LIFE PINNARCA

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Contents

Background

1 SPECIES STATUS AND CURRENT SITUATION

1.1. Biology and Ecology

The fan mussel or pen shell *Pinna nobilis*, as it is known in some areas, is an emblematic large marine bivalve of the order Ostreida, which was first described by Linnaeus in 1758 and belongs to the family Pinnidae. Due to their morphological characteristics, bivalves of this group are classified in a single superfamily, with a single family and three genera: Superfamily Pinnoidea (Leach 1819), Family Pinnidae (Leach 1819), genera *Pinna* (Linnaeus, 1758), *Atrina* (Gray, 1842) and *Streptopinna* (Martens, 1880). Although recent phylogenetic studies consider only two genera, including the genus *Streptopinna* within the genus *Pinna* (Lemer et al. 2014). Pinnidae family, with more than 60 described species, is one of the few bivalve families where all the members are large, with *P. nobilis* being the species with the longest wingspan, exceptionally reaching up to 120 cm in shell length (Zavodnik *et al.*, 1991, Schultz & Huber 2013). Genetic studies suggest that the origin of the Family Pinnidae is possibly the Indo-Pacific, where it diversified and later invaded the Atlantic Ocean and the Mediterranean to the east (Lemer et al. 2014).

It is an endemic species of the Mediterranean Sea, where it has lived since late Miocene, 5 million years ago, in all basins except the Black Sea (Guallart & Templado 2012, Schultz and Huber, 2013). It is considered the largest mollusc in the Mediterranean and one of the largest in the world. In fact, it is an emblematic species that has been well known and appreciated since ancient times, when its populations were exploited for commercial purposes by the Romans and Egyptians. These populations used byssus as a textile material, or the mother of pearl of its shell. Fan mussel exploitation lasted until the 20th century, when it was used for the manufacture of buttons and for culinary and decorative interests in different Mediterranean countries (Basso et al., 2015, Vazquez-Luis et al. 2020).

The fan mussel has an elongated triangular-shaped shell, with a sharp lower end which is partially buried in the sediment, whereas it uses a highly developed byssus specialized in attachment to the substrate. There are important morphological differences between adult and juvenile individuals which respond to increased protection of juveniles in the early stages of life. The colour of the outer part of the shell is light brown, quite uniform. The inner part is brown and pearly. The pearly part occupies at least a third of the shell and shows growth marks of the posterior adductor muscle which some authors have used for age estimation (Richardson et al. 1999, 2004). The animal is large, with wide ctenidia and internally covers the entire shell, it has a reduced foot and the two adductor muscles are unequal in size as a result of anchoring adaptation (García-March 2005, Basso et al., 2015). Large individuals may contain pearls (Schultz and Huber, 2013). Small decapods can often be found inside the pallial cavity which they live in association with *Nepinnotheres pinnotheres* or *Pontonia pinnophylax* are some examples of crustaceans having this association.

It is a sessile bivalve whose distribution differs from a range of coastal environments, although it largely prefers *Posidonia oceanica* meadows. An endemic species that covers large areas of

Mediterranean seabed. Its network of underground rhizomes provides an ideal substrate to produce the byssus, while the exposed part of the shell is protected from the hydrodynamic of the leaf canopy, providing camouflage (García-March et al. 2007, Hendriks et al. 2011, Guallart & Templado 2012, Vázquez-Luis et al. 2014, Deudero et al. 2015). The bathymetric range of the fan mussel matches with that of the seagrass meadows, presenting higher abundances between 10-20 m (Vázquez-Luis et al. 2014b, 2015a), but it can also be found at greater depths in coastal bottoms or in maërl beds (Guallart and Templado, 2012). Individuals have been found down to 60 m depth (Templado 2001) although at deeper levels their abundances are lower, possibly associated with the disappearance of their preferred habitat (Vázquez-Luis *et al*., 2014a, 2015a). At shallow depths they are only found in areas where the waves are weak, such as coastal lagoons (De Gaulejac 1995, Russo, 2017) and estuaries (Addis et al. 2009, Prado et al. 2014, 2020a). These areas are often dominated by soft-bottom habitats such as meadows of other seagrasses such as *Cymodocea nodosa* and *Zostera marina* or macroalgae such as *Caulerpa prolifera* (Guallart and Templado, 2012, Kersting & García-March, 2017), which serve as a substrate to attach the byssus.

The thermal range of *P. nobilis* is not exactly known, but it is perceived that it lives in waters with temperatures between 7 to 32ºC (Butler et al., 1993, Ramos-Esplá et al. 2011). Regarding salinity, although a narrow range of optimal salinities (34 to 40 psu) has been described (Butler et al., 1993), abundant populations have recently been described in areas that exceed this range, with values reaching 45 psu (Giménez-Casalduero et al. 2020) or below it with values below 30 psu (Prado et al. 2021). These are coastal lagoons and estuarine bays respectively, in salinity conditions that *a priori* are suboptimal for the development of the species (Belando et al. 2017, Prado *et al.*, 2021). Therefore, it is a high tolerant species able to cope with temperature and salinity variations. They can adapt well to lagoon environments although this may influence their abundance, distribution, age structure and vulnerability to severe climatic phenomena (Butler *et al.*, 1993, Guallart and Templado, 2012, Prado *et al*, 2021).

Fan mussels are filter feeders, species able to adapted to oligotrophic environments that feeds preferentially on detritus and organic remains from the *P. oceanica* meadows (Butler et al., 1993, Guallart and Templado, 2012, Alomar et al., 2015). Their diet varies significantly throughout their lives and differs depending on their size. The juveniles are detritivorous, feeding mainly on the suspending detritus from the meadows, while an important part of the diet in adults is made up of planktonic organisms, both phytoplankton and zooplankton (Cabanellas-Reboredo et al., 2010; Davenport et al., 2011; Alomar et al., 2015). Despite the size of the animal, detritus makes up almost half of the filtered organic matter, being the main source of carbon for the species in some areas (Trigos et al. 2014). The resuspension occurring in the canopy meadow increases the food availability and supply, explaining the close association between *P. nobilis* and *P. oceanica* meadows (Duarte et al. 1999, Basso et al. 2015). Although detritus seems to be important in their diet, recent studies with captive specimens seem to indicate that phytoplankton is their main nutritional resource (Prado et al. 2020b, 2021)

The fan mussel is a successive hermaphroditic species, the development of both sexes is not synchronous to avoid self-fertilization (de Gaulejac, 1995, Deudero et al., 2017), although this process cannot be completely excluded (Trigos et al., 2018; Prado, Andree, et al., 2020). It reaches sexual maturity approximately at two years old (Richardson *et al*., 1999) and, in open waters, reproduction occurs in the summer months (May-August) (de Gaulejac, 1995, Deudero

et al. 2017, Kersting and García-March, 2017). Earlier reproduction has been observed in coastal lagoons (around late April-mid to May in Delta del Ebro, Garcia-March and Prado, pers. obsr.) Similarly to other bivalves, it has an external fertilization. Larvae are planktonic, and the duration of the larval phase has been estimated from 10 days (Butler *et al.*, 1993; De Gaulejac & Vicente, 1990), to up to 1 month (Deudero et al. 2017, Kersting & García-March 2017, Trigos et al. 2018). After that period, the larvae sink and attach to the substrate. Larval settlement extends from July to October (Cabanellas-Reboredo et al. 2009, Kersting & García-March 2017). In the early stages, juveniles suffer high mortality due to predation, mainly by octopuses (Fiorito & Gherardi 1999), sparid fish (Butler *et al*., 1993), and gastropods such as *Hexaplex trunculus* (Kersting and García-March 2017).

It is a long-lived species that can live up to 50 years (Rouanet et al. 2015), with very rapid growth during the first years of life, which slows down as the animal grow in size (Hendriks et al. 2012, Kersting & García-March 2017). Its growth varies depending on environmental conditions and has been shown to differ among populations (Siletic & Peharda 2003, García-March et al. 2007, Katsanevakis 2007, Hendriks et al. 2012, Kersting & García-March 2017, García-March et al., 2020). Recently, three more general growth models have been proposed, differentiating growth between exposed areas, protected areas and coastal lagoons (García-March et al. 2020). Hydrodynamics seems to be a determining factor in the ecology of this species, influencing it directly or indirectly. Growth, food availability, spatial and size distribution, orientation, and survival also influence the mussel's growth (García-March et al. 2007, Hendriks et al. 2011, Coppa et al. 2013, Basso et al. 2015).

1.2. Distribution and abundance

Until late 2016, *Pinna nobilis* was present throughout the Mediterranean Sea coastal areas. The distribution included the North African coast, up to the eastern coast of the Iberian Peninsula, where it had its western limit of distribution. However, the species has been relatively little studied. For instance, its abundance was known only from some areas, mostly where the species had abundant populations such as bays and coastal lagoons. Abundant populations have been only reported in the Aegean Sea, Adriatic Sea, Tunisia and the Tyrrhenian Sea, and Cabrera National Park, as well as east and south Spain, but generally there was a lack of data on its global distribution (Basso *et al*., 2015). In Spain, *P. nobilis* is a species living throughout the Mediterranean coast, being frequent and abundant in the Balearic archipelago and in the southeast of the peninsula, between Cabo San Antonio and Cabo de Gata. Towards the north, important populations have been seen in the Columbretes Islands, in Medes Islands and in Ebro Delta. Its western distribution limits in the eastern zone of Cádiz, specifically in Algeciras bay (García-Gómez 1983, Templado 2001, Barea-Azcón et al. 2008, Junta de Andalucía, 2017, Prado et al. 2014, Vázquez-Luis et al. 2017, Kersting & García-March 2017, García-March et al. 2020). In the Spanish Mediterranean coasts of North Africa, it has been identified in the Chafarinas Islands (Guallart & Templado 2012) (Figure 1).

