

LIFE PINNARCA

LIFE NAT/ES/001265



DELIVERABLE DA.1

PLANNING CORRECTION MEASURES REPORT IN THE MAR
MENOR LAGOON

FECHA 20/01/2022



Universidad de Alicante
CIMAR-UA



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University of Alicante

Carretera San Vicente del Raspeig s/n

03690 San Vicente del Raspeig - Alicante

Tel. 96 590 3400 - Fax 96 590 3464

ACTION A.1. DELIVERABLE LIFE PINNARCA

Date: [20/ January/ 2022](#)

RESEARCH ACTIVITY CONDUCTED WITH THE SUPPORT OF:



AUTHORS: Prof. F. Giménez Casalduero and E. Cortés Melendreras



1. Background

The fan mussel *Pinna nobilis* Linnaeus, 1758 is a Mediterranean endemic filter-feeding mollusc, the biggest Mediterranean bivalve and one of the biggest in the whole world, attaining lengths up to 120 cm (Zavodnik et al. 1991). According to Butler et al. (1993), it is settled in coastal areas with salinity ranging from 35 psu to 42 psu, at depths between 0.5 and 60 m, and mostly in soft-sediment areas overgrown by meadows of the seagrasses *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera marina* or *Zostera noltii* (Zavodnik 1967, Zavodnik et al. 1991) or sandy bottoms (Katsanevakis 2007). The population of *P. nobilis* in the Mediterranean Sea has been greatly reduced during the last few decades as a result of recreational and commercial fishing and incidental killing by trawling and anchoring (Rabaoui et al. 2011). But this pressure increased markedly from early autumn 2016, when a mass mortality event (MME) was detected, impacting *P. nobilis* throughout the western Mediterranean due to a parasite, *Haplosporidium pinnae* (Darriba, 2017, Catanese et al. 2018, Cabanellas-Reboredo et al. 2019), probably in synergy with mycobacteria a bacterial pathogen from the *Mycobacteria* group (Carella et al., 2019). Specimens of all sizes, depth ranges and habitat types were affected and the mortality reached 100% in some populations (Vázquez-Luis et al. 2017). The rapid collapse of the population caused concern and a change of status from the "Vulnerable" category to "Critically endangered" with a serious risk of extinction (Orden TEC / 1078/2018). At present, the mollusc is catalogued as critical danger of extinction (BOE 10/17/2018) and included in the Spanish List of Threatened Species (CEEAA) (Order TEC / 596 / 2019, of April 8) and in the IUCN red book of threatened species (Kersting et al., 2020) within the category of 'Critically Endangered'.

After the expanding of the pathogenic infection throughout the Mediterranean, coastal lagoons have become the main refuge enclaves for the fan mussel populations (Tsatisris et al. 2018, Panarese et al. 2019). Currently, there are only two populations of shellfish left on the Spanish coast: i) In the Ebro Delta (Catalonia) where, despite human pressure, thousands of specimens continue to be preserved, and ii) in the Mar Menor (Murcia), where the population is in a serious situation of regression, mainly due to the increasing eutrophication events that have occurred since 2016.

The Mar Menor is a hypersaline coastal lagoon, located in a semi-arid region of Southeast Spain (Figure 1) and isolated from the Mediterranean by a sandy bar. Its isolation and geomorphological conditions caused particular environmental conditions such as temperature stress, high salinity, etc, and consequently a landscape dominated by euryhaline and eurythermal species (Mas et al. 2017). However, the connection with the Mediterranean, due to the creation of an artificial channel associated with the construction of a marina in 1976, led to changes in the hydrodynamic regime of the lagoon, altering the water renewal rate. Consequently, the salinity and temperature decreased (Baraza et al. 2003, Lloret et al. 2005). At the beginning of the 80s the settlement of the first specimens of the fan mussel in the lagoon was confirmed, when the salinity had dropped to 45 ups (Rodríguez Babio & Navarro Tárrega 1983). Within a few years this bivalve became a key species within the Mar Menor lagoon reaching colonize about 65% of the lagoon surface until 2014 (Gimenez Casalduero et al, 2020). The successive episodes of eutrophication crises that started in 2016 have seriously decimated the *P. nobilis* lagoon population.

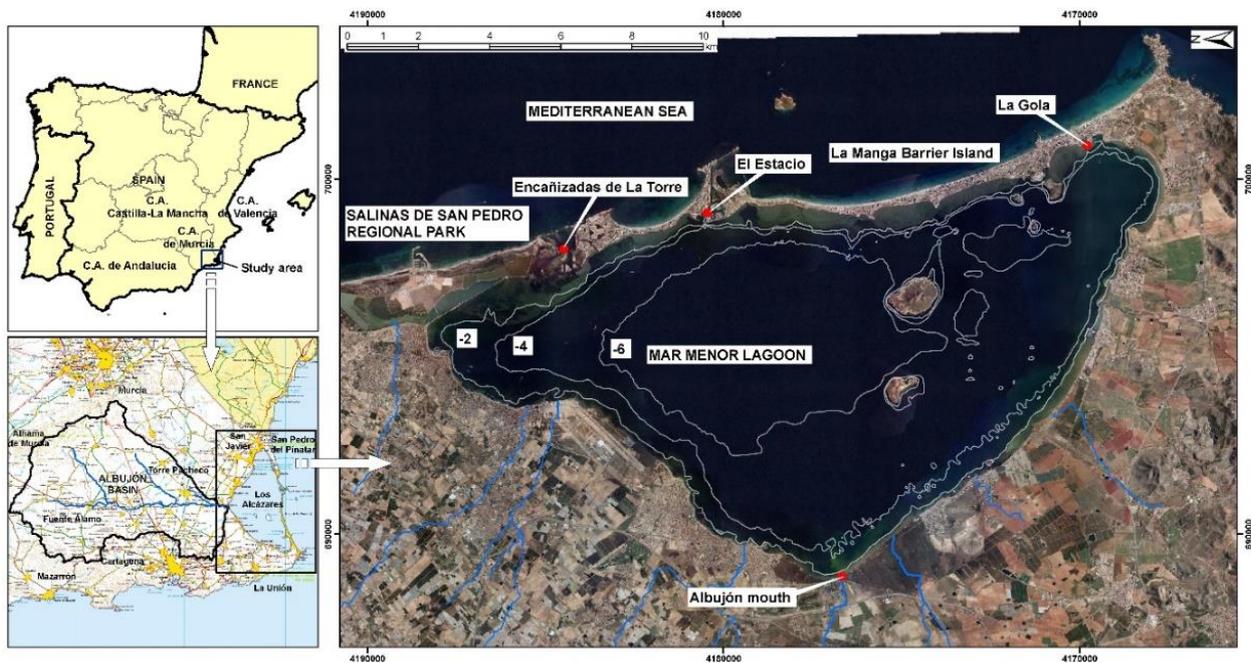


Fig. 1. Location of Mar Menor coastal lagoon.

2. List of local stakeholders

Name	Position	e-mail	Contact phone	Task/Observations
María Monteagudo Albar	Technician in the Unidad de Litoral -Mar Menor. Consejería de Agua, Agricultura, Ganadería, Pesca y Medio Ambiente. Dirección general de Medio Natural. Subdirección General de Patrimonio Natural y Cambio Climático.	maria.monteagudo2@carm.es murcianatural.carm.es	(+34) 968 228910	Technician Responsible of Nacra Working team for the Mar Menor population. Processing of permissions to manipulate <i>P. nobilis</i> in Mar Menor
Emilio Cortés Melendreras	Head and Curator of Murcia University Aquarium	ecortes@um.es	(+34) 609972721	Research " <i>in situ</i> " in the monitoring of fan mussel populations in the Mar Menor and " <i>ex situ</i> " in the development of maintenance and reproduction protocols.
Emilio Maria Dolores	Head of the Servicio de Pesca y Acuicultura. In the Consejería de Agua, Agricultura, Ganadería, Pesca y Medio Ambiente. Dirección General de Ganadería, Pesca y Acuicultura.	emilio.mariadolores@carm.es	(+34) 968326634	<p>a) Management and promotion of the fisheries and aquaculture sector.</p> <p>b) Research and technical assistance in the field of fisheries, aquaculture and shellfish farming.</p> <p>(c) drawing up and implementation of plans for the maintenance and improvement of the coastline and fishery resources.</p> <p>d) Management and maintenance of the Geographic Information</p>

				System within the scope of the Directorate General's competences. e) Proposing regulations and issuing reports on matters within its competence.
Francisco José Espejo García	Head of Dirección General de Ganadería, Pesca y Acuicultura.	http://www.carm.es/web/pagina?IDCONTENIDO=49525&IDTIPO=100&RASTRO=c672\$m	+(34) 968362756	It assumes the competences and functions related to aquaculture and shellfish farming, development and implementation of the Common Fisheries Policy and Integrated Maritime Policy, in particular the actions of integrated maritime surveillance, collection and analysis of data from the marine environment and oceanographic information from the coastline of the Region of Murcia,
Miriam Pérez Albaladejo	Dirección General del Mar Menor	miriam.perez@carm.es	+(34) 968366367	Head of the General Directorate of Mar Menor
Juan Faustino Martínez Fernández	Subdirección General de Patrimonio Natural y Cambio Climático	juanf.martinez@carm.es	+(34) 968 228848	Responsible of the study, planning, execution and development of projects and actions in the Mar Menor related to the environmental protection and regeneration of its ecosystem,
Pedro Luengo	Ecologistas en Acción	murcia@ecologistasenaccion.org		Provides support in volunteers activities
Jose Luís Alcaide Sanjurjo	Chairman of the association	alcaide68@gmail.com	+(34) 626272816	Provides support in volunteers activities.

