LIFE PINNARCA

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SCIENTIFIC PUBLICATIONS RESULTING FROM THE PROJECT

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INDEX

1. Abstract

Dissemination activities through scientific publications is an important goal within the project. Research articles published in impact and open access journals, and posted on the project website, are a way to reach both the scientific community and the general population. For this reason, the initial project proposal calls for the publication of at least 8 scientific publications and their dissemination on the project website.

In this sense, the research activity carried out by all partners has resulted in the publication of 11 scientific articles in indexed journals in the field of biology (one of them is under review). Therefore, with the publications presented in this deliverable, the commitment indicated in the initial proposal of 8 publications would be fulfilled.

In addition, it is noteworthy that all the publications presented are within quartiles 1 and 2 in the area of biology and related fields, indicating the high quality and scientific rigor of the research carried out by the members of the consortium.

2. Introduction

As described in the approved proposal, dissemination activities play an important role in the objectives of the project, since one of the main objectives of the project is to increase public awareness of the status of *Pinna nobilis* and the effect that climate change and human behaviour are having on its survival.

Another major objective within the dissemination activities of the project results is to provide valuable scientific information to the scientific community in order to replicate those conservation actions implemented for this specie in different locations or species.

To achieve these objectives, at least 8 scientific publications are planned to be published in the proposal during the project. In addition, all publications must be published on the project website.

This deliverable shows the original scientific articles published in indexed journals in the field of biology up to the time of submission of this document. All the articles listed in the following section are open access so anyone interested can access them through the DOI of the article. In addition, each of the articles included, along with other related articles, are collected and accessible on the project's website:

https://www.lifepinnarca.com/scientific-documents

3. Scientific articles from the project

The main scientific contribution of the project is shown below. In this sense, the activities have resulted in the publication of a total of 11 original articles (one of them under review), all of them published in high impact indexed journals and framed within quartiles 1 and 2. Particularly, of the total number of publications, 45% of them correspond to high impact journals located in quartile 1 and with impact indices ranging between 2.4-5.8 and the rest of the publications are also in high impact journals in the area of marine sciences (quartile 2) with impact indices of 2.8-4.5. This fact highlights the high quality and rigor of the research carried out by the different members of the consortium.

In addition, it exceeds the initial proposal (8 scientific publications), demonstrating the consortium's commitment to the scientific dissemination of the results derived from the project.

Below are the different articles listed in chronological order (from the least to the most) and the summary with the main results:

1. Cortés-Melendreras, E., Gomariz-Castillo, F., Alonso-Sarría, F., Giménez Martín, F. J., Murcia, J., Canales-Cáceres, R., Ramos Esplá, A. A., Barberá, C., & Giménez-Casalduero, F. (**2022**). **The relict population of *Pinna nobilis* in the Mar Menor is facing an uncertain future**. Marine Pollution Bulletin, 185, 114376. https://doi.org/10.1016/J.MARPOLBUL.2022.114376

Pinna nobilis is undergoing one of the most dramatic events suffered by an endangered species. An emerging disease has relegated its populations to coastal lagoons or estuaries with salinities beyond the 36.5–39.7 psu range. The Mar Menor is one of two such locations on the Spanish coastline. Poor environmental conditions and eutrophication and anoxia events, that became critical in 2016, 2019 and 2021, have reduced its population in >99 %. In this work, the spatial distribution of the species within the lagoon and the factors determining its survival along the successive crises of eutrophication are studied using a two-stage (presence/absence estimation and density modelling) Species Distribution Model. A potential area of 200.97 ha and an average density of 1.05 ind.100 m2 is estimated for 2020. The viability of the Mar Menor population depends on management actions designed both for the species and to improve the lagoon environmental state.