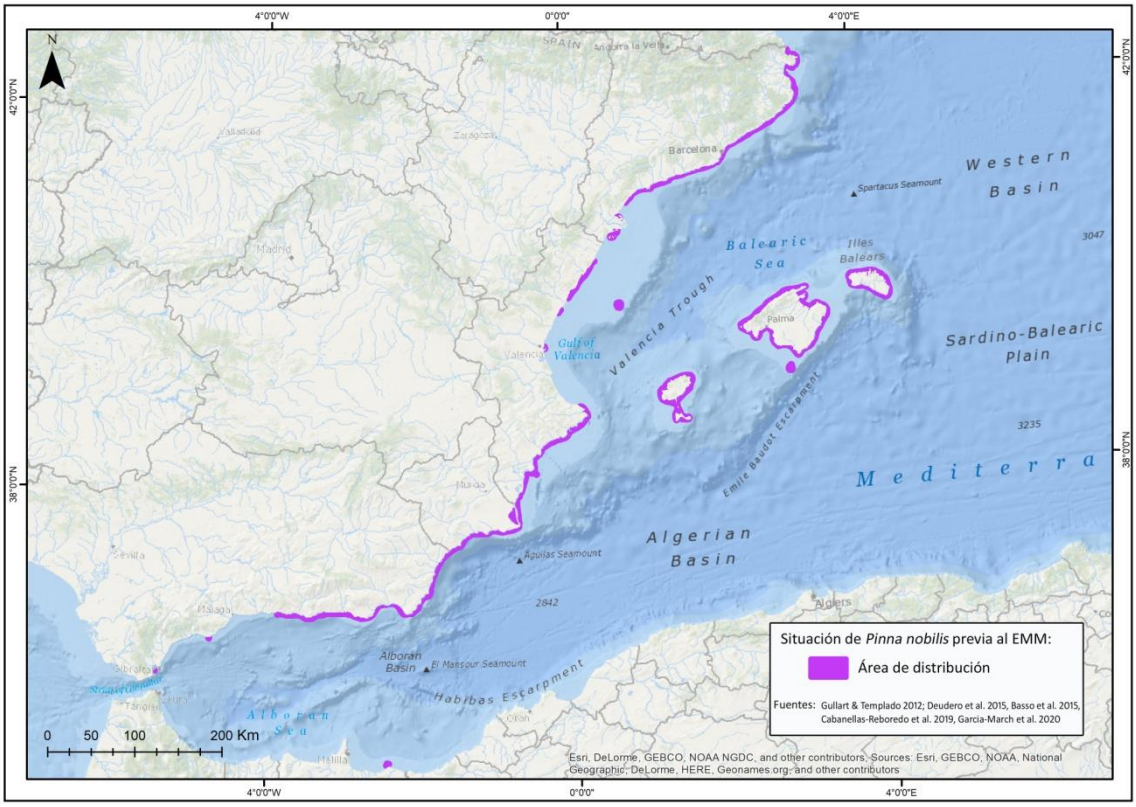

Figure 1. Fan mussel (*Pinna nobilis*) distribution around the Mediterranean coast before the Mass Mortality Event (MME) of 2016 from MITECO 2022. Sources: Gullart & Templado (2012), Deudero et al. (2015), Basso et al. (2015), Cabanellas-Reboredo et al. (2019), Garcia-March et al. (2020).

Along the Mediterranean coasts, global abundance data showed low distribution rate up to 2016, with densities below 1 ind/100 m^2 (Guallart and Templado, 2012). Density distribution rates were higher in protected areas with low hydrodynamics, such as bays, lagoons coastal and marine protected areas, with maximums up to 130 ind/100 m^2 (Basso *et al.*, 2015). In the Spanish coastal areas, high densities have been reported in the coasts of Alicante, Castellón, Murcia and Balearic Islands, places including protected areas such as Tabarca, Columbretes Islands, Isla Grosa or Cabrera National Park. Additionally, high density of individuals was also observed in sheltered areas with low hydrodynamism, estuaries and coastal lagoons such as the Mar Menor and the bays of the Ebro Delta, Fornells or Addaia, with maximum densities up to 37 ind/100 m^2 observed in Cabrera National Park in *Posidonia oceanica* meadow (Templado 2001, Prado et al. 2014, Vázquez-Luis et al. 2014b, Basso et al. 2015). Unfortunately, we do not longer have similar abundance data and these should only be seen as reference values for the *P. nobilis* population in the Spanish Mediterranean coastal habitats.

The current distribution of the species has changed radically in recent years throughout the Mediterranean coast. Most Mediterranean populations of *Pinna nobilis* have disappeared, including those on the Spanish coast, due to a Mass Mortality Event (MME) that has spread throughout the Mediterranean, which has brought the species to the brink of extinction.

1.3. Mass Mortality Event (MME) and current situation in Spanish coast

In autumn 2016, an episode of mass mortality of *Pinna nobilis* associated with an epizootic was detected for the first time in several locations in the southeast of the Iberian Peninsula and the Balearic Islands, with mortalities reaching almost 100% of the individuals in the affected populations. The epizootic spread to the north during 2017, persistently reaching nearly all populations of this mollusc on the Spanish Mediterranean coast (Vázquez-Luis *et al*. 2017a). The mortality was dispersed with the general direction of the currents towards the east, and reached the rest of the fan mussel populations in the Mediterranean Sea during the following 5 years, from Spain to the eastern basin (Katsanevakis et al. 2019, Panarese et al. 2019, Čižmek H. 2020). Until now, it seemed that only the populations of fan mussels in the Marmara Sea were free from the pathogen (Katsanevakis et al. 2021), however mass mortality has already been detected recently in the Dardanelles Strait and its waters (Özalp & Kersting 2020, Çinar et al. 2021).

The recently discovered species *Haplosporidium pinnae* (Catanese et al. 2018, Grau et al. 2022), is probably the responsible pathogen for this panzootic ((Darriba 2017, Catanese et al. 2018, Box et al. 2020), with a high infectious capacity rate. It should be noted that recent studies point to the existence of other opportunistic pathogens such as bacteria of the genus *Mycobacterium* spp, of terrestrial origin, as the cause of mass mortality (Carella et al. 2019), or whose infection together with *H. pinnae*, can increase mortality of fan mussel (Box et al. 2020, Šarić et al. 2020). Although it should be mentioned that this bacterium has also been found in healthy specimens of *P. nobilis* before the EMM (Box et al. 2020, Tiscar et al., 2021, Mihaljević et al., 2022, Grau et al. 2022). Other studies point to a multifactorial disease caused by multiple coexisting pathogens (Scarpa et al. 2020). However, recent studies have shown that *H. pinnae* has the greatest role as the causative agent of MME (Grau et al., 2022), with infections caused by *Mycobacteria* related to the age of the specimens, and those caused by other bacteria Gram negative related to sampling time after death.

It is estimated that the total population size has decreased more than 95% in recent years (Kersting et al. 2019, Katsanevakis et al. 2021) and this percentage rate may increase, as the threatening pathogens are still present in the marine environment (Kersting *et al.*, 2019). However, there are still a few populations that were not affected by the pathogen; they mainly are geographically isolated, in coastal lagoons or estuarine environments, with very specific environmental conditions, often with limited exchange with the open sea (García-March et al. 2020, Katsanevakis et al. 2021, Prado et al. 2021, Nebot-Colomer et al. 2022). Currently in Spain, the mortality of the populations of *P. nobilis* in the open sea is estimated to be approximately 100% (Vázquez-Luis *et al*., 2017, García-March *et al*., 2020). Only the population living in Ebro Delta and the coastal lagoon of the Mar Menor remained intact (Figure 2). These populations are essential for the conservation of the species, however they are critically vulnerable due to the water contamination in the area they inhabit (Cabanellas-Reboredo et al. 2019, García-March et al. 2020). The remaining pen shells are not parasite-resistant, but thanks to the environmental conditions of the area, they remained uninfected (Catanese et al. 2018). Nonetheless, in the past few years we started seeing that even these populations were exposed to infectious pathogens. (Prado *et al*. 2021; Nebot-Colomer *et al*., 2022).

Thanks to citizen science collaboration, some other surviving individuals in the Mediterranean coastal waters -excluding those from the Ebro Delta and Mar Menor population individuals-, have been located and have been monitored on the Spanish coast (in the Balearic Islands and Catalonia coasts, Figure 2) (García-March et al. 2020, Vazquez-Luis et al. 2020, Prado et al. 2021). In this case, these isolated individuals are pathogen-resistant individuals, since they have been exposed to the parasite and managed to survive (García-March et al. 2020). Although few, these individuals are vitally important for the survival of the species.

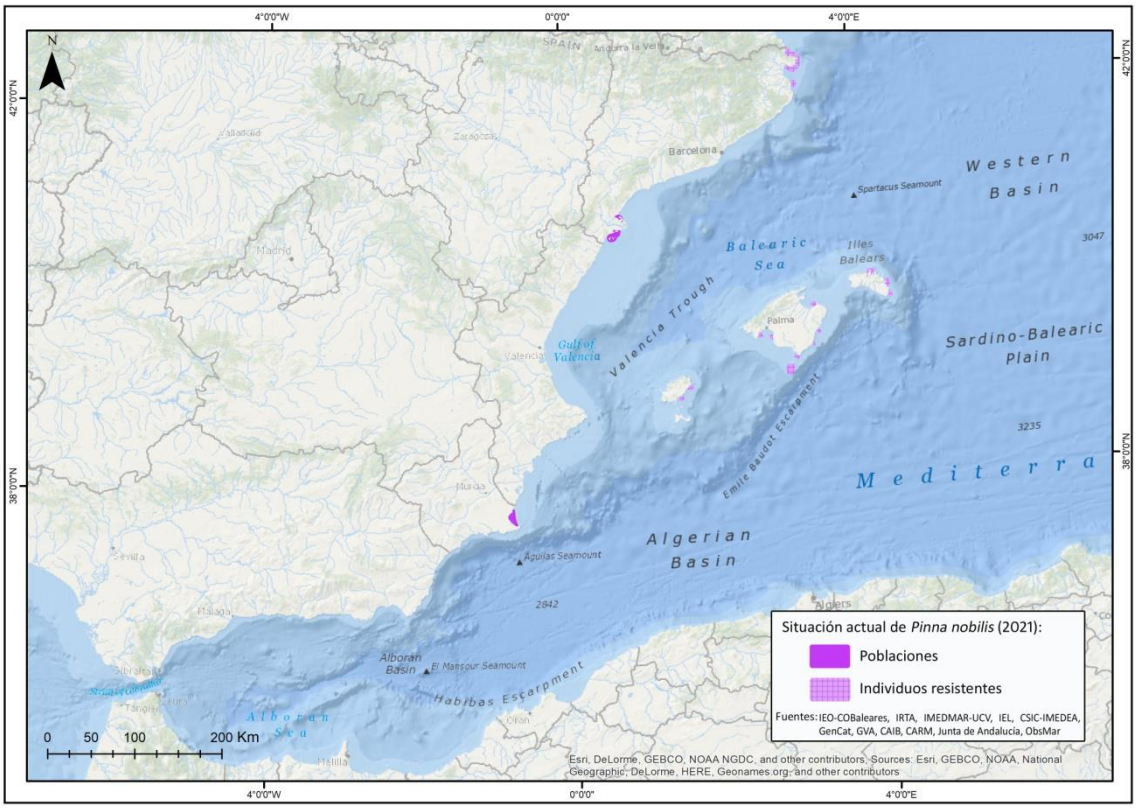

Figure 2. Spain fan mussel (*Pinna nobilis*) distribution in 2021. The figure shows existing and survival populations up to 2017, after the Mass Mortality Event (MME) from MITECO 2022. Source: COBaleares-IEO, IRTA, IMEDMAR-UCV, IEL, IMEDEA-CSIC, GenCat, GVA, CAIB, CARM, Junta de Andalucía, ObsMar.