	Hippocampus			
Manuel Ballesta Paredes	Cofradía de Pescadores de San Pedro	cofradisanpedrodelpinatar@gmail.com	(+34) 968594215 968595528	Head of the fishermen's association of San Pedro del Pinatar
Pedro Jiménez	GRIS	club.gris@gmail.com		Provides support in volunteers activities
Mercedes Varela	Posidonia Ecosport	mvarela@posidoniaecosports.com	+34 665908763	Provides support in volunteers activities
Isabel Rubio	Pacto por el Mar Menor	marmenormarmayor@hotmail.es		Provides support in volunteers activities

3. Recent actions in the Mar Menor coastal lagoon

3.1 Review of the colonization of *Pinna nobilis* in the Mar Menor lagoon previous to 2016 (UA-UMU)

The first record of *Pinna nobilis* in the Mar Menor (Figure 1) dates back to the beginning of the 80's in the 20th century. It was located at 6 m depth in a mixed meadow of *Caulerpa prolifera* and *Cymodocea nodosa* (Rodríguez Babio and Navarro Tárrega, 1983). The presence of this first specimen could be the consequence of the larvae viability coming from the huge population existing in the *Posidonia oceanica* meadow on the next Mediterranean margin, after the fall in salinity from the opening of the Estacio channel in 1974. (Rodríguez Babio and Navarro Tarraga, 1983).

In just a few years, the fan mussel became an important faunal element in the Mar Menor. Within the framework of the BURSATELLA project (MA/12/AYU/746), in 2013 the population in the entire lagoon was sampled and an occupation surface of more than 7000 Ha was considered, which constitutes 49% of the population. surface of the Mar Menor (Giménez Casalduero et al., 2013). The estimated density of *P. nobilis* in the lagoon before 2016 was 1609943 ± 3309 (mean \pm SE) individuals. (Giménez Casalduero et al, 2020).

In order to understand the process of *P. nobilis* colonization in the Mar Menor, it is necessary to construe the influence of the main factors that determine the colonization process. The artificial opening of the Estacio channel (communication channel between the Mar Menor and the Mediterranean) was the cause of the successive entry of numerous species into the lagoon, some non-native and/or invasive (Gimenez-Casalduero et al. 2016). However, Giménez Casalduero et al (2020) described that the natural channel “Encañizada” has also been an important gateway to the lagoon (Figure 2). Originally, the dispersion of *Pinna nobilis* in the lagoon was assumed as an anthropic process caused by the fall in salinity (Giménez Casalduero et al, 2020).

The salinity tolerance range for *Pinna nobilis* had been described in previous studies between 35 and 42 psu (Schlieper et al. 1960, Butler et al. 1993). It is reasonable to think that this species could not support the lagoon high levels of salinity (average values of 52 psu) existing before opening the Estacio channel (Aravio-Torre & Arévalo 1971). But the presence of the fan mussel since the early 80s, (a few years after opening the communication channel between the two water bodies and just as the salinity in the lagoon fall to reach an average value of 44.5 psu according to Azzati et al. 1987), demonstrate that the upper limit of tolerance of this bivalve is above the previously described values (Schlieper et al. 1960, Butler et al. 1993). Subsequently, *P. nobilis* colonized the lagoon widely, withstanding a salinity range between 44 and 45 psu for several years before the eutrophication crisis occurred (Giménez Casalduero et al, 2020).

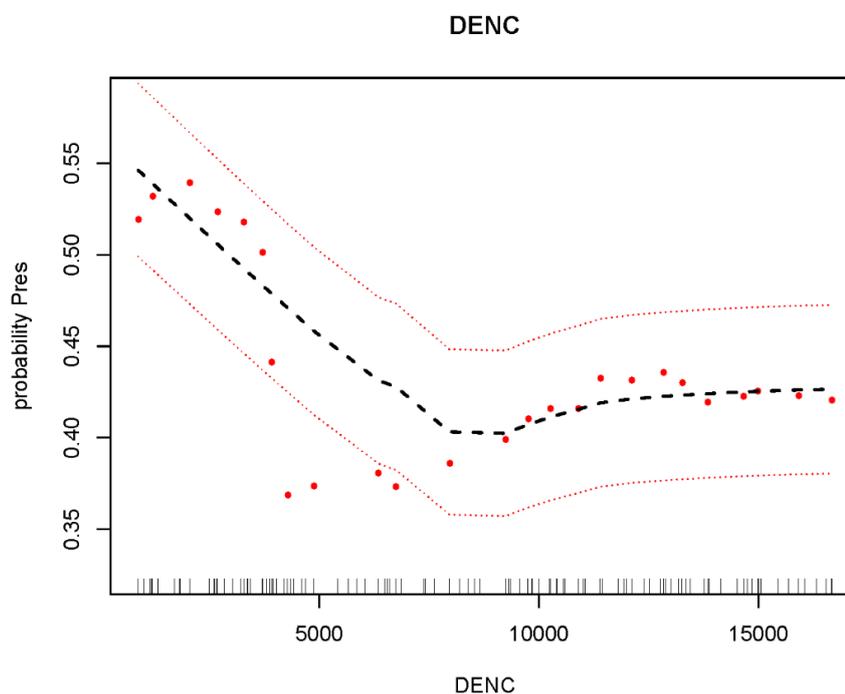


Figure 2: Effect of the Regression Kriging model of distance to the Encañizadas channel on the probability of *Pinna nobilis* presence in the Mar Menor (Giménez Casalduero et al 2020)

From the first moment of colonization until the early 2000s, the distribution of individuals was determined by the distance to the entry points to the Encañizadas and Estacio channels, which implied the influence of the larval

contribution coming from the extensive population existent in *Posidonia oceanica* meadow of the Mediterranean side (Figure 2,3).

