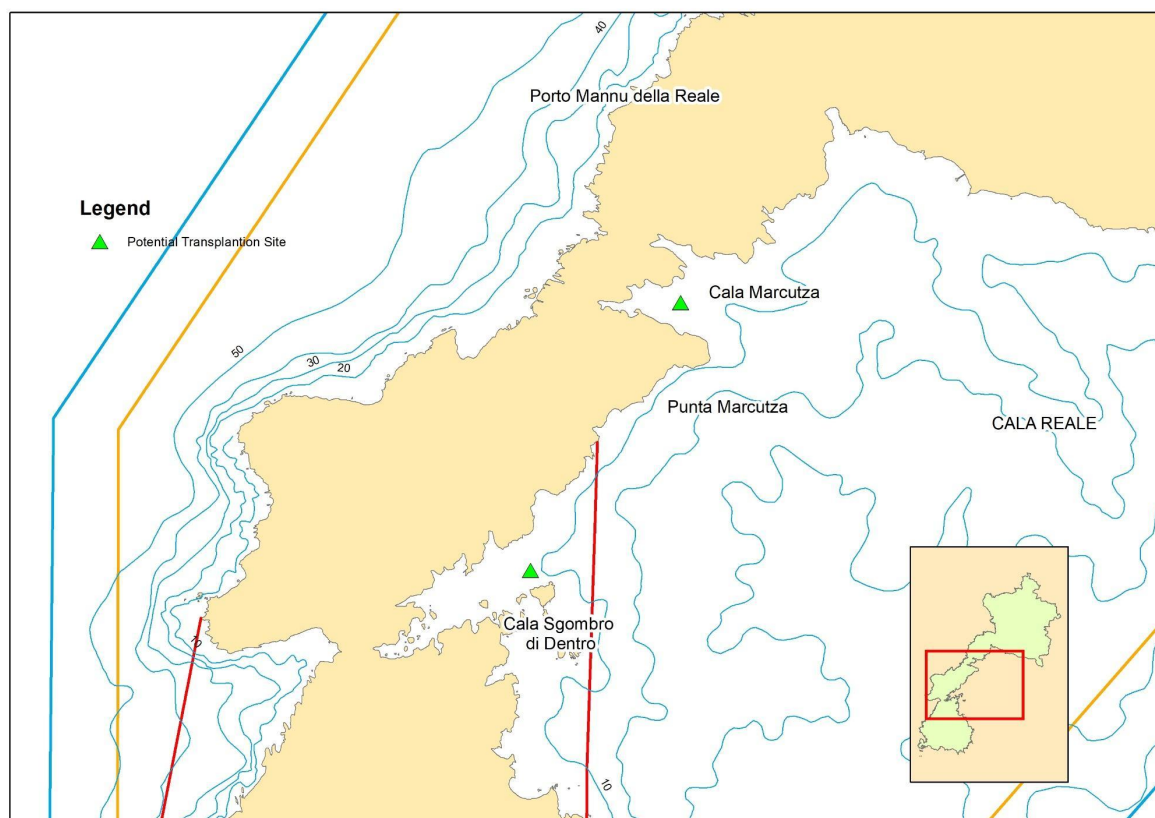


Figure 4.1.



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