2 CONDUCTED ACTIVITIES

2.1. Change of species legal status

Due to the decline of its populations in the last decades of the 20th century, the species was protected at national and European level through the implementation of European laws and directives. The national and international legal provisions where the species appears are the following:

International

- As a species of community interest that requires strict protection, it is included in Annex IV of the Habitats Directive (92/43/EEC, of May 21, 1992 on the conservation of natural habitats and wild fauna and flora, DO L 206, of July 22, 1992).
- As an endangered or threatened species, in Annex II of the Protocol on Specially Protected Areas and Biological Diversity (RAC/SPA), of the Barcelona Convention (Monaco, November 24, 1996, ratified in RD 22/1999, of December 17, BOE, 302, of December 18, 1999).
- As a strictly protected species in Annex II of the Bern Convention (1979, ratified in the RD of May 13, 1986, BOE 235, of October 1, 1986; amendment of December 1996).
- As a critically endangered (CE) listed species on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Kersting et al. 2019).

National (Spain)

- As a species of community interest that requires strict protection, included in Annex V of Law 42/2007 of December 13, which transposes the Habitats Directive into our legal system.
- It is declared a species "**in a critical situation**" because it is in imminent risk of extinction as stipulated in Law 42/2007 (Order TEC / 1078/2018, of September 28).
- Included in the Spanish Catalogue of Endangered Species (RD 139/2011), in the category "**critically endangered**" (Orden TEC/596/2019, of April 8).
- **Conservation strategy** for the fan mussel (*Pinna nobilis*) (undergoing and expected to be approved in 2022).

Regional

The species has been included in the Regional Catalogues of Endangered Species in three of the five communities in which the species is present:

Included in the Andalusian Catalogue of Endangered Species with the category of Endangered Extinction (Law 8/2003, of October 28, on wild flora and fauna; BOJA, 219, of November 15, 2017).

- Included in the Balearic Catalogue of Endangered Species and Special Protection of the Balearic Islands with the category of Endangered (Decree 75/2005, updated on 12/02/2019).
- The project for the Decree of the Catalogue of threatened autochthonous wild fauna is in process, which includes the fan mussel with the category Endangered Extinction, which is currently in the process of being processed for approval (public exhibition July 2020).
- The species is included in the Red Book of Threatened Invertebrates of Andalusia with the category of Vulnerable (Barea-Azcón et al. 2008) and in the Red Book of Marine Invertebrates of the Balearic Sea in the category of Vulnerable (Álvarez 2016).

Regarding the **recovery plans** prepared by the autonomous communities, in three of the five communities they have prepared or are in the preparation phase, and one of them has already approved it:

- **Andalusia**: Agreement of November 7, 2017, of the Governing Council, approving the Plan for the Recovery and Conservation of Endangered Invertebrates and Phanerogams of the Marine Environment, BOJA, 219, of November 15, 2017.
- **Balearic Islands**: has included the Recovery Plan for the fan mussel in a joint plan together with two other species of protected molluscs: Conservation Plan Recovery of marine invertebrates in the Balearic Islands. Pending approval in 2021
- **Catalonia**: the elaboration of the Recovery Plan has been defined in the decree pending approval for the modification of the category of the fan mussel in the Catalogue of threatened fauna. The Recovery Plan is currently in the preparation phase.

2.2. Actions performed for the species conservation

2.2.1. Research projects

Pinna nobilis was not the object of research in Spain until the end of the 20th century and the beginning of 2000, when it began to be included in the Marine Environment Monitoring Programs and was the object of study by several research groups (UV, IMEDMAR-UCV, UB, IEO, CSIC-IMEDEA, among others). There have been few research projects focusing on *Pinna nobilis* before the MME, some of them developed with little funding. Among those studies, some were focused on age and growth, gaping activity and distribution and densities mainly in marine protected areas (MPA). In Valencian Community, studies were performed in Moraira, Tabarca Islands and the Columbretes Islands MPA (Kersting & García-March 2017), the Nacra Project in the Tabarca MPA, Alicante (2009-2011, carried out by the IEL-Jiménez-Gutiérrez et al., 2010) and the PINNA Project in Cabrera National Park (2011-2013, executed by IEO-COBaleares, IMEDEA, LIMIA and CAIB, Vázquez-Luis et al. 2015a). Other projects, such as one carried out by IMEDMAR-UCV, the Oceanogràfic of Valencia and the "Marina Real" of Valencia in collaboration with the Paul Ricard Oceanographic Institute and PINNASPOT project, funded by Alberto II of Monaco Foundation, studied the possibilities of maintenance and reproduction of *P. nobilis* in captivity in semi-open circuits (2012-2015) and have been very useful in recent years. It should be noted that although research on the species had increased considerably in the last decade, there was still a great lack of knowledge about the biology and ecology of the species, as well as the dynamics of its populations (Basso et al., 2015).

The cooperation that has occurred between research groups to evaluate this phenomenon and its scope stands out as a result of the MME. From the outset, several collaborative initiatives between the research groups MITECO and CCAA were launched to assess the scope of mass mortality, some being without funding. From the first meeting organized by the IEO-COBaleares (with representatives of MITECO, regional administrations of the Balearic Islands, the Valencian Community and Andalusia), the collaboration between research groups, field sampling, citizen-science observations, and sample collection, has generated a large amount of information in a relatively short period. This collaboration led also to the first emergency project funded by the Spanish administration (MITECO) to recue an animal species In Spain "Rescate de 215 ejemplares de nacra (*Pinna nobilis*) y su mantenimiento en 5 centros especializados en el marco

del Proyecto UFE IP-PAF INTEMARES (LIFE15 IPE ES 012), "Gestión integrada, innovadora y participativa de la Red Natura 2000 en el medio marino español"". Altogether alerted other countries of the phenomenon (Vázquez-Luis et al. 2017, Kersting et al. 2019, García-March et al. 2020), and allowed for the location of the responsible infectious agent (Darriba, 2017, Catanese et al., 2018), as well as the creation of a model of the expansion pattern of the pathogen as it is dispersed through surface currents and pointing out a first hypothesis of the importance of salinity and temperature modulating the infection (Cabanellas-Reboredo et al., 2019).

The research work carried out so far has corresponded with the following lines:

- Improve knowledge of the biological requirements of the species in captivity (diet, welfare).

- Improve the living conditions of captive fan mussels (stress, prophylactic treatments).

- Closing the ex situ biological cycle of the fan mussel.

- Larval recruitment and obtaining larvae/juveniles from the environment.

- Improve genetic knowledge of the pathogen and develop methods for its detection.

- Advances in genetic knowledge: taxonomic identification, connectivity.

<u>Research projects that have received funding:</u>

There are currently 5 ongoing research projects financed by MITECO (Biodiversity Foundation, Marine Strategy Program, Future DGBBD commission) and two LIFE projects. The topics of these research projects aim to: (1) address reproduction in captivity, (2) improve the conditions of the individuals in captivity and carry out curing tests, (3) identify areas for the reintroduction of the species, (4) identify the environmental parameters that determine the optimal survival of the species, (5) monitor the presence of the pathogen in the environment and in surviving individuals, (6) studies on genetic connectivity, (7) pilot tests for the reintroduction of seed in the environment, (8) carry out coordination actions, (9) monitor and manage the populations of the Mar Menor and the Ebro Delta, as well as international collaboration actions:

- Nacras Project: “Evaluation of the magnitude of a mortality event combining extended scientific censuses with citizen science observations” (2017-2018). Special Action financed by the Balearic Government with European Funds.

- PinnaSpot Project: “The study, protection and possible breeding of pen shell (*Pinna nobilis*) in the Boka Kotorska Bay”. Funded by the Prince Albert II Foundation of Monaco (2016-2019)

- PinnaSpat Project: “Reproduction of *Pinna nobilis* in captive conditions and identification of areas free of *Haplosporidium pinnae* for the reintroduction of juveniles using sentinel seed” (2020-2021). IMEDMAR-UCV. Project funded by the Biodiversity Foundation.

- NeuPinna Project: “Use of reproductive neuropeptides to induce gonadal maturation and laying of *Pinna nobilis* and *P. rudis* under captive conditions” (2021-2023). IMEDMAR-UCV. Project funded by the Biodiversity Foundation.

- Recupera_Pinna Project: "Monitoring, recovery and citizen science actions in the remaining populations of *Pinna nobilis* in the Ebro Delta and the Mar Menor" (2021-2022). IRTA, UA. Project funded by the Biodiversity Foundation.

- Marine Strategies Program (EEMM), in Chapter 2, Action 5, Task 3 "Coordination for the study and monitoring of *P. nobilis* mortalities" (2019-2022). CSIC-IEO-COBaleares,"

- Future DGBBD-IEO assignment. Nacra actions (2022-2025), CSIC-IEO-COBaleares. INTEMARES LIFE. Action C1.1. "Habitat and species conservation projects", during Phase II, "Actions for the conservation of the fan mussel, *Pinna nobilis*, in the Mar Menor" (2018-2024). CSIC-IEO-COBalearic Islands

2.2.2. Cooperation actions

During the last few years, education and training actions focussed to the transfer of knowledge, in terms of field procedures and methodologies between Spanish research groups and with other countries have been conducted.

One of the actions that has been carried out is the creation of the Network of larval collectors. All the groups interested in participating, which include technicians from the Autonomous Communities and researchers from other countries, have contacted the responsible researchers (IMEDEA-CSIC, UB) to participate. They have been provided with the material and the protocol for the installation of larval collectors (Kersting & Hendriks 2019), as well as the protocol to follow for the revision of the collectors after their collection.
Mention should also be made of the agreement between IMEDMAR-UCV and the Pula Aquarium (Croatia), through which they are collaborating in the training of personnel, transfer of protocols and knowledge for the maintenance of fan mussels in captivity that they are carrying out in that country. The work being done by the CSIC-IEO-COBaleares group is included, collaborating with researchers from France and Italy, regarding the training of the procedure for taking samples via biopsies of living individuals.

On the other hand, intense collaboration has been carried out on the rapid detection of the pathogen in tissue samples. Since the detection of *H. pinnae* in 2017 (Darriba, 2017, Catanese *et al*., 2018), numerous analyses have been carried out on live and diseased fan mussels to confirm the presence of the parasite in *P. nobilis*. In this sense, the Marine Research and Aquaculture Laboratory (LIMIA) of the Balearic Government stands out, as it has developed an effective method for detecting the parasite in tissue and has so far been in charge of both histological (150 individuals) and genetic analyses and molecular biology (1100 individuals) of *P. nobilis* from Spain and other Mediterranean countries such as France, Monaco, Italy, Greece, Cyprus and Turkey. They have also carried out the analysis of about 50 samples of *P. rudis,* to confirm the absence of the parasite in this species. LIMIA is currently working on the development of early detection of the parasite in the water column, and on the study of the genetic variability of the pathogen. A new non-destructive method aimed at the taxonomic identification of small size juvenile (<50 mm) fan mussels, has also developed using environmental DNA, because morphological differences between *P. rudis* and *P. nobilis* are not evident on these individuals. This method will help in the early identification of *P. nobilis* without harming the individuals (Catanese et al., *in press*).