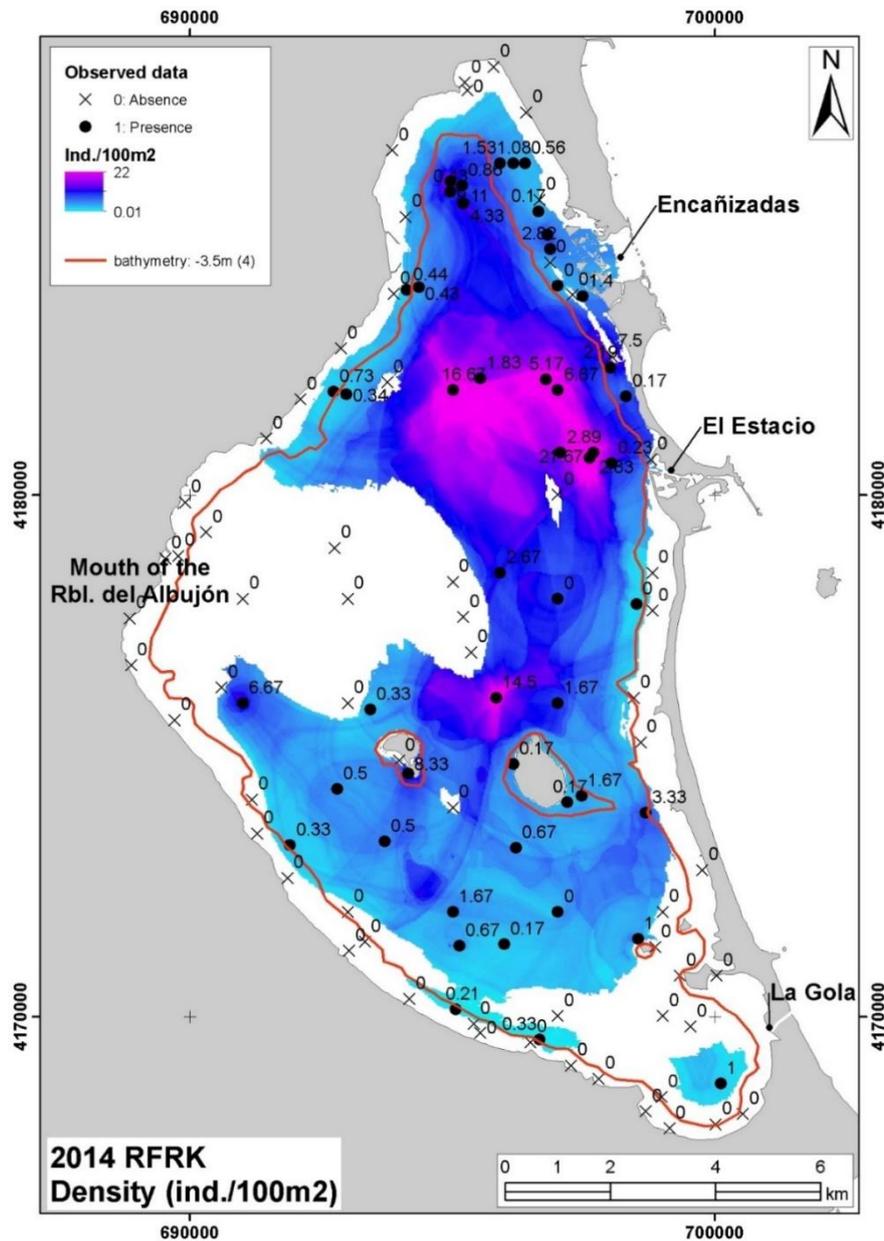


Figure 3: Potential distribution of the population of *Pinna nobilis* before 2016. The estimate is based on a Regression Kriging model, using a Random Forest model and ordinary kriging to estimate the relationship between observations and covariates to estimate their spatial relationship. The densities of individuals/100 m2 are represented for the surveyed points. The red line marks the 3.5 m depth level (Giménez Casalduero et al, 2020).

One of the main factors that explains the distribution of the population previous to 2015, is the presence of gravel in the sediment (Figure 4) which it can be explained by the fact that the juveniles needs fixed to large particles, seagrass rhizomes or solid substratum by its byssus filaments (Katsanevakis & Thessalou-Legaki 2009). The Mar Menor is characterized by the presence in many areas of a high concentration of particles such as gravel of conchiferous origin.

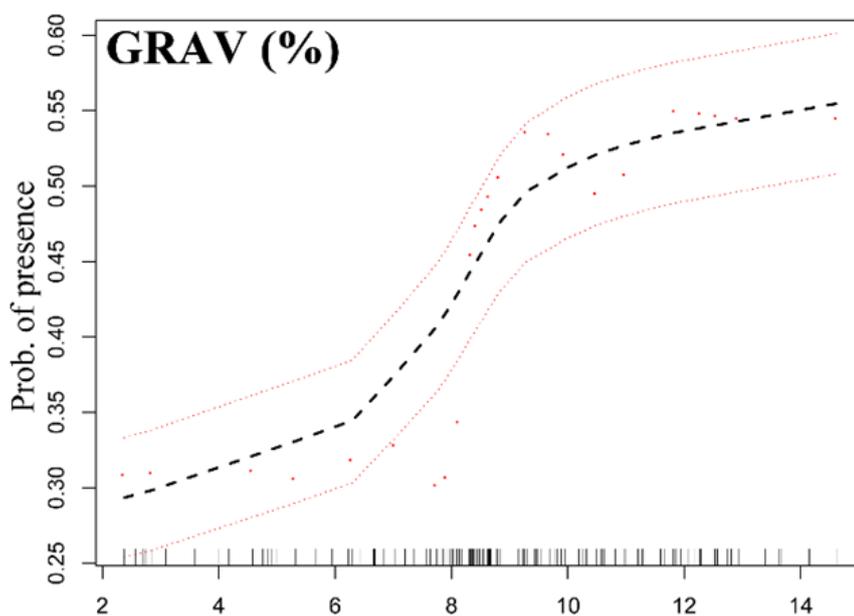


Figure 4: Effect of the Regression Kriging model of gravel concentration in the sediment on the probability of *Pinna nobilis* presence in the Mar Menor (Giménez Casalduero et al 2020).

3.2 Status of *Pinna nobilis* in the Mar Menor lagoon since 2016 (UMU-UA)

The processes of colonization and establishment of *P. nobilis* in the lagoon were interrupted by two simultaneous events in the Mar Menor area and the Mediterranean during 2016: i) the dramatic episode of eutrophication within the lagoon and; ii) the masive mortality event of *P. nobilis* in Mediterranean Sea that caused the disappearance of the large population that for years had been a source of larvae towards the lagoon.

At the start of the second half of 2015, a massive proliferation of phytoplankton began, and a Harmful Algal Bloom (HAB) event was detected, reaching total concentration values of 250,000,000 cells/litter (Aguilar and Giménez-Casalduero, 2016, Aguilar et al., 2016). In the spring of 2016, the process achieved the severe eutrophication status and "environmental collapse" was reached (Figure 4). The excess of nutrients caused an explosive growth of unicellular algae and the high cell concentration caused a decrease in light in deep areas, preventing photosynthesis in these areas. The vegetation located below the new photic threshold dies, and the decomposition of the organic matter causes a high consumption of oxygen (Mar Menor Scientific Advisory Committee, 2017). The absence of oxygen caused benthic organisms to die in masse, including the population of *Pinna nobilis*.



Figure 5: Massive algal bloom (MAF) in the Mar Menor lagoon during spring 2016. Photograph by J. Murcia

A survey conducted between April and June 2017 confirmed a mortality of more than 99 % of adult fan mussel in the lagoon. Two different situations can be observed. First, no living individuals were found below 2.5 m. in depth, even in areas where, until 2014, there had been a strong likelihood

of individuals appearing (Giménez Casalduero et al., 2020). This depth threshold coincides with that described by Belando et al. (2017), which confirmed the total disappearance of benthic macrophytes below an average of 2.5 m depth in the whole lagoon, after the eutrophication episode of 2016 and its replacement by an extremely slimy and anoxic sediment. It is highly probable that in these areas, the loss of light produced by the high concentration of phytoplankton in the water column was responsible for the death of the macrophytes, while the decomposition of the organic matter led to intense anoxia processes that caused the death of the macrofauna, including *P. nobilis*. However, in the population located above 2,5 m depth, a survival rate of 10% was estimated, which is higher than that described for populations affected by *Haplosporidium pinnae* in the Mediterranean (Catanese et al, 2018). The potential distribution in the lagoon was reduced to 934 Ha, taking up less than 11% of the potential distribution area existing before 2016. The surviving specimens were located in the surface area, with the highest densities being observed in areas close to the Isla del Barón and La Perdiguera (Cortés and Giménez Casalduero, 2019, 2020). Analysis of one of the surviving individuals during 2017 confirmed the absence of *Haplosporidium pinnae* in the Encañizadas channel in the lagoon (Catanese et al. 2018). It is very likely that the death observed in the surface areas of the lagoon perimeter up to 2017 was due to an episode of eutrophication in the lagoon and not to the presence of the pathogen. After sampling in early 2017 that ruled out the presence of *Haplosporidium* sp. in the lagoon, a series of meteorological confluences came together that caused a significant inflow of water from the Mediterranean through the Encañizadas.

During 2017, an Isolated Upper Level Atmospheric Depression (DANA) caused a drop in salinity in the lagoon coincided with intense storms of easterly winds that introduced a large amount of water into the lagoon. After these episodes, the mortality of all the *Pinna nobilis* specimens in the Encañizadas area was confirmed (probably due to the entry of *H. pinnae*). After these episodes, a rapid rise in salinity was observed, which left the relict pathogen in the area (Cortés et al, 2021).