Journal ranking: Marine Pollution Bulletin (Q1, Aquatic Science and Oceanography)

Impact factor 2023: 5.8

2. Zotou, M., Papadakis, O., Catanese, G., Stranga, Y., Ragkousis, M., Kampouris E., T., Naasan Aga - Spyridopoulou, R., Papadimitriou, E., Koutsoubas, D., & Katsanevakis, S. (2023). **New kid in town: *Pinna rudis* spreads in the eastern Mediterranean**. Mediterranean Marine Science, 24(3), 709–721. https://doi.org/10.12681/mms.35343

In the Mediterranean Sea, the genus *Pinna* encompasses two large fan-shaped bivalve species, *Pinna nobilis* and *Pinna rudis*. Historically, both species coexisted in the western Mediterranean until a mass mortality event (MME) brought *P. nobilis* to the brink of extinction. Notably, *P. rudis* remained unaffected by the MME, and its recent successful recruitment and further spread have been hypothesized to be linked to the local extinction of *P. nobilis*. Although *P. rudis* has been sparsely recorded in the eastern Mediterranean Sea (with some of these records being doubtful), reports emerging in the summer of 2023 from researchers and citizens have unveiled its sudden spread in the region. This study documents the recent presence of *P. rudis* within Greek waters through a dedicated survey and molecularly confirmation through two distinct molecular methods. Information derived from a citizen science initiative, following photo-identification of the species was also included. Furthermore, an updated review of the distribution of *P. rudis* in the Mediterranean Sea, integrating data from literature and online repositories is provided. This research confirms the recent spread of *P. rudis* in Crete, the Greek Ionian Sea, and Cyprus (first verified records of the species in the regions), in marine areas where *P. nobilis* has become extinct, further strengthening the hypothesis that *P. rudis* has benefited from the collapse of *P. nobilis* populations.

Journal ranking: Mediterranean Marine Science (Q2, Aquatic Science and Oceanography)

Impact factor 2023: 2.8

3. Papadakis, O., Mamoutos, I., Ramfos, A., Catanese, G., Papadimitriou, E., Theodorou A., J., Batargias, C., Papaioannou, C., Kamilari, M., Tragou, E., Zervakis, V., & Katsanevakis, S. (2023). **Status, distribution, and threats of the last surviving fan mussel populations in Greece**. Mediterranean Marine Science, 24(3), 679–708. https://doi.org/10.12681/mms.35384

Since the first confirmed records of mass mortality events (MME) in the Aegean Sea in 2018, *Pinna nobilis* populations in Greek seas have been decimated. To bolster recovery efforts, this study aimed to assess the status of fan mussel populations in the Aegean and Ionian seas and investigate potential recolonization through natural recruitment. From May 2022 to May 2023, 163 independent underwater visual surveys were conducted across various locations and depths along the Greek coastline. A total of 4348 *P. nobilis* individuals was recorded, of which 87.3% were found dead and 12.7% were alive. The sole surviving fan mussel populations were located in the semi-enclosed gulfs of Amvrakikos in the Ionian Sea and of Kalloni in the Aegean Sea, with estimated recent mortality rates (excluding potential poaching) of 7.7% and 6.3%, respectively. To track potential new recruitment, a network of larvae collectors was deployed in multiple locations. Additionally, an ocean circulation model (OCM) was developed to predict the export and fate of larvae from the surviving populations in the Marmara Sea towards the Aegean Sea. Beyond the MME, this study identified several other threats, which significantly endanger fan mussel survival. The findings of this study underscore the urgent need to implement protection measures and restoration actions to enhance the chances of *P. nobilis* survival and recovery in the Greek seas.