As previously mentioned, given the situation of the species from the MME, *Pinna nobilis* is the object of a joint and coordinated action between the different regional and state administrations, and the different research entities. Cooperation between all actors is essential for the proper management of living population or individuals and the necessary fluid exchange of information between all of them, given the dynamism that has characterized the process during this period. Several meetings are held on a regular basis between the different actors, and since the creation of the Nacra Critical Situation Working Group by MITECO in 2019, actions have been carried out in a consensual manner for the management of the populations, as well as the establishment of the priority lines of work. The result of this collaboration has resulted in the publication of more than 40 papers between 2017-2021, numerous technical advisory documentation, more than 20 technical reports, methodological protocols (Alvarez et al. 2017, Kersting & Hendriks 2019, Nebot-Colomer et al. 2020) technical reports (Kersting et al., 2019), and Action Plan proposals (Vazquez-Luis et al. 2017).

The most important meetings held at national level were:

- In March 2017, the first *P. nobilis* mortality Expert Workshop organized by the CSIC-IEO-COBaleares was held, with the participation of representatives from MITECO and the regional administrations of the Balearic Islands, the Valencian Community and Andalusia, and research groups from these communities. Given that in just 6 months most of the populations of *Pinna nobilis* at the national level had disappeared, in this workshop all the experts agreed that the situation of the species was critical and that it was urgent to carry out field sampling to assess the impact of mortality to which all groups were committed.

- April 2017, the SGPM, with the advice and agreement of the researchers, began the procedures for the inclusion of the species in the category "Endangered" (Order TEC/596/2019, of April 8). In July 2017, the Environment Sector Conference approved the declaration of “critical situation” for fan mussel (*Pinna nobilis*) (Order TEC/1078/2018, of September 28). This declaration has as a consequence that the works and projects aimed at the recovery of this species will be considered of general interest and their processing will be urgent as established in article 60.2 of Law 42/2007, of December 13, of the Natural Heritage. and Biodiversity.

- September 2017, MITECO coordination meeting with the 5 Autonomous Communities involved in the management of the species and representatives from researchers, for the adoption of measures to try to avoid the extinction of the fan mussel. Conditions of the project were determined: Rescue of 215 individuals that took place in November of that same year.

- September 2019. First meeting of the Working Group on the critical situation of *Pinna nobilis*, constituted by MITECO and attached to the State Commission for Natural Heritage and Biodiversity, and the Wild Flora and Fauna Committee. Formed by representatives of MITECO and the 5 Autonomous Communities involved, scientist who work directly with the species were invited to participate as advisers. In this coordination meeting, a list of proposed actions was established to be carried out by the Autonomous Communities and MITECO, which must be prioritized: in situ conservation actions, ex situ actions and coordination actions.

- September 2020. Second meeting of the Working Group on the critical situation of *Pinna nobilis*. In this meeting the priority lines of action were approved and the proposed financing mechanisms were established.

During this period, regional Working Groups has been also created in the communities of Murcia, Catalonia and the Balearic Islands, made up of representatives of the departments responsible for the management of the species, the management of protected areas, and scientific advisers who are working with the species in each region.

In terms of international cooperation, it should be noted that Spain was the first country to be affected by the MME, and once the scope of the problem was revealed at the end of 2017, it carried out dissemination actions with national institutions and research organizations from the rest of the Mediterranean countries:

- Madrid, March 2018. Meeting organized by MITECO and IUCN, with the assistance of representatives of the national marine biodiversity monitoring programs of France, Italy and Morocco, to carry out effective collaboration between countries.

- Tunisia, September 2018. Workshop promoted by the Coastal Planning and Protection Agency, with the collaboration of the IUCN and SPA / RAC, to launch a surveillance and rescue network for fan mussel.

- Barcelona, January 2020. Bilateral meeting promoted by CSIC-IEO-COB between research groups from France and Spain in order to seek common points of collaboration, avoid duplication of efforts and work in a coordinated manner by sharing knowledge.

- In November 2020, the IUCN organized an international online meeting with scientific representatives from some Mediterranean countries such as Spain, France, Monaco, Italy and Croatia, in order to share information on keeping *Pinna nobilis* in captivity and its reproduction.

2.3. Surviving and resistant fan mussels

During these years, an active search for *P. nobilis* survivors has been carried out by the autonomous communities involved, MITECO and collaborating research groups (CSIC-IEO, IMEDEA-CSIC, IRTA, UA, UMU, IMEDMAR-UCV). To this end, information and dissemination campaigns, volunteer programs and citizen science (*Observadores del Mar*, https://www.observadoresdelmar.es/Projects/View/14) have been carried out.

The search for surviving individuals in the open sea, it has been carried out on the entire peninsular coast and the Balearic Islands, in most cases integrated into the different monitoring programs for the marine environment, but also within crowdfunding programs (e.g. Salvar la nacra of IMEDMAR-UCV). In the case of the Valencian Community and the Region of Murcia, also through the monitoring of the *Posidonia* meadows carried out by the IEL and the IEO-COMurcia (Jiménez-Gutiérrez, *et al*., 2017, GEAM-IEO, 2019) respectively, and in Andalusia through the Sustainable Management Program for the Andalusian Marine Environment (JA, 2008-2020). In all these cases, resistant individuals have not been detected from 2018. In addition, in the Valencian Community and the Region of Murcia, searches for specimens are also carried out annually in the Marine Reserves of the Columbretes, Tabarca and Cabo de Palos

Islands by UB, IEL and IEO-COBaleares, respectively, in collaboration with SGP staff. In the Columbretes Islands MPA, within the MPA global change monitoring program (UB), some surviving specimens and hybrids have been located, of which only the latter survive today (D. Kersting-UB *com pers*).

Between 2017-2021, a total of 129 surviving individuals have been monitored in the open sea and have been located year after year (M. Vázquez-Luis, IEO-COBaleares. *com pers*). More than 40 of them did not survive the second infectious wave between 2017-2018, and on the coast of Andalusia, Murcia and the Valencian Community no individuals were have been found since then. On the coast of Catalonia and the Balearic Islands, survivors have continued to be located every year to date, thanks to social participation through citizen science platforms (*Observadores del Mar*), through which most of the individuals have been identified over the years.

In the case of the Balearic Islands, isolated resistant specimens have been located every year. The CAIB has been monitoring these specimens in collaboration with the IEO-COBaleares, within the framework of own projects (ARES and ARES II project), although it should be noted that it has been uneven and in some years, such as 2020, it has not been carried out. In this period, 51 individuals have been monitored, located in all the islands except for Formentera. Most of them died during one of the mortality waves. At the beginning of 2021 there were 9 surviving individuals in Balearic Islands. In addition, in some cases, to ensure their survival, it has been decided to transfer some individuals located in frequented areas and with interaction with human activities to other sheltered areas. It has been the case of some resistant individuals in the Balearic Islands (7) due to the threat caused by the anchoring of boats (CAIB, M. Vázquez *pers. comm.*) (Figure 3). In all cases, translocations were carried out successfully (2017-19), fan mussels survived the transfer (CAIB, M. Vázquez-Luis- IEO-COBaleares *com pers*.).

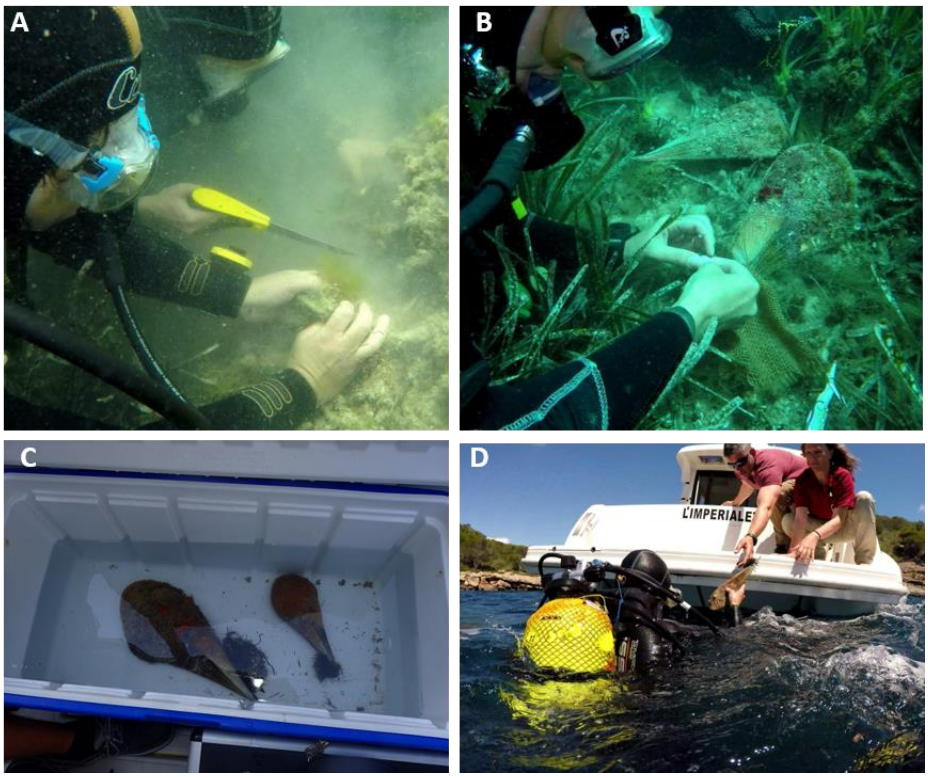

Figure 3. Translocation of *Pinna nobilis* in the Cabrera National Park. A. Extraction of the specimens in the locality of origin. B & D. Installation of the specimens in the receiving locality. C. Transfer of specimens of *P. nobilis.*

In Catalonia coast, 35 individuals have been monitored in this period, 23 of whom succumbed in the first period (2017-2018) and the rest, have found through volunteers in the area of Cap de Creus (ObsMar) in the last years 2020 and 2021. These resistant individuals, around 10, have not been periodically monitored *in situ*, but it will be carried out soon within the framework of the Marine Strategy Framework Directive monitoring program (EsMarEs, MITECO) by the CSIC-IEO-COBaleares team. Furthermore, in the Ebro Delta translocation of individuals have been done due to the danger of collisions with boats and/or poaching or because they are located in an area that is too shallow to guarantee their survival (P. Prado-IRTA *pers. comm.*).