Between 2016 and 2018, after the eutrophic crisis, a slight recovery of vegetation in the shallow areas of the lagoon and a primary recolonization of invertebrates in the sediment were detected (Ruiz et al., 2019). However, during 2019, the chlorophyll a concentration in the water column progressively increased to levels similar to those recorded during 2016 (Ruiz et al., 2019). This situation worsened in October 2019, when a heavy flood caused a massive inflow of fresh water into the lagoon, resulting in stratification of the water body (i.e., high salinity with active eutrophication processes in the deeper waters and lower salinity and high nutrient levels of the flood runoff in the shallower waters). This event caused euxinic conditions in the deeper water of the lagoon (Ruiz et al., 2019). Euxinic conditions occur when the water is anoxic, and hydrogen sulphide (H₂S) levels increase (Schouten et al., 2001). In the Mar Menor the euxinic process caused the death of macrofaunal community below 3m, due to the lack of oxygen and the presence of toxins as a result of the decomposition of organic matter by anaerobic bacteria (Meyer and Kump, 2008; Ruiz et al., 2019, 2020, Martínez Fernandez & Giménez Casalduero, 2020). The drop-in salinity values also could be the cause of the expansion of the *Haplosporidium* sp pathogen in localities such as Pueblo Calido close to the area where it could be relict since 2017 (Cortes et al, 2021). The presence of *Haplosporidium pinnae* in this locality was later confirmed by Nebot-Colomer et al (2021)

Deep water mass emerged in the northern area due to the effect of the easterly winds, in October 12, 2019, causing the flight to the shore of millions of specimens of mobile species (fish, crabs, prawns and various invertebrates), where they died in masse cornered and affected by the mass of anoxic and toxic water. In the same way, superficial *Pinna nobilis* population located in that area and that had survived in previous episodes, died (Cortés et al, 2021).

Just weeks after the 2019 Mar Menor euxinic episode, large aggregations of polychaetes from the family Serpulidae Rafinesque (1815) (Annelida,

Polychaeta), concentrated in the central coastal region, reappeared. Serpulid reefs were observed on the sedimentary bottom, and some fan mussel shells (*Pinna nobilis*; Linnaeus, 1758) were also covered with serpulid aggregations (Figure 6) causing the death of some specimens in the enclaves most affected by the change in environmental conditions (Sandonnini et al 2021).



Figure 6: Fan mussel shell (*Pinna nobilis*) covered by *Hydroides dianthus* polychaete tubes, colonized by a *Gobius paganellus*. Photography by Javier Murcia.

It is estimated that around 20% of the surviving specimens from the 2016 episode died in 2019 (Cortés Melendreras & Giménez Casalduero, 2020; Cortés et al, 2021).

3.3. Meeting and workshop focused on the situation of *Pinna nobilis* population in the Mar Menor.

<https://transparencia.carm.es/-/comite-de-asesoramiento-cientifico-del-mar-menor>

- 2018 Specific meeting of the "Lagoon Ecology Working Group" of the Scientific Advisory Committee of Mar Menor, to analyse the situation of *Pinna nobilis* in the coastal lagoon (E. Cortés Melendreras and F. Giménez Casalduero).
- 2019 Specific meeting of the "Lagoon Ecology Working Group" of the Scientific Advisory Committee of Mar Menor, to analyse i) the Current situation of the lagoon populations of *Pinna nobilis* and ii) actions carried out by the *Dirección General del Mar Menor*. (E. Cortés Melendreras and F. Giménez Casalduero)
- 2019 Specific meeting of the Scientific Advisory Committee of Mar Menor, carried out by the *Dirección General del Mar Menor*. (E. Cortés Melendreras and F. Giménez Casalduero).
- 2020 Specific meeting of the "Lagoon Ecology Working Group" of the Scientific Advisory Committee of Mar Menor, to analyse i) the Current situation of the lagoon populations of *Pinna nobilis* and ii) actions carried out by the *Dirección General del Mar Menor*. (E. Cortés Melendreras and F. Giménez Casalduero).
- 2020 (23, 24 January) Scientific Meeting in Barcelona.
- 2021 Specific meeting of the "Lagoon Ecology Working Group" of the Scientific Advisory Committee of Mar Menor, to analyse i) the Current situation of the lagoon populations of *Pinna nobilis* and ii) actions carried out by the *Dirección General del Mar Menor*. (E. Cortés Melendreras and F. Giménez Casalduero).
- 2022 Specific meeting of the "Lagoon Ecology Working Group" of the Scientific Advisory Committee of Mar Menor, to analyse i) the Current situation of the lagoon populations of *Pinna nobilis* and ii) actions carried

out by the *Dirección General del Mar Menor*. (E. Cortés Melendreras and F. Giménez Casalduero).

Funding: Fees for travel and assistance own funds AQUARIUM-UMU/CIMAR-UA

3.4 Project *Pinna nobilis* tagging from The Mar Menor Special Area of Conservation (SAC) and Study the epibiosis community and its influence on survival of the specie (CIMAR-UA/ AQUARIUM-UMU)

The Management Integral Plan of the Special Conservation Areas (ZEC) (Decree No. 259/2019, of October 10), of Mar Menor and the Mediterranean coastline of the Region of Murcia responds to the need to guarantee the conservation the Mar Menor ecosystem and it establishes a total of 58 actions for conservation, linked to each of the general and operational objectives described in its corresponding section.

The project is part of the SA.5^a. Evaluation and monitoring of key lagoon and marine species Action. This action advances in knowledge about the distribution of existing populations. On the other hand, on 01/31/2020 the following actions were declared an emergency in relation to the recovery of the population of *Pinna nobilis* (Fan mussel):

1. Management, extraction and translocation of specimens from the natural environment.
2. Maintenance and conservation "ex situ" of the species.
3. Monitoring and diagnosis of the population of *Pinna nobilis* in the Laguna del Mar Menor.

Preliminary observations pointed that the presence of a large number of epibionts has been detected on numerous shells of *Pinna nobilis* and this could generate a possible risk to the population. As part of the actions necessary to achieve the objectives described above it considered the

necessity to assess the state of the specimens of *P. nobilis* through a descriptive study on epibionts and a preliminary diagnosis on this new threat.

The achievement of these objectives is carried out through the coordinated and collaborative work of technicians and specialists from the University of Alicante, specifically the Marine Biology Research Group of the Department of Marine Sciences and Applied Biology, the Aquarium of the University of Murcia and the technical staff of the CARM through the company TRAGSATEC.

The project had three main objectives: i) Tag and monitor *Pinna nobilis* specimens; ii) Characterize the epibionts present in the valves; iii) Study of the influence of epibionts on the survival of the fan mussel and proposal of management measures.

The design of tagging labels was tested in an initial stage. The results laid the foundations to analyse their effectiveness and helped define size, materials, colour, size number, etc., looking for resistant and cheaper materials (Figure 7).



Figura 7: Numbered label to tag *Pinna nobilis* specimen

During the period covered by this project (2019) 590,000 m², or 76 Has have been sampled during 77 sampling days. A total of 686 specimens were georeferenced and 321 tagged in two localities (El Barón and Puerto Romano).

In order to obtain the distribution of the *P. nobilis* population after 2016 eutrophication crisis, a extensive survey was carried out throughout the lagoon perimeter and presence and absence data have been obtained. A potential distribution map of *P. nobilis* has been obtained, after 2016 (Figure 8). During the 2016-2020 period, 94 campaigns and nearly 200 sampling surveys have been carried out throughout the lagoon. As a result of this joint work between the *Consejería de Agua, Agricultura, Ganadería, Pesca y Medio Ambiente (Dirección General del Mar Menor y Dirección General de Medio Natural)*, the University of Murcia and the University of Alicante, a total of 820 specimens have been recorded, of which more than

88% have been georeferenced and labelled. Of this surviving population, death has been verified in these years (including the population nucleus that disappeared after the euxinic episode of 2019) of less than 3% of the total copies previously counted. To this percentage we must add the 8% of specimens that have disappeared due to actions of vandalism and poaching with illegal extraction of healthy specimens, intentional or accidental removal of specimens due to malpractice of some fishermen.

The probability of occurrence has been estimated from the presence/absence and density data obtained from the entire lagoon surface.

The results indicate the presence of 5 important population settlements between 2017 and 2020, set up by survivors of the 2016 crisis: i) Lo Pagán; ii) Pueblo Cálido; iii) Cabo Romano; iv) Baron Island; v) Perdiguera Island. The rest of the lagoon perimeter at depth of less than 3 m present loose specimens of fan mussel, without observing gregarious settlements. No living specimen were detected below 3 m depth. It should be noted that the nucleus described in Lo Pagán was disappeared due to the outcrop of euxinic waters that occurred after the 2019 DANA (Giménez Casalduero et al., 2020).