Journal ranking: Mediterranean Marine Science (Q2, Aquatic Science and Oceanography)

Impact factor 2023: 2.8

4. Moro-Martínez, I., Vázquez-Luis, M., García-March, J. R., Prado, P., Mičić, M., & Catanese, G. (2023). ***Haplosporidium pinnae* Parasite Detection in Seawater Samples**. Microorganisms, 11(5). https://doi.org/10.3390/microorganisms11051146

In this study, we investigated the presence of the parasite *Haplosporidium pinnae*, which is a pathogen for the bivalve *Pinna nobilis*, in water samples from different environments. Fifteen mantle samples of *P. nobilis* infected by *H. pinnae* were used to characterize the ribosomal unit of this parasite. The obtained sequences were employed to develop a method for eDNA detection of *H. pinnae*. We collected 56 water samples (from aquaria, open sea and sanctuaries) for testing the methodology. In this work, we developed three different PCRs generating amplicons of different lengths to determine the level of degradation of the DNA, since the status of *H. pinnae* in water and, therefore, its infectious capacity are unknown. The results showed the ability of the method to detect *H. pinnae* in sea waters from different areas persistent in the environment but with different degrees of DNA fragmentation. This developed method offers a new tool for preventive analysis for monitoring areas and to better understand the life cycle and the spread of this parasite.

Journal ranking: Microorganisms (Q2, Microbiology and Virology)

Impact factor 2022: 4.5

5. Labidi, S., Vázquez-Luis, M., Catanese, G., Grau, A., Khammassi, M., ben Youssef, S., & Sghaier Achouri, M. (2023). **First detection of the invasive protozoan *Haplosporidium pinnae* in the critically endangered bivalve *Pinna nobilis* in the Southern Mediterranean Sea (Bizerte Lagoon, Tunis) and update of its current status**. Mediterranean Marine Science, 24(3), 470–481. https://doi.org/10.12681/MMS.31664

Pinna nobilis (Linnaeus, 1758) populations have been severely damaged in the last few decades, and since early autumn 2016, a mass mortality event (MME) has drastically impacted populations in the Mediterranean Sea. Accordingly, the aim of the present study was to improve the knowledge on the status of *P. nobilis* populations in the Bizerte Lagoon (Tunisia) between 2016 and 2022. Before the MME, *P. nobilis* was found in the lagoon at depths from 1.5 to 6 m, with a density ranging from 2 to 30 ind/100 m². After the MME, mortality reached 100% in the monitored area, except in the eastern part of the Bizerte Lagoon near the Menzel Jemil site, where some living specimens were detected. Moreover, in 2022, successful recruitment was observed in the lagoon. Additionally, our results revealed the presence of *Haplosporidium pinnae* and *Mycobacterium* sp. in some living specimens sampled in the Bizerte Lagoon after the MME. This finding constitutes the first detection of both pathogens in Tunisia and in the entire Southern Mediterranean Sea.

Journal ranking: Mediterranean Marine Science (Q2, Aquatic Science and Oceanography)

Impact factor 2023: 2.8

6. Hernandis, S., Ibarrola, I., Tena-Medialdea, J., Albentosa, M., Prado, P., Vázquez-Luis, M., & García-March, J. R. (2023). **Physiological responses of the fan mussel *Pinna nobilis* to temperature: ecological and captivity implications**. Mediterranean Marine Science, 24(2). https://doi.org/10.12681/mms.31050

The fan mussel *Pinna nobilis* is experiencing a mass mortality event throughout the Mediterranean Sea. The survival of the remaining isolated populations and the maintenance and reproduction of individuals in captivity could determine the future of the species. This paper examines the clearance rate (CR) and oxygen consumption (VO2) of fan mussel individuals measured under 5 different temperatures (8.5, 14, 18, 23 and 28ºC). The measurements of both physiological variables revealed a threshold limit of thermal tolerance at both extreme temperatures, indicating the critical situation of the remaining populations, which are located in coastal lagoons and shallow bays where these extreme temperatures usually occur. Besides, the high clearance rates observed highlight the significant ecosystem service provided by the species in terms of water filtration, especially in confined waters with low renewal rates. Routine control of the clearance rate as an early warning system is proposed for detecting ailing individuals. Such a system could also be used to improve captivity conditions.

