LIFE PINNARCA

Life Pinna

IX INTERNATIONAL SYMPOSIUM ON MARINE SCIENCES
VALENCIA. JULY 10-12

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Valencia (Spain), 10-12 July 2024

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FIGHT FOR SURVIVAL: CONTRIBUTION TO THE STUDY OF *PINNA NOBILIS* POPULATION IN KERKENNAH ARCHIPELAGO

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Abstract: *Pinna nobilis*, the largest bivalve in the Mediterranean Sea, is a well-known and important endemic marine invertebrate, considered as a flagship species in the region. Since 2016, dramatic mass mortality of this species has been observed in several areas including the Kerkennah archipelago (southern Tunisia). The density of *Pinna* has decreased significantly due to the destruction of its natural habitat by trawling, the presence of new predators such as the blue crab, and the epizootic. This study is carried out as part of the PP-KER project "Ecological restoration for the conservation of heritage species (*Posidonia oceanica* and *Pinna nobilis*) in the future AMCP of the Kerkennah Archipelago", financed by CEPF in 2023. It aims i) to characterize the remaining population, ii) to take measures to protect living *Pinna* specimens and restore the population, and iii) to highlight the importance of *Posidonia* meadows and *Pinna* through awareness raising activities with local communities.
Several diving and snorkeling surveys were conducted from April to November 2023, resulting in the tagging of 8 live specimens over 2km². Examination of 35 dead mussels revealed that the total height and maximum width of dead mussels ranged from 24-44 cm and 10-16 cm respectively. The age of the dead individuals ranged from 5 to 9 years. As part of the study, 24 *Pinna* larval collectors were installed at depths between 1 and 2 meters, with 3 to 4 collectors being retrieved and sorted every three months. Unfortunately, no *Pinna nobilis* larvae were captured. However, the most abundant taxonomic groups captured in the larval collectors were molluscs, annelids, crustaceans, and ascidians. Additionally, 4 protective cages were placed over 4 live *Pinna nobilis* individuals at one of the surveyed sites. Awareness-raising workshops were held with stakeholders to ensure that they were aware of the importance of the issue.

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Key words: Survey, Restoration, Larval collectors, Conservation

Acknowledgments: This work was supported by the Critical Ecosystem Partnership Fund and BirdLife International, PP-KER project: Ecological restoration for the conservation of heritage species (*Posidonia oceanica* and *Pinna nobilis*) in the future AMCP of the Kerkennah Archipelago. We would like to thank the Regional Activity Centre for Specially Protected Areas (RAC/SPA) for their technical and financial support. We thank also the scientific expert Saul Ciriaco (WWF Italy Miramare AMP) for his valuable inputs.

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LARVAL COLLECTORS AND EX-SITU MAINTAINANCE FOR JUVENILES OF *PINNA NOBILIS* IN THE GULF OF TRIESTE (NORTHERN ADRIATIC SEA) AS PART OF EUROPEAN PROJECT LIFE PINNA

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Abstract: In the period 2022-2023, larval collectors for *Pinna nobilis* were set up in the Gulf of Trieste as part of the LIFE Pinna project: 38 lines were installed (24 in shallow coastal waters, 13 in mussel farms, 1 in a shallow lagoon), a total of 175 bags. In addition, juveniles were collected in areas threatened by the human activity (mussel farms, floating protections, docks, mooring and anchoring areas). The objectives of this work were twofold: *(i)* to obtain data on the reproductive potential of the species and *(ii)* to promote natural recruitment of the species, aided by keeping juveniles under controlled conditions. The individuals retrieved were kept in aquaria in two different facilities: Santa Croce (Shoreline) and MBP Piran (NIB). In the first case, they were kept in a closed system, with UV lamp and filter, the water temperature was 18 °C and they were fed with live phytoplankton and commercial food. In the second case, they were kept in an open system with a UV lamp at the same temperature as the seawater and fed with commercial food.
A total of 77 live juveniles of *P. nobilis* were found: 39 in the larval collectors in shallow coastal waters, 15 in collectors in mussel farms and 23 in endangered areas. In addition, 84 empty shells of pinnids were found in the collectors. The living specimens were between 0.5

and 8 cm long and were mainly found in the first 6 m depth.
Four years after the first detection of *Haplosporidium pinnae* in the Gulf of Trieste (Manfrin *et al*., 2023), our results confirmed the presence of larvae of *P. nobilis* in this area and thus the presence of a source population of spawning individuals. However, the parasite is still present as genetic testing confirmed that some dead individuals were positive for *H. pinnae* and/or *Mycobacteria* spp.