During 2017, 2018 and 2019 some measurements have been carried out in order to increase the survival rate of living individuals in Catalonia such as the use of predator exclusion cages (2017-2018). The trial of exclusion cages did not have the expected success rates and no differences were found in the survival outcomes (García-March *et al*., 2020).

The most remarkable of individuals that have survived the disease “waves” of warmer summer periods, is that they seem to have some kind of resistance to the disease. Resistant individuals carry out highly resistant genes, which make them extremely important for the survival of the species.

2.4. Recruitment

On the other hand, with the intention of evaluating larval recruitment, a pan-Mediterranean network of larval collectors has been created, coordinated by CSIC-IMEDEA and UB, in which all the research groups, the Autonomous Communities and MITECO participates. Annually since 2017 in the reproductive period (May-October) more than 50 larval collectors have been installed in 37 locations in Spain, France, Italy, Croatia and Algeria and more than 300 larvae of *P. nobilis* and *P. rudis* have been collected (Kesting *et al*., 2020, Garcia-March *et al*., 2020). Although during 2017 there was a significant recruitment peak and a large number of juveniles were collected in some locations such as the Columbretes Islands, the number of larvae collected has decreased notably in the following years and in most locations they have not been recruited. In addition, it should be added that the collected fan mussel larvae were infected by the parasite, and although they were transferred to the *ex situ* facilities, none survived after 6 months (Kersting *et al.*, 2020, Garcia-March *et al.*, 2020).

2.5. Fan mussel in captivity: *ex-situ* conservation programs for individuals

From the declaration of the species in a critical situation, funds were obtained to carry out emergency measures in the face of the serious mortality situation suffered by the species in Spain. A rescue project funded by INTEMARES LIFE Project was launched trying to save as many individuals as possible from mass mortality: "Rescue of 215 individuals of fan mussel (*Pinna nobilis*) and their maintenance in 5 specialized centres within the framework of the LIFE IP-INTEMARES Project (LIFE15 IPE IS 012)" coordinated by IMEDMAR-UCV with the participation of 11 entities including regional administrations, research centres and foundations. The rescue took place between November and December 2017, and the molluscs were taken to 5 specialised centre in shellfish farming: IRTA, IEO-Mazarrón, IMEDMAR-UCV, IFAPA and Oceanogràfic. Fan mussels were extracted from two areas, 115 of them from an area supposedly not yet affected by the parasite, in the Portlligat area (north of Catalonia), and the remaining 100 from the Alfacs bay in the Ebro Delta. Although the transfer of the animals was successful and only one loss associated with the transfer was recorded; the Portlligat pen shells were already infected by the parasite, and shortly after they began to show symptoms of the infection. Measures were taken to try to stop the infection by means of a temperature and salinity treatments, which managed to slow it down but it did not stop it. In September 2018, only 10.9% (12) of the total individuals extracted from Portlligat (115 individuals) remained alive (García-March *et al*. 2020). Those individuals ended up dying throughout 2019.

The individuals extracted from Alfacs and kept at the IRTA have suffered high mortality due to other causes than infection by the *H. pinnae* parasite, since they were not infected. They showed problems linked to long-term housing associated with an infection by *Vibrio mediterranei*. This bacterium is naturally present in the shellfish of the Delta and is favoured by the stress conditions associated with housing and the lack of an adequate diet. The summer temperature rises above 22°C caused a mortality peak associated with the bacteria, which was 34% at 12 months and 88% at 18 months of its *ex situ* maintenance (Prado, 2019, Prado *et al*., 2020b, García-March *et al.*, 2020). The individuals received treatments to fight the infection, such as the use of hydrogen peroxide and antibiotics (florfenicol) and prophylactic treatments (vitamins and controlled temperature) (Prado, 2019, Prado *et al*., 2020b). The surviving individuals were kept until the end of the project and were returned to the estuarine environment (Alfacs Bay)

in April 2020 (P. Prado-IRTA *pers. comm.*). In all cases, for indoor maintenance, it has been observed that filtering the seawater to 1µ and passing it through UV light (> 100 $mWs^{-1}cm^{-1}$) maintains it free of *H. pinnae*.

Regarding reproduction in captivity, there are currently two groups (IMEDMAR-UCV and the UMU Aquarium) in collaboration with the rest of the groups (IRTA, IEO, UA, LIMIA). Within the framework of projects financed by the Biodiversity Foundation, they are carrying out trials for the maturation of individuals in captivity in order to close the biological cycle of the species and obtain viable seeds. They are working with individuals from the Ebro Delta (20) and the Mar Menor (30), respectively.

Maturation in captivity has already been achieved on 4 occasions (September 2020, April, September and December 2021) (JR. García-March-IMEDMAR-UCV, E. Cortes-UMU, *pers. comm.*), although it has not yet been possible to close the reproduction cycle of *ex situ* fan mussels given the high larval mortality observed during the first 10 days of cultivation. On the other side, IMEDMAR-UCV has been the recipient of most of the larvae obtained in the larval collectors during this period, housing more than 200 juveniles of *P. nobilis* and *P. rudis* between 2017 and 2018. Unfortunately, all the *P. nobilis* were infected and died after few months.

The UMU Aquarium expects to have a maximum of 60 fan mussels in its facilities within the project of the Bank of Emblematic Species and of Singular Importance of the Mar Menor (General Directorate of the Mar Menor of the CARM), which began in February 2020 and IMEDMAR-UCV will host between 10 and 15 individuals uninterruptedly.

2.6. Recent advances in fan mussel captivity maintenance

Recent (*submitted to review*) advances have been made in understanding the energetic physiology of the fan mussel using the congeneric species *Pinna rudis* as a model. Work has been done on the impact of temperature, and it has been seen that, in conditions of high temperatures, shellfish stop feeding and consume their reserves (M. Albentosa-IEO-CSIC, JR García-March-IMEDMAR-UCV, *com pers*). In addition, work has been done on the development of an optimal diet for the species that seems to be giving good results, using different species of phytoplankton and zooplankton based on their size so that to establish nutritional profiles.

With all the information obtained in this period, the research groups are preparing methodological protocols for the *ex situ* maintenance of the species, which are expected to be available shortly.

Proposals for conservation

3 MAIN THREATS IDENTIFIED

- Panzootic

The current mass mortality event is the most worrying and widespread threat to the conservation of *Pinna nobilis* throughout its distribution range (Vázquez-Luis et al. 2017, Katsanevakis et al. 2019, Kersting et al. 2019, Panarese et al. 2019). The epizootic has rapidly spread from Spain to the rest of the Mediterranean basin in less than 3 years, causing mortality rates between 80-100% among populations.

Several studies have suggested that a parasitic protozoan, *Haplosporidium pinnae,* a new species of Haplosporidia was the main etiological agent causing the epizootic among *P. nobilis* (Darriba *et al*., 2017, Catanese *et al*., 2018, Box *et al*., 2020). These types of protozoa parasitize marine and freshwater invertebrates and are known to be highly infectious. In this case, *H. pinnae* affects the fan mussel digestive gland reducing food absorption and possibly leading to several digestive dysfunctions, starvation and ultimately death (Catanese *et al*., 2018, Box *et al*., 2020). Additionally, it seems like *H. pinnae* only affects *P. nobilis* and it has not negative effects on other invertebrates, including the congeneric species *P. rudis* (Catanese *et al.*, 2018).

Throughout their life cycle, these types of parasites present various morphologies including uninucleate cells, plasmodia, and spores. Morphological (histological and ultrastructural) and molecular analyses showed the presence of the whole life cycle in the same host, which is not common in other species of the same group. In addition, the appearance of uni- and binucleate stages in the samples indicates that there is direct transmission from infected to healthy fan mussels (Catanese *et al*., 2018). This finding resulted vital during fan mussel captivity maintenance programs.

An important characteristic of this parasite is the production of spores, a stage of latency or resistance, allowing it to remain in the environment for long time but for which it requires a host to survive, as it has been seen for other parasite species of the same genus (e.g. *H. nelsoni* and *H. costale*) (Haskins & Andrews 1988). Currently, we still do not know which organisms act as a host when fan mussels are not present in the environment, but if they are kept in unfiltered water, they get infected (García-March pers. obs.). The presence of the pathogen in the marine environment makes highly difficult the natural recovery of the population in the sea proposing a worrying scenario for the species.

Models based on the western Mediterranean scale suggest that the pathogen is dispersed through surface currents, and that the optimal conditions for infection are temperatures above 13.5^{o}C and salinities between 36.5-39.7 psu, typically for the Mediterranean Sea (Cabanellas-Reboredo, *et al*., 2019). Only the populations located in specific areas such as lagoons, estuaries or closed bays, with a salinity significantly higher or lower than the open sea managed to survive the mass mortality events occurred during each summer-autumn since 2016 (Catanese *et al*., 2018, Kersting *et al*., 2019, García-March *et al.,* 2020, Katsanevakis *et al*., 2021).

- Habitat loss and destruction

Although seagrass meadows are key habitat for fan mussel populations, they are going through an alarming decrease throughout the Mediterranean coast (Boudouresque et al. 2009, Marbà

et al. 2014). This is occurring as a result of human activities such as coastal works, dredging, port construction, pollution, eutrophication, illegal trawling, etc. (Duarte 2002). *Pinna nobilis* became largely vulnerable to seagrass meadows loss. Activities such as coastal works, dredging, mooring and anchoring boats on shallow areas are still causing severe consequences upon *P. nobilis* population (Hendriks et al. 2013, Prado et al. 2014, Deudero et al. 2015, Vázquez-Luis et al. 2015b). These activities need to be accounted for when managing the existing and affected fan mussel populations. In addition, it is essential to underpin that fan mussel recruitment is also negatively affected by habitat loss, degradation or fragmentation, due to the protecting role seagrass meadows offer to juvenile individuals (Basso *et al.* 2015).

- Physical damage and not-natural mortality

Currently, the unnatural mortality of individuals occurs in most cases accidentally, due to interaction with human activities, even though there have also been reported cases of direct mortality of fan mussels. Despite its protection, fan mussel populations have been harvested in some eastern Mediterranean countries, where commercial fishing caused significant mortality rates (Katsanevakis et al. 2007, 2011, Öndes et al. 2020). In Spain, since its protection began, mussel collection decreased and was not considered a major threat any longer. Nevertheless, in the past years live individual collection in the Ebro Delta, Mar Menor and the Balearic Islands has been reported (Moreno et al 2017a b, Prado-IRTA and Giménez-UA, *pers. comm*).

Regarding the mortality associated with fishing activities, this was solely associated to gillnets, since the other nets are deployed below 50 m where *P. nobilis* are rarely found anymore. However, although effects of gillnets on fan mussel populations have been studied (Basso *et al*., 2015), these are limited to shallow areas such as Mar Menor, where fishing activities were more intense and the population more abundant (see Mar Menor report).