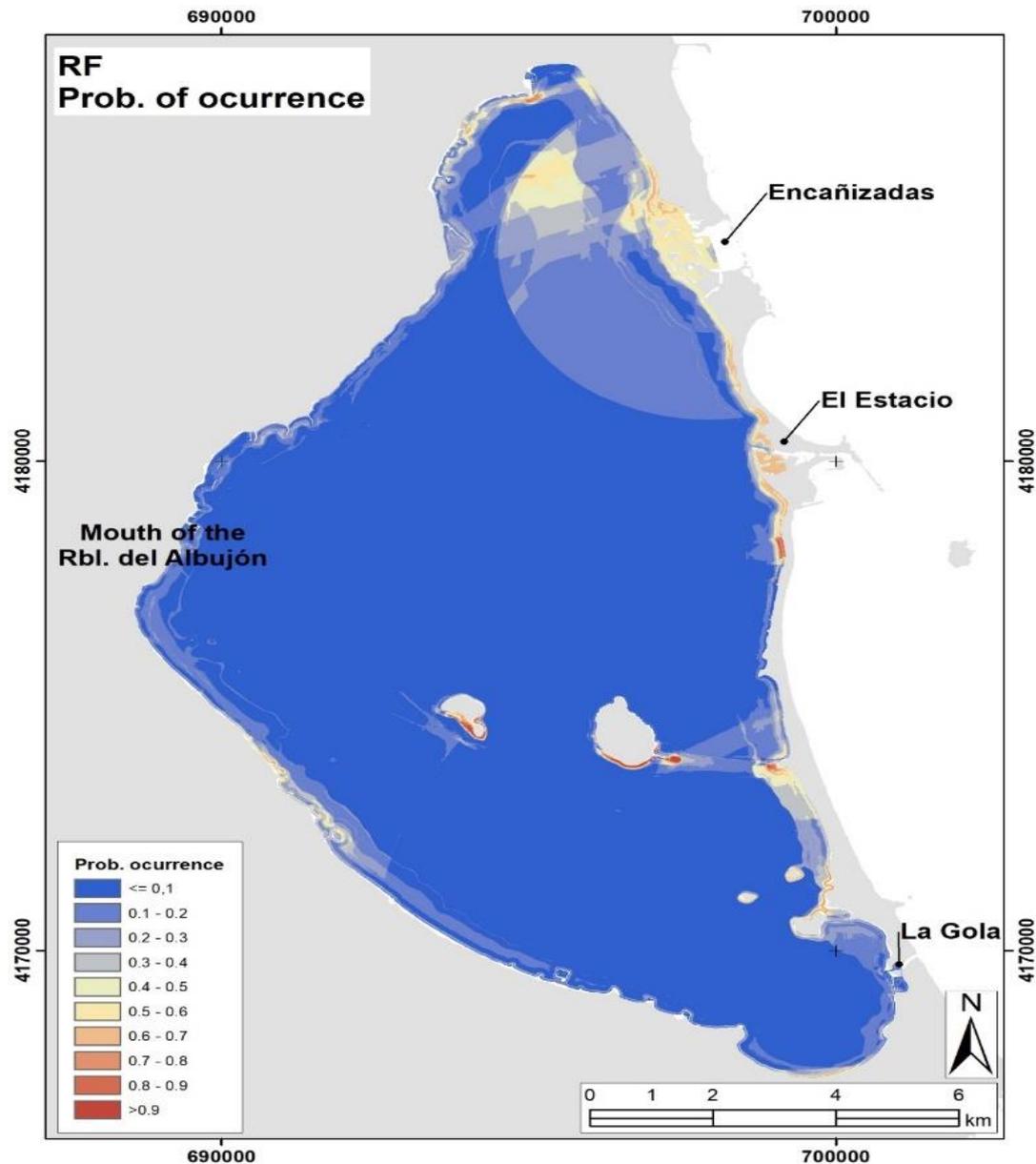


Figure 8: Probability of *Pinna nobilis* occurrences after 2016 in the Mar Menor coastal lagoon.

3.5 Establishment of citizen science project to report observations Fan mussel specimens

Murcia: “Nacranet blogspot” This is a specific informative and participative blogspot where information about the specie *Pinna nobilis*, mass mortality and the eutrophic crisis of the Mar Menor is described and where citizens can participate providing information about the lagoon specimens

Funding: CIMAR-UA/ AQUARIUM-UMU project "*La nacra en el Mar Menor: una responsabilidad histórica*" financed with FEDER funds from the Dirección General del Mar Menor.

3.6 Management and *ex situ* conservation of *Pinna nobilis* specimens from the Mar Menor. Project duration 10/12/2018 - 08/07/2019.

Project performed by the Aquarium of the University of Murcia, a centre integrated in the *Vicerrectorado de Investigación de la Universidad de Murcia*, in coordination with the Dirección general del Mar Menor, belonging to the Consejería de Agua, Agricultura, Ganadería, Pesca y Medio Ambiente, dedicated to the conservation of specimens collected in the natural environment because their viability was considered risky in the areas where they were located. Fundamentally, these were specimens in bathing areas that were often already lying down and in unfavourable conditions.

Based on these specimens, the protocols for the *ex situ* maintenance of *Pinna nobilis* began to be developed at the facilities of the Murcia University Aquarium

3.7 Proposal for a research study on the population of fan mussel (*Pinna nobilis*) in the area proposed for the location of anchorages in the islands of the Mar Menor. 2018/123141. Project duration 08/02/2019 - 09/12/2019.

Research carried out by the Aquarium of the University of Murcia, in coordination with the *Dirección general del Mar Menor*, belonging to the *Consejería de Agua, Agricultura, Ganadería, Pesca y Medio Ambiente*, dedicated to carrying out a study on the presence of fan mussel in areas intended for the installation of ecological anchorages, designed to avoid the

problems associated with the use of anchors in areas with the presence of fan mussel.

A diagnosis was made of the existing specimens in the proposed areas of the larger islands of the lagoon, with the aim of estimating the population density in these locations, data that increase knowledge about the population of the Mar Menor under monitoring.

Information was also obtained on the recruitment potential of the species in the selected areas. By studying the data as a whole and the characteristics of the habitat, potential areas for recruitment of the species were sought. The identification of these areas is of particular importance to ensure their protection in relation to anchorages and thus allow for future colonisation.

3.8 Emergency actions for the rescue of *Pinna nobilis* specimens in the Mar Menor. 2019/072853. Project duration 05/12/2019 - 05/03/2020.

Project performed from the Aquarium of the University of Murcia, in coordination with the *Dirección General de Medio Ambiente y Mar Menor*, belonging to the *Consejería de Empleo, Universidades y Medio Ambiente*, whose main objective was to carry out work dedicated to the rescue, conservation and restoration of the fan mussel populations of the Mar Menor, after the state of emergency caused by the climatic episode of the DANA that took place in September 2019.

Different actions were carried out for this purpose:

- 1 Location and monitoring of the specimens georeferenced previously and inclusion in the protocol of the new individuals found during the process.
- 2 Transfer of a certain number of specimens to new "*in situ*" locations free of possible threats and to closed system "*ex situ*" study tanks, designed to faithfully reproduce the conditions found in their natural

environment and to continue working on the development of maintenance and reproduction protocols in a closed system.

3 The fan mussel specimens destined for *ex situ* conservation have been under continuous observation since then and continue to be monitored according to the species' maintenance protocol, which determines the elements necessary to generate the stability of the appropriate physic-chemical parameters of the system, as well as the optimisation of diets that allow them to maintain their usual rates of growth and maturation.

3.9 Monitoring of *Pinna nobilis* breeding event in the Mar Menor ZEC, aimed at collecting larvae for study in the facilities of the University of Murcia Aquarium. 2020/023654. Project duration 20/07/2020 - 29/12/2020.

Project carried out by the Aquarium of the University of Murcia in coordination with the *Dirección General de Medio Natural*, belonging to the *Consejería de Agua, Agricultura, Ganadería, Pesca y Medio Ambiente*. This project has been very useful in locating in time the reproductive event of fan mussel in the different colonies of the species in the Mar Menor.

To achieve this, parameters indicating the proximity of the reproductive event were monitored in order to locate it over time.

Once located, gametes and larvae were collected to study their development under laboratory conditions.

3.10 *Ex situ* breeding of *Pinna nobilis* and creation of the Bank of Protected and Singular Species of the Mar Menor. Decree nº 65/2020. Dates 17/11/2020 - 30/06/2022

From the Aquarium of the University of Murcia, a centre integrated in the Vicerrectorado de Investigación de la Universidad de Murcia, in coordination with the *Dirección General del Mar Menor*, belonging to the *Consejería de Agua, Agricultura, Ganadería, Pesca y Medio Ambiente*, the

creation of a Bank of Emblematic Species and Species of Singular Importance of the Mar Menor, was considered as an action of general public interest, which guarantees the permanence over time of the populations of these species, regardless of possible events, occasionally critical in the natural environment.

In addition to ensuring their viability over time, the implementation of the Project is providing a huge amount of data on the biology of these species and on their evolutionary state in relation to other nearby populations.

The project is structured in two phases. The first phase will be carried out in the Aquarium of the University of Murcia, with a duration of 4 years in which reproductive groups of the different species will be formed and the protocols for maintenance and reproduction "ex situ" will be established and optimised.