Key words: Pen shell, Recruitment, Controlled growth, *Haplosporidium pinnae*, Conservation

Acknowledgments: This research was financed mainly by the European Union under the Life project LIFE PINNA, LIFE20NAT/IT/001122. It was co-financed by the Ministry of Environment and Spatial Planning of Slovenia, with financial support from the Slovenian Research Agency (research core funding n.P1-0237).
Special thanks go to the staff of Marine Biology Station Piran and Aquarium Piran (Slovenia), in particular to Ana Lokovšek, Leon Lojze Zamuda, Milijan Šiško, Tihomir. Makovec, Manja Rogelja, and the students Melina Tadić and Urška Kajtna who helped with the preparation, collection and checking of the collectors and with the care of the small pinnids in aquaria.

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IMPACT OF *PINNA NOBILIS* PICORNAVIRUS (PNPV) ON HEMOCYTES: EVIDENCE FROM VIRAL REPLICATION TO HOST IMMUNOSUPPRESSION

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Abstract: Single-stranded RNA viruses affecting immune cells and leading to immune dysfunction have been widely reported in human and animal species (Cusik et al., 2014; Rouse and Horohov, 1986). Here, we present data linking *P. nobilis* mass mortality events to picornavirus (PV) affecting the animal hemocytes (Carella et al., 2023). This study was performed from 2021 to 2024 specimens from wild and captive populations in two regions of Spain, Catalonia and Murcia, and 2 regions in Italy, Venice and Sicily. Each of them was analysed using transmission electron microscopy (TEM) to describe the morphology and self-assembly of virions. Moreover, we used an *in vitro* flow cytometric method using pH-sensitive dyes (*PHrodo*) to investigate the effect of PnPV on phagocytosis and animals immunocompetence. In 100% of the samples from all the areas and years (n=45), ultrastructure revealed the presence of a virus (20 nm) 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. Indeed, Pen shell in captivity had significantly lower Total Hemocyte Count (THC) compared with natural population (mean number of 7-9 x 10^4 vs 2-5 x 10^5 cells/mL, respectively). Flow cytometry-based phagocytosis analysis demonstrate that animals in captivity had scarce or absent ability to phagocyte the two stimuli (*Staphylococcus aureus* and *Zymosan A*) (10.2% - 1.7 of positives) when compared

with natural population in Alfacs Bay (28.5 % - 5.6 of positive). Ultrastructure images showed that PnPV itself can lead to an alteration of the hemocyte cytoskeleton, possibly lowering the capabilities to perform an active phagocytosis and an efficient phagolysosome fusion. Indeed, this viral disease represents an epidemiological threat of unknown proportions for marine animal populations.

Key words: *Pinna nobilis*, Mass mortality, Picornavirus, TEM, phagocytosis

Acknowledgments: supported by the EU LIFE Programme "Project Protection and restoration of *Pinna nobilis* populations as a response to the catastrophic pandemic started in 2016 (LIFE PINNARCA)" (grant number LIFE20 NAT/ES/001265).

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THE EVOLUTIONARY HISTORY OF *PINNA NOBILIS*: IMPLICATIONS FOR CONSERVATION AND RESTOCKING ACTIVITIES

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Abstract: The noble pen shell, *Pinna nobilis*, an endemic species of the Mediterranean Sea, has a rich and intricate history. Since 2016, the species has faced devastating mass mortality events (MMEs), affecting the entire Mediterranean region. The mechanisms and causes of this event, suspected to involve a multifactorial disease, are currently under scientific investigation. In response to this urgent situation, several European Union initiatives, including LIFE PINNARCA and LIFE PINNA, have been launched to implement *ex situ* conservation programs. One of the main results obtained by LIFE PINNA is the