Other important activities are those related to the recreational nautical sector, especially anchoring and mooring of boats, which largely affects the *P. nobilis* population (Hendriks *et al*. 2013, Vázquez-Luis *et al.* 2015b), but also the practice of surf and kite surf. These activities can break fan mussel shells leading to death for some individuals. This should particularly be considered on the Spanish coast, since these recreational activities have grown considerably during the past few years. It is important to establish measures to avoid these activities in areas nearby fan shell mussel population of the Mar Menor and the Ebro Delta. We are also aware that boat collisions in shallow areas have caused the mortality of monitored individuals in Ebro Delta, Alfacs and Fangar (Prado *et al*., 2021) and in the Mar Menor (F. Giménez-UA, *pers. comm.*).

- Climate change

Climate change is one of the threats that can negatively affect fan mussels, in particular its effects in terms of warming water, rising sea levels and the increase in extreme weather events. One of the effects of climate change is sea level rise, which in the medium-long term could involve the permanent flooding of many low-lying areas. Salinity variation is often a vector for marine invasive species to inhabit coastal areas such as the Ebro Delta and the Mar Menor (CEPYC-CEDEX, 2021a, b, Prado *et al.,* 2021). Yet, sea level rises may magnify the effects of storms, increasing the chance of sandbar deterioration and water mass exchanges, as it has already happened in Trabucador, Illa de Buda and Ebro Delta. These areas are also exposed to great pluvial erosivity, associated with a greater frequency of torrential influences (CEPYC-CEDEX, 2021a, b).

- Invasive species

Global change is promoting the establishment of invasive species that represent a threat to biodiversity and can affect sessile species such as *P. nobilis.* It has been observed that some invasive macroalgae such as *Lophocladia lallemandii* and *Caulerpa cylindracea*, use *P. nobilis* as their preferred substrate. This can induce biological stress on *P. nobilis*, reducing food intake and growth (Box et al. 2009, Cabanellas-Reboredo et al. 2010, Vázquez-Luis et al. 2014a, Kersting & García-March 2017). Other invasive species such as *Callinectes sapidus*, a voracious crab that inhabits estuarine and lagoon environments, can become a major threat to juvenile *P. nobilis*, especially in the coastal lagoons, where the presence of this species has been already recorded for few years (García-March et al. 2019, CEPYC-CEDEX, 2021a).

- Predation

Juveniles are the size class greatly suffering predation pressure, so attention needs to be brought when establishing conservational management plans. A high density of predators can be a limitation for the recruitment and survival of juveniles.

- Water quality degradation

Given fan mussels are benthic filter feeder organisms, water contamination, whether direct or indirect, can alter the physicochemical characteristics of the water and affect their populations. Fan mussels have showed to be highly tolerant to the water contamination and degradation. They can bioaccumulate toxins, such as hydrocarbons after an oil-spill or tolerate high levels of heavy metals. However, elevated levels of oxidative stress markers have been detected even months after the oil-spill (Sureda et al. 2013b a, Vázquez-Luis *et a*l. 2016). It is estimated that other environmental contaminants could have inmunotoxic effects on *P. nobilis* (Basso *et al*. 2015) and/or affect shell formation (Vicente et al. 2003).

High levels of eutrophy in coastal lagoons or semi-confined systems can lead to anoxia and even euxinia conditions, which can seriously affect *P. nobilis* populations, as recorded in the Mar Menor in 2020 (Perez-Ruzafa et al. 2019, Giménez-Casalduero et al. 2020, Ruiz et al. 2020). Another consequences of eutrophication are the rapid growth of macroalgae (e.g. *Caulerpa prolifera*; M. Vázquez-Luis-IEO-COBaleares, *com pers*.), which grow on the *P. nobilis* shells and can reduce their food intake and opening of the valves (F. Giménez-UA, *com pers.*).

4 PROPOSAL OF SITES

4.1. Proposal sites for visual censuses to search for survivors

Continental Spanish coasts

Due to the Massive Mortality Event (MME), it is of great interest to look for survivors that may be potentially resistant to the disease and to aggregate them in optimal places with the highest chances of survival. For this reason, in Spain further census should be conducted in shallow (<30 m depth) and deep (30-45m depth) areas. Also, larvae collectors should be installed during the

reproduction period, to assess the occurrence of recruitment, from relict undisclosed populations. For this purpose, scuba diving also aided with propulsion techniques, such as underwater scooters, or seaplanes, should be used, along with georeferencing from a support vessel. Shallow census are recommended in some of the *P. oceanica* meadows of Alicante, Catalonia and Almeria. Deep census are recommended in Islas Hormigas, Columbretes Islands, Medes Islands and Cap de Creus (Figures 4 to 12). All of them in areas previously known to have fan mussel populations. The surveys could be carried out by teams of researchers that could be aided by volunteers or by trained volunteers. Divers will mark any specimens found alive with a buoy attached to the bottom of the sea and easily visible above the sea level. Subsequently, GPS coordinates (WGS84) of each buoy should be georeferenced by staff on the surface. All these actions should be complemented with dissemination actions to increase global awareness of the problem we are referring to and attract more citizen help.

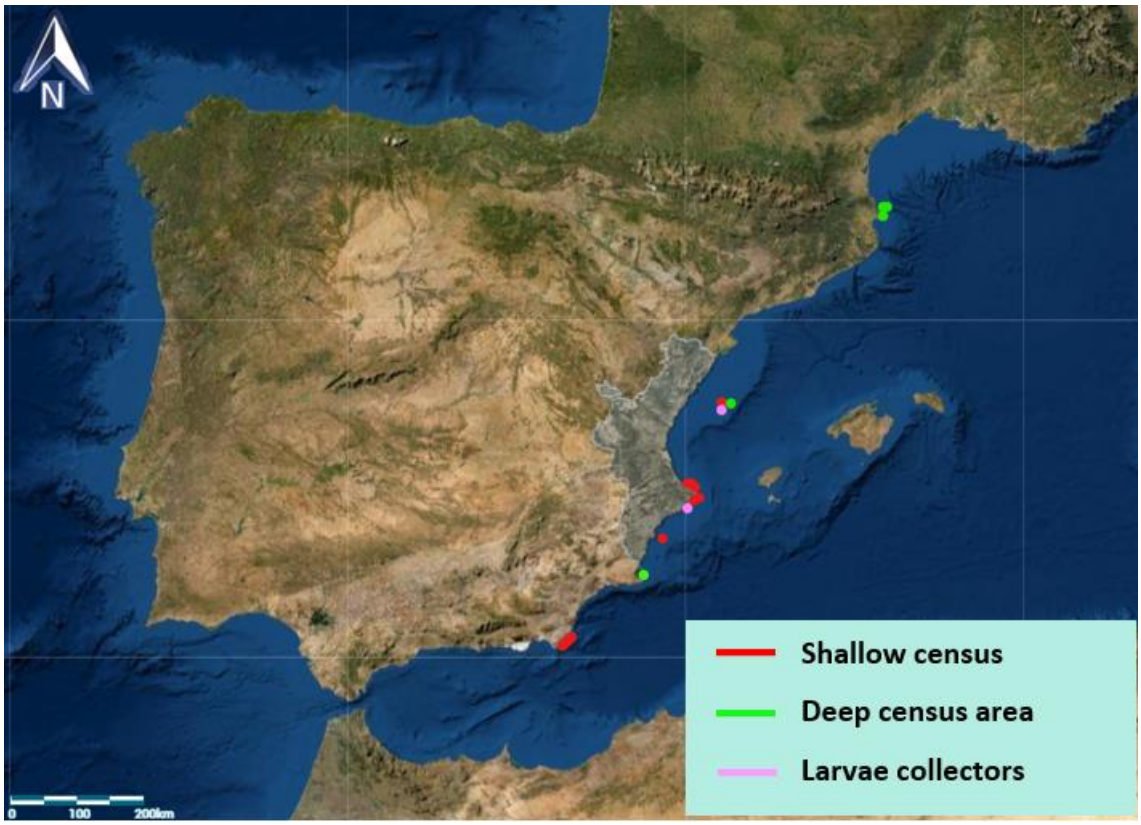

Figure 4. Areas proposed for shallow and deep exploration and for the installation of larvae collectors in Spain

Figure 5. Areas proposed for shallow exploration in Cabo de Gata (Almería)

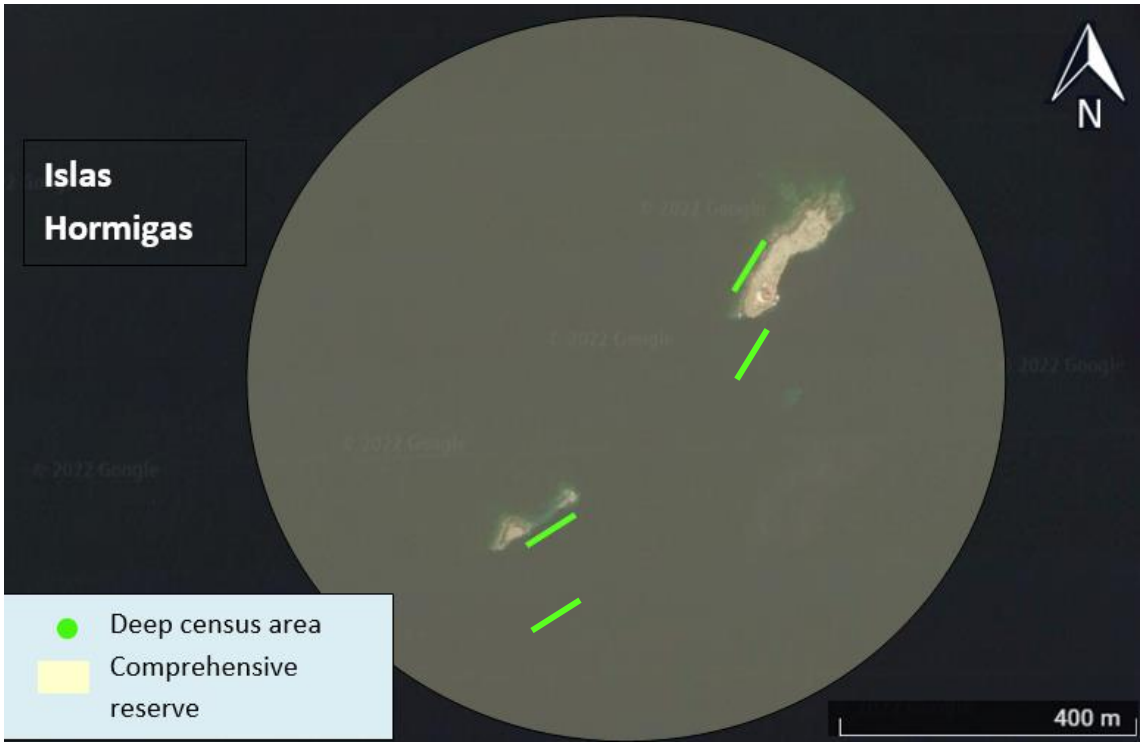

Figure 6. Areas proposed for deep exploration in Hormigas Islands (Murcia)