Journal ranking: Mediterranean Marine Science (Q2, Aquatic Science and Oceanography)

Impact factor 2023: 2.8

7. Foulquie, M., Coupe, S., Vicente, N., & Bunet, R. (2023). **First detection of *Pinna nobilis* infection by *Haplosporidium pinnae* in the sanctuary area of Thau lagoon, France**. Mediterranean Marine Science, 24(3), 569–573. https://doi.org/10.12681/MMS.32300

The noble pen shell *Pinna nobilis* is an endemic and emblematic giant bivalve whose populations have been recently affected by a mass mortality event (MME), primarily due to the spread of

the pathogen *Haplosporidium pinnae*. Since the beginning of the MME in Spain in 2016, nearly one hundred percent of monitored open sea water populations have been decimated around the Mediterranean Sea. The only refuge areas with living *P. nobilis* populations were found in coastal lagoons. Today, the Thau lagoon in France is home to a vitally important population of *P. nobilis* and was thought to be safe from the parasite. Here, we report the first molecular detection of *H. pinnae* (isolate PN1) in tissue samples of moribund individuals from Thau lagoon.

Journal ranking: Mediterranean Marine Science (Q2, Aquatic Science and Oceanography)

Impact factor 2023: 2.8

8. Coupé, S., Giantsis, I. A., Vázquez Luis, M., Scarpa, F., Foulquié, M., Prévot, J. M., Casu, M., Lattos, A., Michaelidis, B., Sanna, D., García-March, J. R., Tena-Medialdea, J., Vicente, N., & Bunet, R. (2023). **The characterization of toll-like receptor repertoire in *Pinna nobilis* after mass mortality events suggests adaptive introgression.** Ecology and Evolution, 13(8). https://doi.org/10.1002/ece3.10383

The fan mussel *Pinna nobilis* is currently on the brink of extinction due to a multifactorial disease mainly caused to the highly pathogenic parasite *Haplosporidium pinnae*, meaning that the selection pressure outweighs the adaptive potential of the species. Hopefully, rare individuals have been observed somehow resistant to the parasite, stretching the need to identify the traits underlying this better fitness. Among the candidate to explore at first intention are fast-evolving immune genes, of which toll-like receptor (TLR). In this study, we examined the genetic diversity at 14 TLR loci across *P. nobilis*, *Pinna rudis* and *P. nobilis* × *P. rudis* hybrid genomes, collected at four physically distant regions, that were found to be either resistant or sensitive to the parasite H. pinnae. We report a high genetic diversity, mainly observed at cell surface TLRs compared with that of endosomal TLRs. However, the endosomal TLR-7 exhibited unexpected level of diversity and haplotype phylogeny. The lack of population structure, associated with a high genetic diversity and elevated dN/dS ratio, was interpreted as balancing selection, though both directional and purifying selection were detected. Interestingly, roughly 40% of the P. nobilis identified as resistant to *H. pinnae* were introgressed with *P. rudis* TLR. Specifically, they all carried a TLR-7 of *P. rudis* origin, whereas sensitive P. nobilis were not introgressed, at least at TLR loci. Small contributions of TLR-6 and TLR-4 single-nucleotide polymorphisms to the clustering of resistant and susceptible individuals could be detected, but their specific role in resistance remains highly speculative. This study provides new information on the diversity of TLR genes within the *P. nobilis* species after MME and additional insights into adaptation to *H. pinnae* that should contribute to the conservation of this Mediterranean endemic species.

Journal ranking: Ecology and Evolution (Q1, Ecology and Nature and Landscape Conservation)

Impact factor 2023: 2.6

9. Carella, F., Prado, P., de Vico, G., Palić, D., Villari, G., García-March, J. R., Tena-Medialdea, J., Cortés Melendreras, E., Giménez-Casalduero, F., Sigovini, M., & Aceto, S. (2023). **A widespread picornavirus affects the hemocytes of the noble pen shell (*Pinna nobilis*), leading to its immunosuppression**. Frontiers in Veterinary Science, 10. https://doi.org/10.3389/fvets.2023.1273521