The second phase of the project will be dedicated to expanding the stock of individuals of each species and their maintenance in semi-freedom conditions, in facilities provided for this purpose in different centres, which will be coordinated through the research group of the Aquarium of the University of Murcia and the *Dirección General del Mar Menor*.

Main objectives of the first phase of the research project.

1. To achieve the correct adaptation of the selected specimens of the different species to the closed systems installed.
2. To form an initial group of breeders that can be the base group for future phases of the species bank.
3. To determine the maintenance protocols for these species "ex situ".
4. Study the breeding behaviour of all the species to be worked on, developing breeding protocols for each of them. This is a simple task for some of the species we are already working on, but complex for others for which there is still no data on reproduction and development to adults.

The selected species are:

- Fan mussel (*Pinna nobilis*).
- Long-snouted seahorse (*Hippocampus guttulatus*).
- River pipefish (*Syngnathus abaster*).
- Mule needle (*Syngnathus typhle*).
- Fartet (*Apricaphanius iberus*).
- Fox (*Gobius niger*).
- Sand goby (*Pomatoschistus marmoratus*).
- Gallerbo (*Salarias pavo*).
- Chirrete (*Atherina boyeri*).
- Dragonlip (*Callionymus pusillus*).

Due to the instability of the populations of species such as the fan mussel and the seahorse, work has begun with these species, with the fan mussel (*Pinna nobilis*) being the main target in this first phase of the project.

As for the fan mussel maintenance protocols, interesting results have already been obtained, ranging from detailed environmental control to the evaluation of different diets and nutritional profiles.

The reproduction protocol is in the optimisation phase. So far, the larval stage of early pediveliger has been reached.

3.11 RECUPERA PINNA project (IRTA, University of Alicante, and IMEDMAR. IRTA1_21T

The research contract “Monitoring services, recovery and citizen science actions in the remaining populations of *Pinna nobilis* in the Mar Menor” within the framework of the project RECUPERA PINNA led by IRTA from June 2021 to January 2023. This project funded by the Fundación Biodiversidad is aimed at evaluating the current status of the populations in the Ebro Delta and Mar Menor, and to gain further understanding on the factors controlling the survival of individuals in these areas. Thanks to this project, we have now considerable information about mortality rates of

specimens associated with episodes of eutrophication and other anthropic actions. Preliminary results have detected a mortality of specimens between 8 and 31% associated directly or indirectly with the eutrophication crisis in the summer of 2021. In addition, two citizen science activities are planned whose objective is the extensive prospecting of areas of survival of specimens. These activities will be carried out with a diving club characterized by its involvement in the conservation of the marine environment and its high technical level. The dates of the activities are scheduled for April 9-10.

4. Monitored areas in Mar Menor

The first record of *Pinna nobilis* in the Mar Menor (Figure 1) dates back to the beginning of the 80's in the 20th century. It was located at 6 m depth in a mixed meadow of *Caulerpa prolifera* and *Cymodocea nodosa* (Rodríguez Babio and Navarro Tárrega, 1983; Murillo & Talavera, 1983) (Figure 10).

In 1988, the Comunidad Autónoma de la Region de Murcia carried out a pioneering study on the coastline (Characterisation, ecological assessment and determination of areas to be protected on the coast of the Region of Murcia) which gave rise to the first bionomic mapping at a scale of 1:50,000 with a high level of precision (Gomariz Castillo & Gimenez-Casalduero, 2012). Based on the previous works, the bionomic cartography is updated again in 2004, in order to be used as base information for the cartography necessary for the application of the Habitats Directive (92/43/CEE), especially in the areas designated as Places of Community Importance (LIC). All these studies are promoted by the Dirección General de Medio Ambiente of the Comunidad Autónoma de la Region de Murcia (CARM) (Gomariz Castillo & Gimenez-Casalduero, 2012). This update includes a database with relevant information for each of the cartographic polygons defined as a first data collection of the populations of *Pinna nobilis* in the Mar Menor, with information on their distribution. This database includes 1646 presence/absence observation points sampled during 2003 and 2004 (Figure 10, 11).

The following research on the species in the Mar Menor focus mainly on the characterization of the species, specifically Garcia March's doctoral thesis "Contribution to knowledge of Biology from *Pinna nobilis* Linneo, 1758 (Mollusca Bivalvia) on the Iberian Mediterranean coastline (2005).

Later, within the framework of the Bursatella project (MA/12/AYU/746), six surveys were conducted over a period of nine months, between March and October 2013 (Fig. 12).

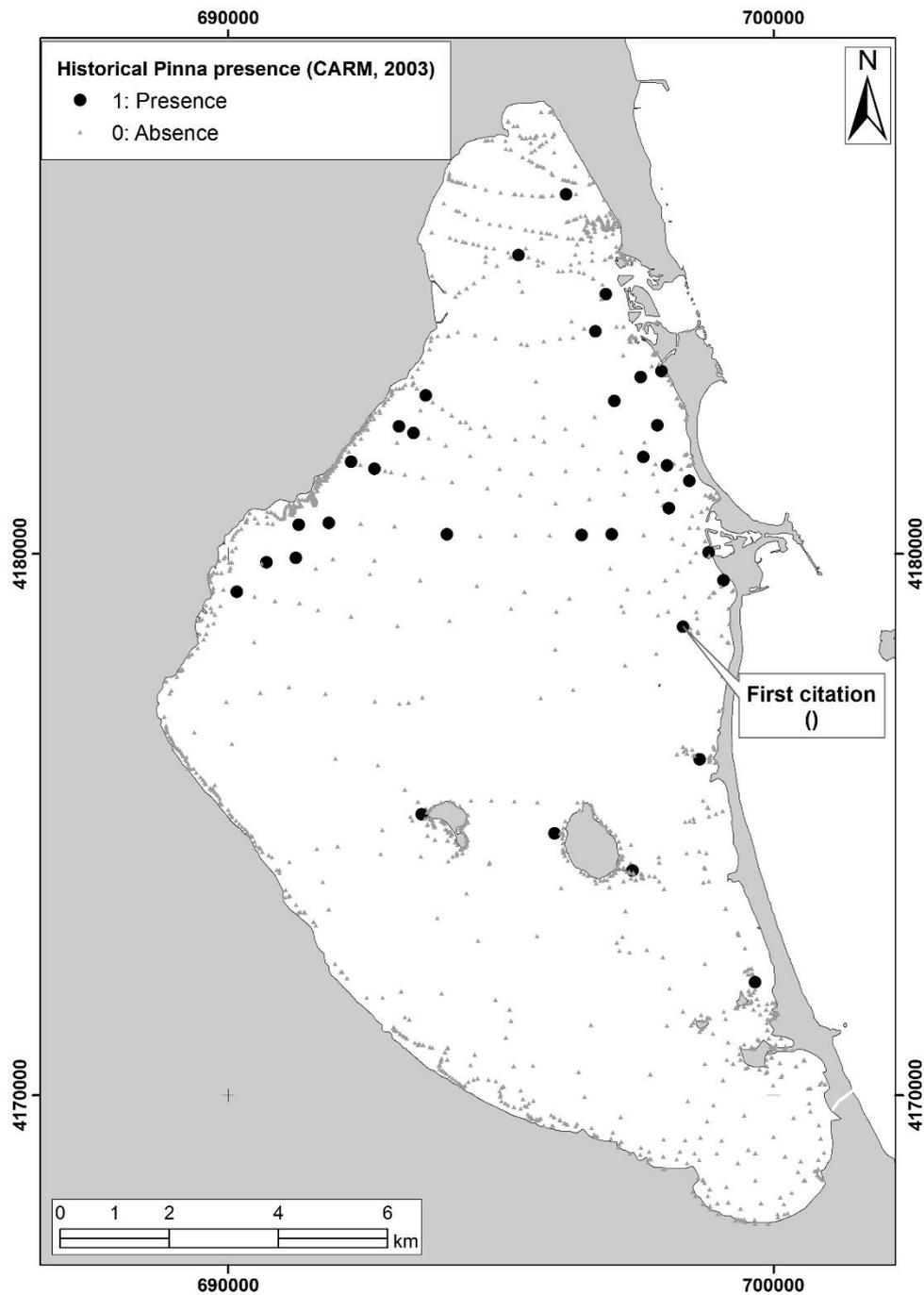


Fig 10: 2003 data of *Pinna nobilis* presence from database of CARM. First record of *P. nobilis* presence cited by Rodríguez Babio and Navarro Tárraga (1983) is marked

The lagoon was divided into a 49 square grid of 2000 m resolution to perform regular sampling to characterize the distribution of the mollusc (Figure 11). Three line transects were made from the epicentre of each square. This regular sampling was complemented with surveys using

aquaplaning by SCUBA diving with 1400 m long and 2 m wide transects in the deepest areas, or by sampling using bathyscaphe from a boat in shallow areas. All individuals within the transect area were counted, and their size was estimated visually. A total of 58 density measurements were made. (Fig. 12).