reconstruction of the evolutionary history of *Pinna nobilis* before the MMEs, aiming to understand its adaptation processes to the Mediterranean Sea over time. This analysis sought to provide a solid foundation for the species restocking activities planned for the project. To achieve these goals, researchers conducted phylogenetic and phylogeographic analyses based on 469 cytochrome c oxidase subunit I (COI) mitochondrial gene sequences, including 100 sequences obtained during the present study from previously overlooked geographic areas. Results highlighted a relevant haplotype sharing and genetic homogeneity between populations from the western Mediterranean and Adriatic Sea. Moreover, the molecular dating analysis revealed that *Pinna nobilis* diverged approximately 2.5 million years ago from an ancestor who entered the Mediterranean Sea during the Zanclean flood around 5.33 million years ago. The spreading of *Pinna nobilis* likely began in the central part of the western Mediterranean basin, with a subsequent population expansion towards Adriatic and the eastern basin. Our data provide a crucial foundation for the development of an effective managing of surviving individuals and successful restocking or transplantation strategies in regions where *Pinna nobilis* faced extinction. Our efforts are devoted to implement local restocking plans aimed at generating new populations of fan mussels with genetic variability similar to that which characterized the now extinct native populations in the same sites before MMEs.

Key words: fan mussel, mass mortality, molecular dating, evolution, mitochondrial DNA

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PINNA RUDIS' UNEXPECTED COLONISATION OF THE GREEK SEAS

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Abstract:
Until recently the only *Pinna* sp. inhabiting Greece was *Pinna nobilis*, while *Pinna rudis*' Mediterranean distribution was limited to the western basin (Gvozdenović et al., 2019). However, since 2019 *P. nobilis*' populations have essentially declined in the Greek seas, and by 2021 the remaining populations were restricted to two separate gulfs (Zotou et al., 2021).
During the summer of 2023, environmentally aware citizens informed our lab about the re-appearance of live *Pinna* individuals in Crete and the Ionian Islands. In addition, photos taken by recreational divers depicting live individuals were uploaded on social media, and on iNaturalist platform, highlighting the presence of surviving individuals from different locations in Greece and Cyprus. Mantle samples were collected from eight individuals found in Crete for molecular analysis to identify the species. The identification of individuals from photos uploaded on social media platforms was based on the evident morphological characteristics.
The molecular analysis (conducted using two distinct methods: Catanese et al., 2022; Vázquez-Luis et al., 2021) verified our estimations, that the individuals found in Crete were indeed *P. rudis*. Morphologically the photographed individuals from sites across Greece and Cyprus shared more similarities with *P. rudis* than with *P. nobilis*.

Overall, 31 individuals were recorded during the visual observations in Crete, and 43 individuals were documented at 28 sites from other locations in Greece and Cyprus.
The findings of our study reveal a broader distribution range for *P. rudis* in the Mediterranean Sea than previously thought (Zotou et al., 2023). These previously, undocumented populations in the Greek and Cypriot seas converge with observations made by Donato et al. (2021) in Italy. These observations and the increased successful recruitment in the western Mediterranean support the hypothesis that *P. rudis* benefited from the collapse of *P. nobilis* populations, following their mass mortality event (Kersing & Ballesteros, 2021).

Key words: Pinnidae, *Pinna rudis*, Distribution, Mediterranean, Molecular identification

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EXPLORING NOVEL PICORNAVIRUS STRAINS IN *PINNA NOBILIS* HEMOCYTES: TRANSCRIPTOMIC INSIGHTS

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Abstract: In recent years, mass mortalities affected Mediterranean populations of *Pinna nobilis*, often attributed to diverse pathogens like *Haplosporidium pinnae*, *Mycobacterium* spp., *Vibrio mediterranei*, and *Perkinsus* spp., hinting at an elusive common cause (Carella et al., 2020). Electron microscopy analyses of *P. nobilis* specimens from Spain and Italy unveiled structures similar to RNA virus particles within hemocytes. Thus, we adopted a transcriptomic strategy to identify and classify RNA viruses infecting noble pen shell hemocytes, aiming to highlight potential links between RNA viruses and *P. nobilis* mass mortalities. Illumina sequencing of cDNA libraries from *P. nobilis* hemocyte samples generated 162,139,300 paired-end raw reads. After trimming and mapping against the *P. nobilis* genome, 20.4% of reads remained unmapped and were assembled, yielding 140,922 contigs. BLASTX analysis against the NCBI viral protein database revealed 43.7% contigs with positive viral protein matches. Focusing on RNA viruses, VirSorter2 (Guo et al., 2021) and VirBot (Chen et al., 2023) identified five Riboviria-classified contigs, mainly Picornavirales, two of which exhibited notable abundance in *Pinna* samples, with one matching *Chaetoceros tenuissimus* RNA virus type II. Genomic organization analysis revealed two prevalent viruses with large ORFs encoding RdRp and RNA helicase, indicative of Picornavirales. After the discovery in 2 populations of Spain, quantitative Real Time RT-PCR was conducted on other populations of *P. nobilis* collected in different Mediterranean countries to check for the presence of the *in silico* identified virus. Most of the examined samples resulted positive to the infection of the *P. nobilis* picornavirus similar to the *C. tenuissimus* RNA virus type II, suggesting this virus could be the primary cause of the noble pen shell mass mortality.