Figure 7. Areas proposed for shallow exploration in Tabarca Island (Alicante)

Figure 8. Areas proposed for larvae collectors in Calpe (Alicante)

Figure 9. Areas proposed for shallow exploration in Moraira (Alicante)

Figure 10. Areas proposed for shallow exploration in Marina Alta, between the towns of Dénia and Jávea (Alicante)

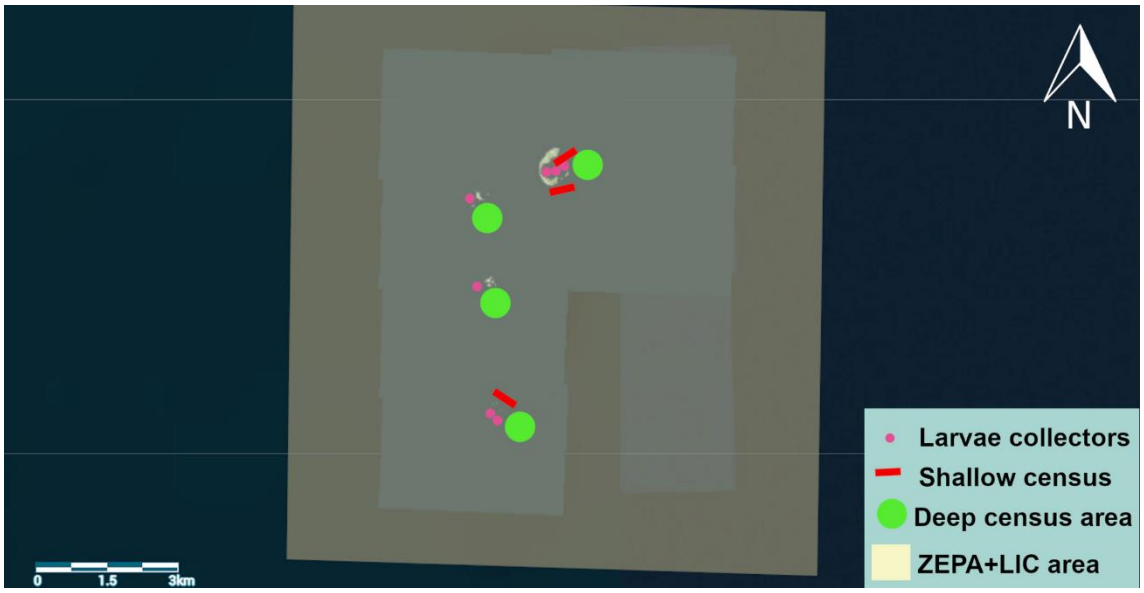

Figure 11. Areas proposed for shallow and deep exploration and for larvae collectors in Columbretes Islands (Castellón)

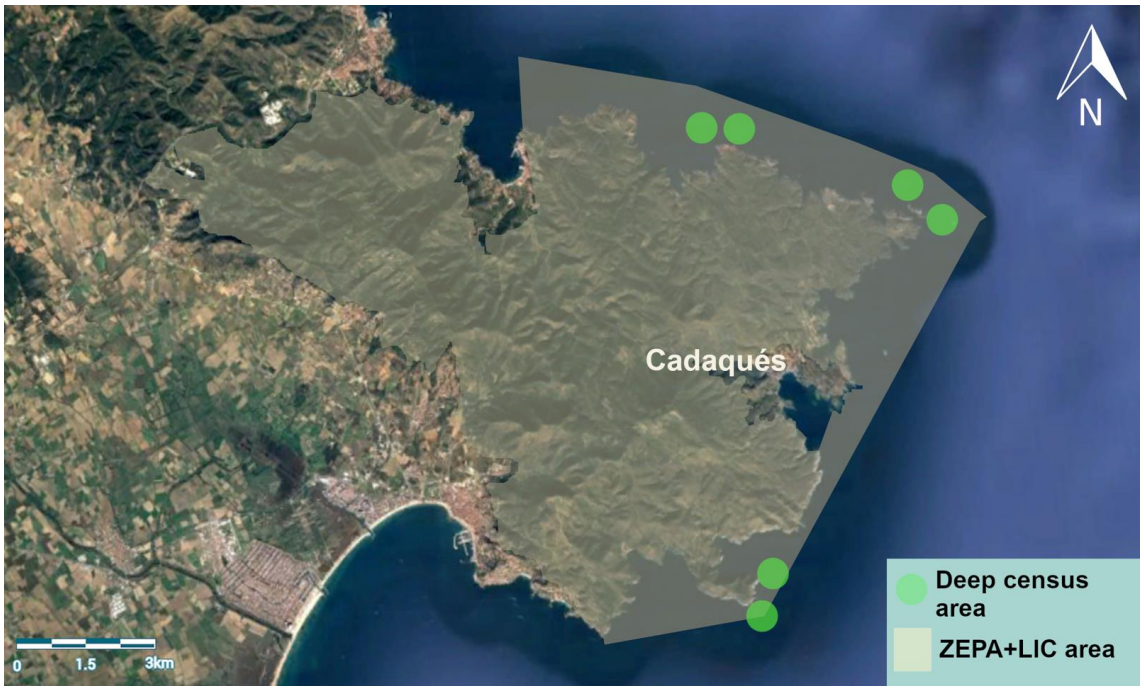

Figure 12. Areas proposed for deep exploration in Cap de Creus (Girona)

Balearic Islands

Previous knowledge on distribution and densities of *Pinna nobilis* across Balearic Islands are available. CSIC-IEO conducted several censuses at five main islands previous the MME: Mallorca, Menorca, Ibiza, Formentera and Cabrera and several islets (Figure 4). This mesoscale study integrates variability among hundreds of kilometres covering an area of around 150,000 km^2 (Deudero et al. 2015, Vázquez-Luis et al. 2015a). A total of 805 visual censuses by scuba diving

were conducted to survey fan mussel's density in different islands of the Balearic Archipelago (Cabrera, Formentera, Ibiza, Mallorca and Menorca), 661 of them at two different depths (10 and 20 m) in *Posidonia oceanica* seagrass meadows, and in addition, in Cabrera MPA other habitats of the MPA (detritic, rocky, sandy) up to 50 m depth were also surveyed with a total of 144 transects.

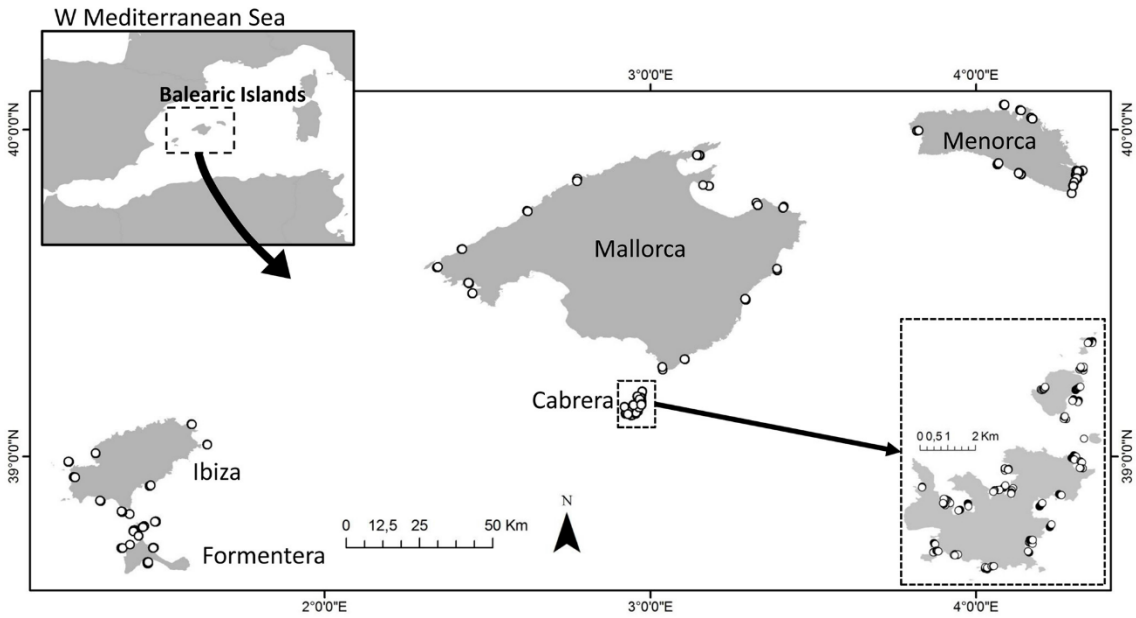

Figure 4 13. *Pinna nobilis* visual censuses carried out in the Balearic Islands (Deudero et al. 2015).

Total observed average densities among islands were 3.21±0.13 ind/100 m^2, varying among islands and among localities. The highest density values were found in Cabrera National Park, with average values of 5.74 ind/100 m^2 in *P. oceanica* meadows and maximum values of 37.33 ind/100 m^2 at 8 m depth. The lowest density values of *P. nobilis* were found in Ibiza island, with mean values of 0.83 mean ind/100 m^2 (Deudero et al. 2015, Vázquez-Luis et al. 2015a) (Figure 5). It should be noted that some localities with high densities were found on each island, as is the case of the N and E coast of Menorca, the E coast of Mallorca and in the area of the strait between the islands of Ibiza and Formentera. Therefore, these localities with high previous densities are taken into account to select the proposed sampling areas.