The widespread mass mortality of the noble pen shell (*Pinna nobilis*) has occurred in several Mediterranean countries in the past 7 years. Single-stranded RNA viruses affecting immune cells and leading to immune dysfunction have been widely reported in human and animal species. Here, we present data linking *P. nobilis* mass mortality events (MMEs) to hemocyte picornavirus (PV) infection. This study was performed on specimens from wild and captive populations. We sampled *P. nobilis* from two regions of Spain [Catalonia (24 animals) and Murcia (4 animals)] and one region in Italy [Venice (6 animals)]. Each of them were analyzed using transmission electron microscopy (TEM) to describe the morphology and self-assembly of virions. Illumina sequencing coupled to qPCR was performed to describe the identified virus and part of its genome. In 100% of our samples, ultrastructure revealed the presence of a virus (20 nm diameter) capable of replicating within granulocytes and hyalinocytes, leading to the accumulation of complex vesicles of different dimensions within the cytoplasm. As the PV infection progressed, dead hemocytes, infectious exosomes, and budding of extracellular vesicles were visible, along with endocytic vesicles entering other cells. The THC (total hemocyte count) values observed in both captive (eight animals) (3.5 × 104–1.60 × 105 ml−1 cells) and wild animals (14 samples) (1.90–2.42 × 105 ml−1 cells) were lower than those reported before MMEs. Sequencing of *P. nobilis* (6 animals) hemocyte cDNA libraries revealed the presence of two main sequences of *Picornavirales*, family *Marnaviridae*. The highest number of reads belonged to animals that exhibited active replication phases and abundant viral particles from transmission electron microscopy (TEM) observations. These sequences correspond to the genus *Sogarnavirus*—a picornavirus identified in the marine diatom *Chaetoceros tenuissimus* (named *C. tenuissimus* RNA virus type II). Real-time PCR performed on the two most abundant RNA viruses previously identified by in silico analysis revealed positive results only for sequences similar to the *C. tenuissimus* RNA virus. These results may not conclusively identify picornavirus in noble pen shell hemocytes; therefore, further study is required. Our findings suggest that picornavirus infection likely causes immunosuppression, making individuals prone to opportunistic infections, which is a potential cause for the MMEs observed in the Mediterranean.

Journal ranking: Frontiers in Veterinary Science (Q1, Veterinary)

Impact factor 2023: 3.2

10. Feria-Rodríguez, A., March, D., Mourre, B., Hendriks, I. E., & Vázquez-Luis, M. (2024). **Sink-source connectivity for restocking of Pinna nobilis in the western Mediterranean Sea**. Marine Environmental Research, 197, 106428. https://doi.org/10.1016/j.marenvres.2024.106428

The critically endangered endemic bivalve *Pinna nobilis* from the Mediterranean Sea suffered a sudden population decline after a mass mortality event in early autumn 2016. Conservation efforts aimed at preventing extinction included safeguarding resistant individuals and implementing a breeding plan to contribute to the repopulation of the species. This study utilized a model combining Lagrangian dispersion and connectivity analyses to pinpoint optimal restocking sites in the Western Mediterranean. Our approach allowed to identify locations capable of sustaining and generating larvae for broader repopulation in key areas of the Western Mediterranean Sea prior to the mass mortality event. Six important repopulation locations from Murcia, Valencia and Balearic Islands were selected for reintroduction efforts. The results obtained in this study show how the network could be self-sufficient and able to self-replenish

itself of recruits. Overall, our work can be used to direct the reintroduction of resistant animals in the Western Mediterranean Sea.

Journal ranking: Marine Environmental Research (Q1, Aquatic Science and Oceanography)

Impact factor 2023: 3.3

11. **Demographic regulation process in Pinna nobilis populations implications for restocking**. Estuarine, Coastal and Shelf Science. The article is currently under review. Once properly published, it will be added as an annex along with other possible publications.

4. ANNEXES

ARTICLES IN PDF