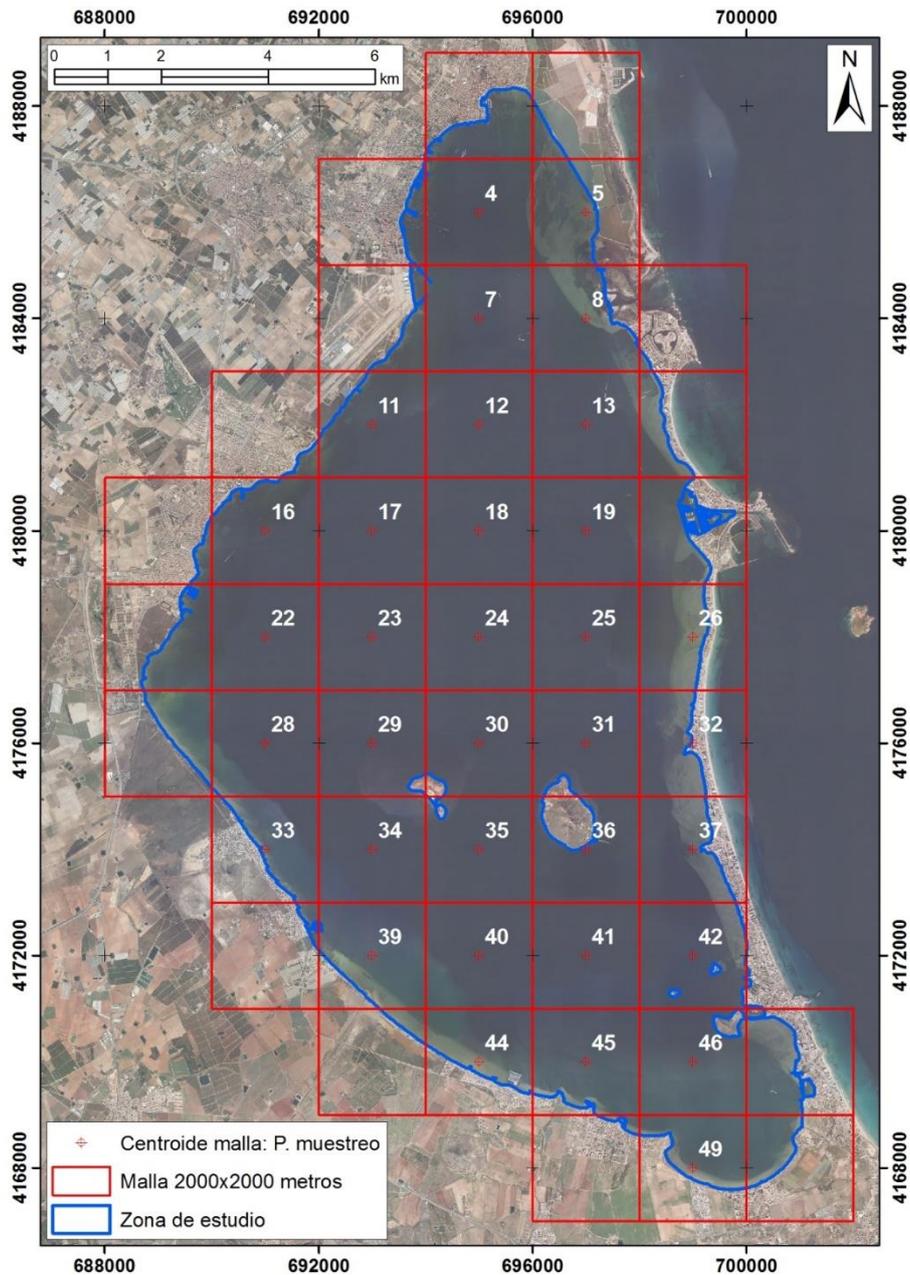


Fig. 11. Sampling units designed to carry out regular sampling in the lagoon since 2013 within the framework of the Bursatella project (2013)

Until 2014, it is estimated that there is an occupation of 8,279 hectares, which corresponds to 61% of the lagoon bottom.

Additionally, durin 2017 after the environmental collapse of 2016 caused by a eutrophication process, a survey was carried out to estimate the surviving specimens, sampling. 17 points

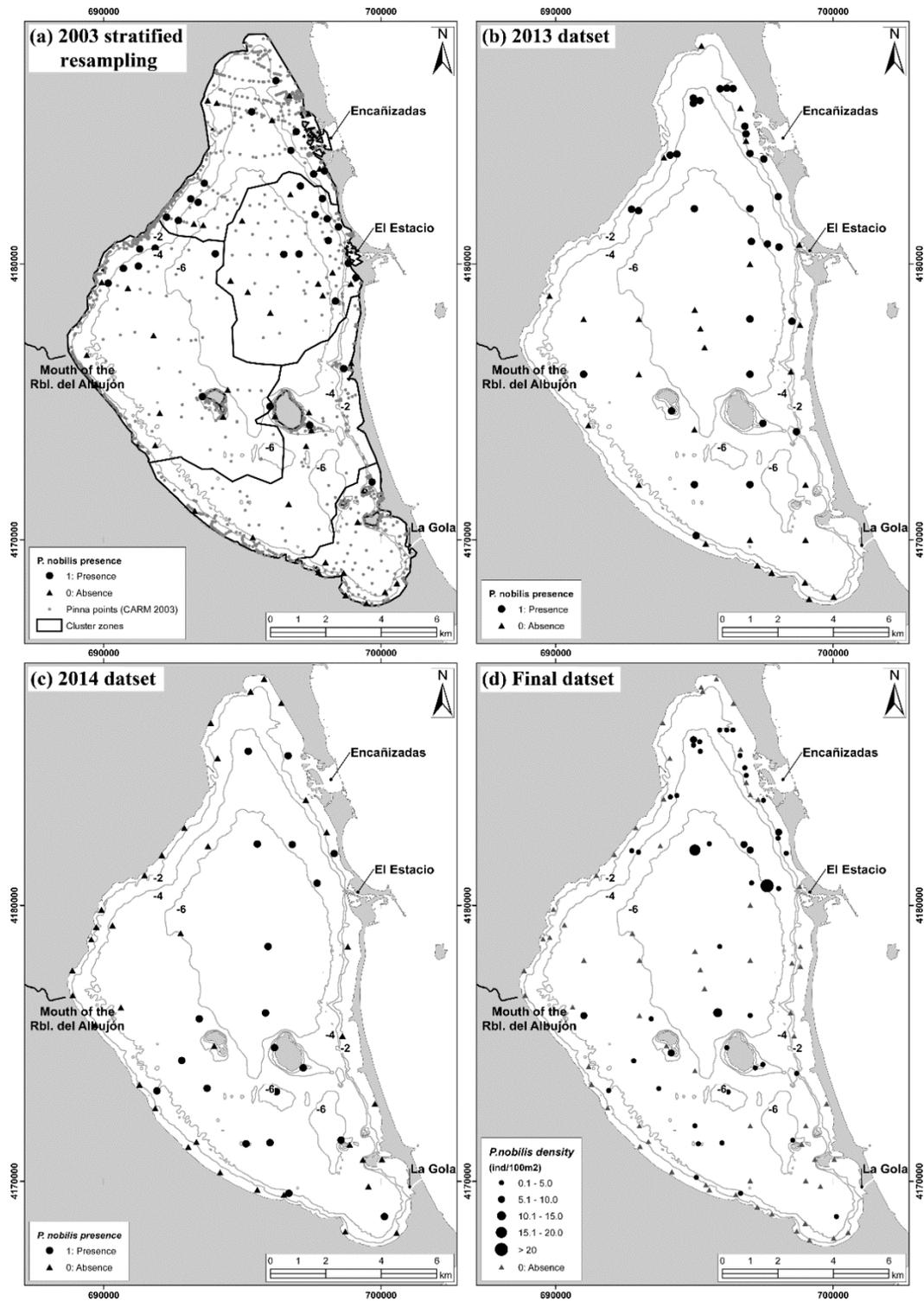


Fig. 12. Sampling survey and presence of *P. nobilis* in 2003, 2013 and 2014 from different sources. (Rodríguez Babio & Navarro Tarrega 1983, CARM 2003, Belando et al. 2014 and Giménez Casalduero et al 2020).