Key words: *Pinna nobilis*, Mass mortality, Picornavirus, Transcriptomics

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PRESERVATION AND CURRENT STATUS OF *PINNA NOBILIS* IN CROATIA

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Abstract: *Pinna nobilis* mass mortality event on the Croatian part of the Adriatic Sea was recorded for the first time in 2019 when also the presence of the parasite *Haplosporidium pinnae* was confirmed in all the sampled individuals (Čižmek et al., 2020). Considering the situation, in 2020, 23 partners coming from different origins (e.g., public institutions, private institutions, and nongovernment organizations, science institutions) grouped under the project "Conservation of the noble pen shell (*Pinna nobilis*) in the Adriatic Sea" to perform coordinated activities for the preservation of the noble pen shell. The project is coordinated by the Ministry of Economy and Sustainable Development and co-financed by the Environmental Protection and Energy Efficiency Fund. The present work aims to summarise all the performed activities and to present future steps under the frame of the project. The main in-situ project activities include the identification, preservation, and monitoring of surviving *P. nobilis* individuals and populations, the installation of larvae collectors following the IUCN guidance (Kersting and Hendriks, 2019), and taking samples for laboratory testing across the Croatian part of the Adriatic Sea. The research on growth and health conditions of juvenile individuals and the possibility of reproduction of adults in controlled conditions is being investigated in Aquarium Pula as part of ex-situ activities. In the last year, there were around 150 reports through citizen science, and only 13 new locations of living individuals have been confirmed. Currently, in the Croatian part of the Adriatic Sea, 25 living individuals are known. The present study provides valuable information regarding the current status of *Pinna nobilis* in Croatia. Future activities will focus on scanning potential locations to find possible shelters for living individuals, analytically determine the origin and genetic morphology of the juvenile individuals, and continue the collaboration with partners across the Mediterranean.

Key words: *Pinna nobilis*, Adriatic Sea, larvae collectors, *Haplosporidium pinnae*

Acknowledgments: This study has been supported by the Environmental Protection and Energy Efficiency Fund under the project "Conservation of the noble penn shell (*Pinna nobilis*) in the Adriatic Sea".

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SEED PRODUCTION OF THE PEN SHELL ATRINA LISCHKEANA (BIVALVIA: PINNIDAE) IN JAPAN: AN APPLICABLE METHOD FOR *PINNA NOBILIS*

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Abstract: Establishment of techniques for seed production of *Pinna nobilis* is an urgent issue in Mediterranean countries. On the other hand, in Japan, the techniques for *Atrina lischkeana*, a Pinnid species taxonomically close to *Pinna*, have been upgraded sufficiently to produce one million juveniles a year. Here, we introduce several important points in the techniques that might lead to the success of *P. nobilis* seed production. (1) A few months before the egg/sperm release induction, each broodstock is numbered and its sex is confirmed by the color of gonads. (2) The broodstocks are suspended in the sea where phytoplankton is rich, to achieve optimum sexual maturity. (3) A few weeks before the induction, the broodstocks are retrieved from the sea, shell surfaces cleaned, and moved to rearing tanks. The males and females are then separately reared with filtered seawater and cultured phytoplankton, to purge each individual. (4) Thermal shock or air exposure was used to induce gamete release, however, injection of a novel peptide (Funayama et al. 2022) has been recently used for the induction with high success rate. (5) A dual tank system, comprised of two 500 L round-shaped tanks connected with a pipe, was used to rear *Atrina* larvae. During the rearing period up to a month or more, continuously one tank is washed and then the other is washed with a three day gap in between, to enable ideal water quality. (6) Two showers are connected to the top of each tank to cause surface disturbance to help larvae sink back into the water column. (7) Aeration is not used, because *Atrina* larvae are quite sensitive against air bubbles. Even without aeration, oxygen exchange is efficiently obtained from step (6).