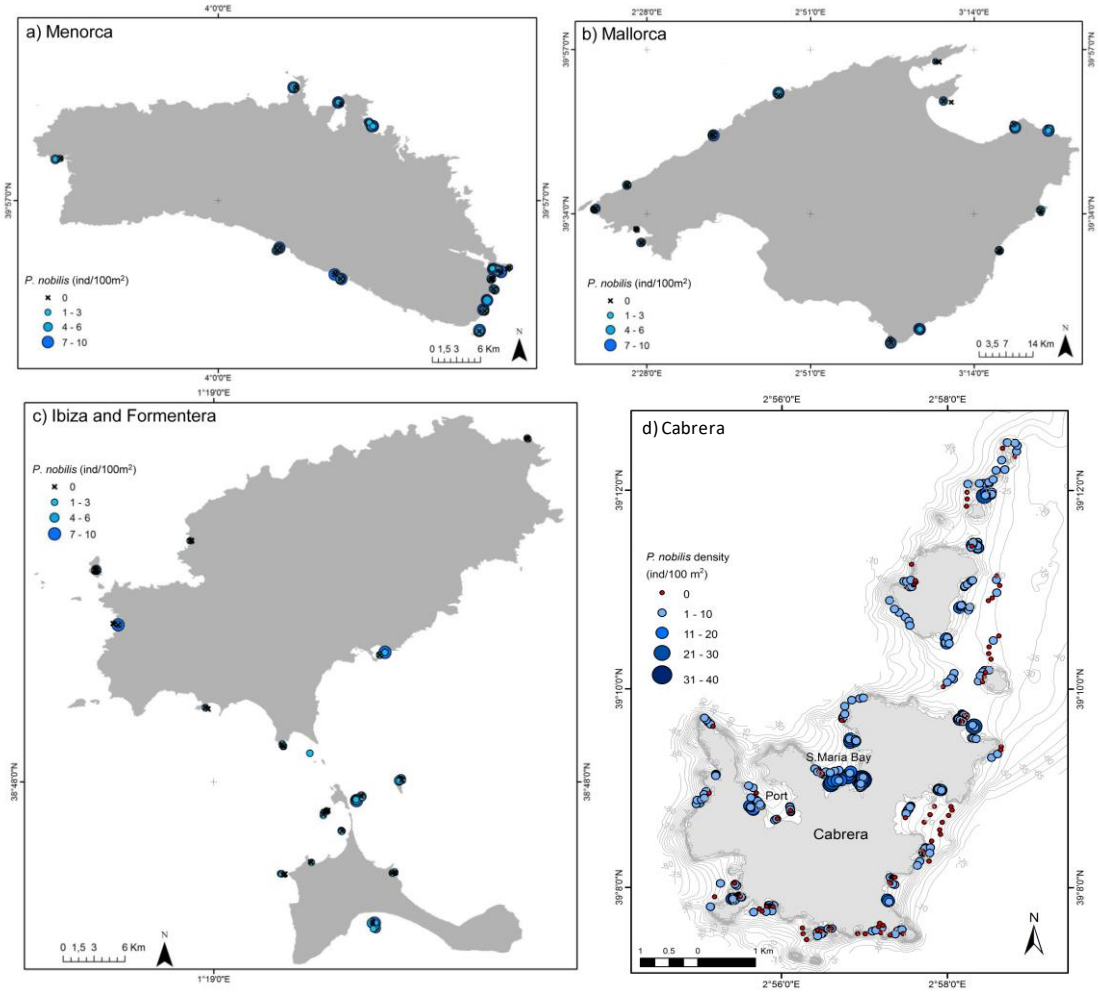

Figure 5 14. Densities of living *Pinna nobilis* (ind/100m^2) found across Balearic Islands. (Note that density scale differs for Cabrera) (Deudero et al. 2015, Vázquez-Luis et al. 2015a).

In the frame of PINNARCA project scientific divers will perform shallow underwater visual surveys (<20 m depth) in sites that presented the highest densities before the MME across the different islands of the archipelago. Moreover, deep underwater visual surveys (at depths between 20 and 40 m) will be also conducted by using and underwater propulsion vehicles (UPV, scooters) (Figure 6). More than one site will be surveyed at each location. In addition, parallel observations by citizen science will be also taken into account and verified in situ as it is proving to be a fundamental source for the detection of survivors in Spain.

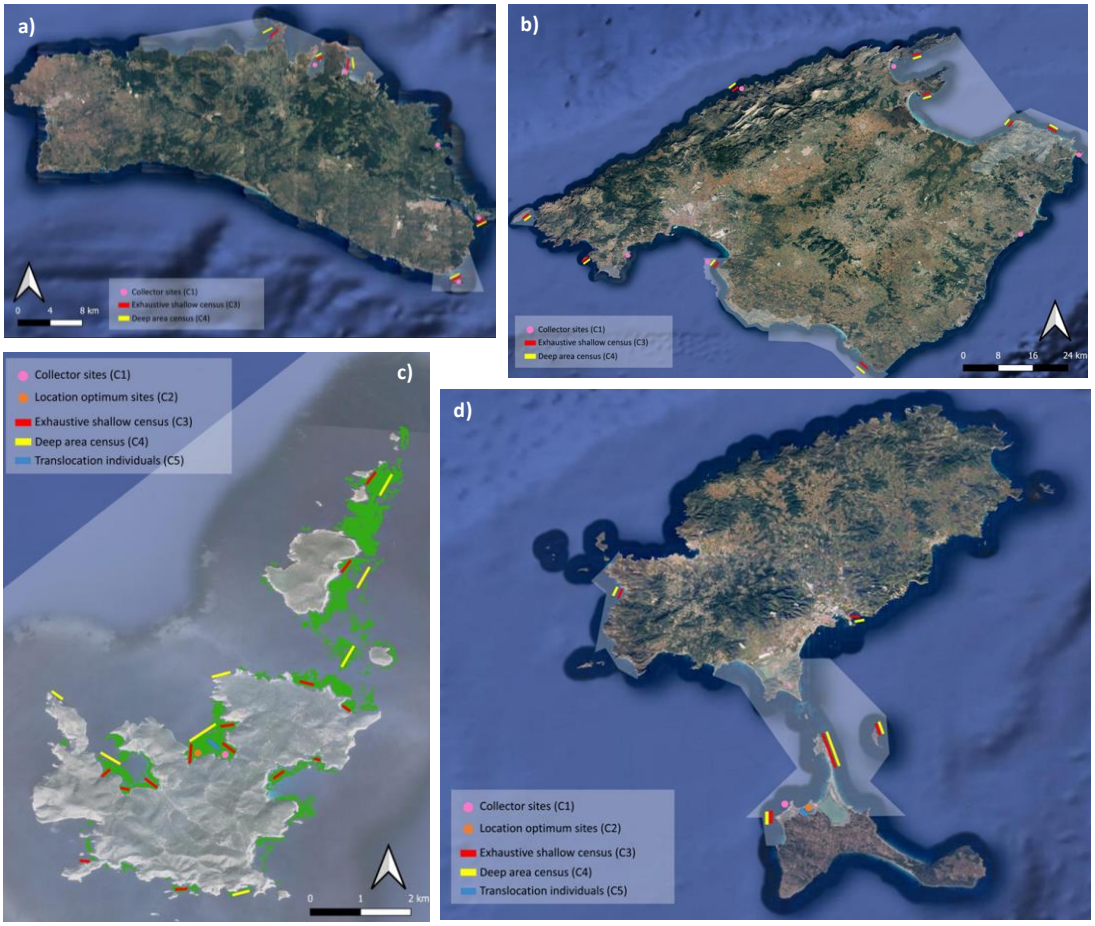

Figure 6 15. Proposed sampling localities for the shallow (red) and deep (yellow) diving visual censuses. The locations proposed for the optimal sites are also indicated (orange) in Cabrera National Park (c) for resistant individuals translocations, and Formentera island (d) for hosting *P. nobilis* populations

4.2. Potential areas to host *Pinna nobilis* population

Optimal sites for translocation of resistant individuals will be identified considering previous data on distribution, density, population dynamics, genetic connectivity of the species and protection level. New areas for the translocation of survival individuals will be identified within the limits of the Cabrera National Park, where there is currently the first optimal site for translocation of resistant's (Figure 615c).

It is also recommended to explore several coastal lagoons including Charcones de Punta Entinas, Levante lake in El Fondó Elx i Crevillent, Albufera of Valencia and areas of Delta del Ebro in the connection channels and el Fangar. Furthermore, there is a network of salt evaporation ponds, not only in Spain, but also in Europe, whose intake channels may host optimum conditions for the survival of *P. nobilis*. The Salinas de Cabo de Gata hosts one of those salt ponds that should be monitored for optimum conditions.

The probability of pathogen-free areas should also be explored in the Balearic Islands which may enhance dispersal capacity and successful settlement. One of this probable sanctuary areas, particularly L'Estany des Peix (small and shallow coastal lagoon located in Formentera island) will be prospected (Figure 6B). Although seems the salinity regime of this coastal lagoon is favourable for hosting fan mussel populations, annually salinity variations are not fully known. For this reason, a study will be carried out either through satellite data or by installing salinity sensors for a proper quantification of temperature and salinity.

Networking

5 SPECIFIC SYNERGIES WITH OTHER PROJECTS AND COUNTRIES

The PINNARCA LIFE Project will aim important synergies with projects, research groups and private entities and non-governmental organizations, such as:

- INTEMARES LIFE Project (2019-2024). Within the framework of the INTEMARES project, the aim is to evaluate the state of conservation of the pen shell population (*Pinna nobilis*) in the Mar Menor, as well as to determine the viability of recovering the population and to propose conservation actions if necessary.
- Marine Strategy Framework Directive (EsMarEs Project). Within the Marine Strategies project (EsMarEs), several actions are being carried out to assess the state of the *P. nobilis* Spanish population in and its degree of affectation by the pathogen *Haplosporidium pinnae.*
- ARES-2 Project. Within the framework of this regional project from Balearic Government, the surviving specimens of *P. nobilis* in the Balearic Islands coast will be monitored.
- MOVxPAIMED Paisajes salvados/paisajes por salvar. Movilización social y preservación del paisaje en el litoral mediterráneo español
- ECESIS (Ecology and impact of the Atlantic blue crab in Spanish Mediterranean coastal lagoons, estuaries, and adjacent waters)
- Sumaeco (Sustainability of marine coastal ecosystems in the context of global change in the mediterranean sea: modeling and simulations)
- National Parc of Port-Cros : Since 2020- Monitoring of residual individuals in the National Parc of Porquerolles and Port-Cros (Convention n°21-008 "Suivi des populations de grandes nacres Pinna nobilis dans le Parc national de Port-Cros -2021") (financed)
- French monitoring network for P. nobilis and P. rudis populations from Perpignan to Monaco and Corsica since 2017 (Nardo Vicente et al.)
- EuConexus (European University for Smart Urban Coastal Sustainability)
- H2020 SwafS – EuConexus-RFS Research for Society
- Pinores: 2021-2022 Genome sequencing of resistant, sensitive and hybrid individuals of *P. nobilis* across Europe (samples collected from Spain, Greece, Italy and France.
- LIFE REEF – Research of marine protected habitats in EEZ and determination of the necessary conservation status in Latvia
- CRIOBE (France). Centre for Island Research and Environmental Observatory is a Research Laboratory from École Pratique des Hautes Études (EPHE), the National Centre for Scientific Research (CNRS) and the University of Perpignan Via Domitia (UPVD). Dr. Serge Planes's team is a worldwide reference group on conservation genetic studies of the marine environment, larval dispersal and marine connectivity.
- The National Research Council of Italy – Institute of Anthropic Impact and Sustainability in Marine Environment

– Aquarium Pula (Croatia)

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