During 2016-2020 period, 94 sampling campaigns and nearly 200 sampling surveys have been carried out throughout the lagoon between the Consejería de Agua, Agricultura, Ganadería, Pesca y Medio Ambiente (Dirección General del Mar Menor y Dirección General de Medio Natural), the University of Murcia and the University of Alicante. As a result of this joint work, a total of 820 specimens have been recorded. For this period, of which more than 88% have been georeferenced and labeled (Figure 10). Of this surviving population, death has been verified in these years (including the population nucleus that disappeared after the euxinic episode of

From the surviving population of the 2016 eutrophication episode, the death in recent years of less than 3% of the total number of previously recorded specimens has been confirmed. (including the population group that disappeared after the 2019 euxinic episode). To the previous percentage must be added 8% of specimens that have disappeared due to actions of vandalism and poaching with illegal extraction of healthy specimens, intentional or accidental removal of specimens due to malpractice of some fishermen.

5. Evaluation of risk areas for pathogen infection

Based on the available evidence, it seems unlikely that there was an entry of the pathogen in the Mar Menor before 2017. The fan mussel mortality pattern observed in 2016 coincides with the effect of the eutrophication process that occurred in the lagoon that year (Giménez Casalduero et al, 2020).

At the beginning of 2017 a PCR analysis was performed on a specimen, and it tested negative to the presence of *Haplosporidium pinnae* (Catanese et al. 2018). No analyses of mycobacteria were carried out in that period. No infective cells were detected in the digestive gland, or haemocytosis that might suggest the existence of infection (A. Grau pers. comm.). However, an infection of the gills by ciliated protozoa (saprophytic opportunistic parasites) was observed, possibly as a consequence of the existing high organic load (Giménez Casalduero et al 2020).

According to some authors, the drop-in salinity occurred in the lagoon due to an extreme rainfall event (ERE) during September 2019, facilitated the entry of the parasite, affecting the fan mussel (Nebot et al, 2022). However, this hypothesis for the entry of the pathogen into the lagoon during this meteorological event does not coincide with the existing data. (Cortes et al *in press*). The evidence indicates that it was in 2017 when the pathogen entered the lagoon (Cortés Melendreras et al 2021).

The possible expansion of the pathogen in the rest of the lagoon depends mainly on salinity levels. A drop in salinity due to contributions from torrential rains or an increase in the connectivity between the lagoon and the Mediterranean will cause an increase in the mortality of the surviving *P. nobilis* specimens.

6. Evaluation of other local risks

The main threat to the species, along with the possible entry and spread of the pathogen within the Mar Menor, is the degradation of water quality due to the serious eutrophication crisis to which the lagoon is subjected. The lagoon has been affected for years by continuous inputs of water with high concentrations of nutrients from agricultural and sewage source (Martínez Fernández and Esteve, 2000; Pardo et al., 2004; Carreño et al., 2008). As a consequence of this massive supply of nutrients, midway through 2015, the water quality showed a radical change due to a massive proliferation of phytoplankton (Aguilar et al., 2016). The Mar Menor reached a stage of severe eutrophication that ended in an “environmental collapse” which resulted in 85% of seagrass meadows lost (Belando et al., 2017). The absence of oxygen caused a massive mortality of benthic organisms (molluscs, polychaetes, etc.). Critically endangered species like the bivalve *Pinna nobilis* suffered a drastic reduction in their populations (Kersting et al., 2020; Giménez-Casalduero et al., 2020; Sandonnini et al., 2021a).

In recent years the Mar Menor has suffered three major eutrophication crises that have had a high impact on the populations of fan mussel (2016, 2019 and 2021). In 2016 began an episode of ecosystem disruption algal bloom (EDAB) that caused anoxia in the lagoon and caused the massive death of benthic organisms (Aguilar et al., 2016, Belando et al., 2018), including the fan mussels populations (Giménez-Casalduero et al., 2020). During 2019, a new eutrophication process was aggravated by the effect of an ERE (an extreme rainfall event) generating euxinic conditions in the lagoon basin with lethal effects for organisms from a depth of 3 m (Ruíz et al., 2019). The results of the mortality rates associated with the last eutrophication crisis that occurred in the summer of 2021, are currently being processed for publication.

As a consequence of the conditions generated by the observed eutrophy, massive growths of serpulids have been found on the shells of the fan mussel. Since 2017, aggregations of the polychaeta *Hydroides dianthus* have been observed on live and dead *P. nobilis* shells in different areas of Mar Menor, and numerous remains of partially “dissolved” shells that were covered with polychaete aggregations have been found in deep areas of the lagoon, below 3m depth (Sandonnini et al 2021a) (cover photography). These aggregations of *H. dianthus* could be a consequence of eutrophication in the lagoon. Mar Menor reached pH values below this limit during the eutrophication event in 2016 (Álvarez Rogel et al., 2016). Thus, the rapid formation of *H. dianthus* carbonate aggregations may have been at the expense of the affected *P. nobilis* shells, due to the sudden change in pH associated with this eutrophication crisis (Sandonnini et al 2021).



Figure 13: Artisanal shellfish extraction instrument found in the Mar Menor during the shellfish density study -Fotography J. Giménez (Cortés et al, 2021).

A significant percentage of surviving specimens of the eutrophication process have died due to actions of vandalism and poaching with illegal

extraction of healthy specimens, intentional or accidental removal of specimens due to malpractice by some fishermen (Figure 13) (Cortés et al, 2021).

Recreational activities in the nautical sector, such as anchoring boats or sailing, especially at shallow levels, can pose a risk to the surviving specimens. In addition, the great accessibility of the areas with populations from the bathing areas, increases the pressures and the risk of extraction and breakage of specimens. It has been proven that one of the most important threats to the populations of *P. nobilis* in the Mar Menor has been related to tourist and recreational activities (direct impact of the anchors of recreational boats on individuals or mooring), and the fishing activity, both recreational and professional (especially with nets) (Cortés et al, 2021). In many of these cases, the cause has its origin in the "ignorance" of the general public, and of the economic sectors most related to the conservation of shellfish, therefore, it is proposed to carry out a campaign of informative talks to the different social actors that can interact with the species, and transmit good practices that avoid the degradation of the specimens and their habitats.

Another noteworthy threat is the contribution of sediment from runoff that causes hypersedimentation and an increase in turbidity that ends up depleting the gill system of the mollusk. Something similar occurs with the movements of sediments in the actions of supply of sand on beaches or movements of sediments for different reasons. Salinity variations due to increased exchange with the Mediterranean would cause an increased risk of pathogen entry.

In 2021, for the first time after the 2016 crisis, recruitment juveniles were located this year, which is an important milestone that sheds some light on the viability of a future survival of the population of shellfish in the Mar Menor. In recent years, the population of the gastropod *Hexaplex trunculus* has increased exponentially, due to its great resistance to variations in water quality and its mode of feeding as a predator and scavenger. *H.*

trunculus seems to be exerting significant pressure on juvenile *Pinna nobilis* (Cortés et al, 2021) (Figure 14).



Figure 14: Specimens of juveniles of *P. nobilis* with *Hexaples trunculus*. Photography: J. Murcia

7. Recommendations

IN SITU ACTIONS:

- Reduction of anthropic pressures suffered by existing populations.
- Cleaning epiphytate specimens. Derived from the "Study of epibiosis and its influence on the survival of the species", it was concluded that the lagoon specimens were suffering different threats. The massive growth of serpulids on the shell of the nacra is one of the causes of mortality of specimens in some areas. There is evidence that the local conditions of the adjacent sediment may be having a determining effect on the massive growth of serpulids.
- Comprehensive population diagnosis of the *Pinna nobilis* population after episodes of eutrophication and extreme meteorological events.
- Recruitment program using larval collectors and actions to obtain gametes, associated with an "ex situ" breeding and maintenance program for the species.
- Continuous monitoring of the population, which will allow reacting to any indication of mortality in the population. Study to know the stock and the dynamics of the unaffected populations (Mar Menor).
- Identification of optimal areas for recruitment, development and translocation.
- Definition of action protocols for translocation and/or rescue to ex situ maintenance centers in situations of environmental crisis.
- Carry out detection tests for the possible identification of the pathogen in the surviving specimens using slightly aggressive techniques with *P. nobilis*
- Activate a protocol for the rescue of specimens, so that in the event of detecting a risk of mass mortality, proceed urgently to the "ex situ" translocation of specimens. The rescue processes must include maintenance in adequate facilities (that meet the requirements to ensure the survival of the specimens) for the necessary time, probably years.

EX-SITU ACTIONS:

- “Ex situ” breeding plan.
- Streamline projects for the development of standardized protocols for the maintenance of *Pinna nobilis* specimens “ex situ”.
- Approval of *Pinna nobilis* Conservation Strategy based on scientific consensus.
- Boost the captive breeding project for the Mar Menor specimens at the UMU Aquarium facilities (the procedure for signing a UMU-CARM/ UA-CARM agreement is currently underway).

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