Key words: broodstock maintenance, sex confirmation, dual tank system

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MANAGEMENT OF THE NOBLE PENN SHELL JUVENILES IN AQUARIUM PULA AND NEW INSIGHTS INTO THE OCCURRENCE OF *HAPLOSPORIDIUM PINNE* IN THE ADRIATIC SEA

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Abstract: Mass mortality events affecting the noble penn shell (*Pinna nobilis*) in Croatian Adriatic Sea were first noticed during 2019. Similarily to the rest of the Mediterranean these events were associated with *Haplosporidium pinne* infections. To adress this crisis and support the research and conservation efforts, Aquarium Pula Ltd. established the Noble Sanctuary in June 2019, a quarantine unit designed to house individuals under controlled conditions for extended periods. The early care experiments enabled progress in multiple techniques, such as managing water quality in a closed circulation system with continuous filtration and sterilization, temperature regulation, food production and optimization of nutrition.

In November 2023 Aquarium received 81 juveniles gathered from collectors deployed on the west coast of Istria. Unfortunately most of them died, with currently 24 still alive in the Aquarium. Ten dead juveniles were sent for diagnostics to LIMIA, Spain, with five being positive for the pressence of *H. pinne* and all negative for *Mycobacterium* sp. Molecular diagnostics was performed in the Aquarium on another 23 dead juveniles using the HPNF3/HPNR3 primers for *H. pinne*, with 19 being positive, and Tb11/Tb12 and 246/264 primers for *Mycobacterium* sp., with 17 and 12 positive, respectively.

During the years 2018 and 2019 samples of *P. nobilis* were collected throughout the Croatian Adriatic Sea as part of the project „DNA barcoding of Croatian faunal biodiversity“. In April 2024, DNA extracted from these samples was made available to Aquarium Pula. Samples from three out of eight locations were positive for the presence of *H. pinne.* Most interestingly, all individuals sampled in November 2018 at Telašcica location (N=10) which was then under periodic monitoring scheme, were found positive, but the first mortalities were observed in September 2019. Detailed results will be shown as part of the presentation.

Key words: *Pinna nobilis*, conservation, controlled conditions, mortalities

Acknowledgments: Management of *P. nobilis* juveniles in Aquarium Pula is partly financially supported by The Environmental Protection and Energy Efficiency Fund under the project "Preservation of noble pen shells (*Pinna nobilis*) in the Adriatic Sea" coordinated by the Ministry of Economy and Sustainable Development. We would like to thank Mauro Štifanić and Neven Iveša from the University of Pula for providing us with samples of *P. nobilis* from 2018 and 2019.

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PINNA NOBILIS POPULATIONS' ASSESSMENT, MONITORING AND RESTORATION ACTIONS IN GREECE

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Abstract: Since 2018, when the first confirmed records of mass mortality event (MME), caused by the parasite *Haplosporidium pinnae* in the Aegean Sea were reported (Katsanevakis *et al.*, 2019), *Pinna nobilis* populations in the Greek seas have been decimated (Zotou *et al.*, 2020; Zotou *et al.*, 2021; Papadakis *et al.*, 2023). 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 Kalloni in the Aegean Sea, with estimated recent mortality rates (excluding potential poaching) of 7.7% and 6.3%, respectively. In Kalloni Gulf, the presence of *H. pinnae*, combined with an extreme and prolonged heatwave that struck Greece in July-August 2023, appears to have triggered a new catastrophic MME, which was recorded in subsequent opportunistic surveys. To track potential new recruitment, a network of larvae collectors was deployed in multiple locations in the North Aegean Sea and Amvrakikos Gulf. Beyond the MME, this study identified several other threats, which significantly endanger fan mussel survival, such as illegal fishing activities, poaching and surviving individuals in shallow waters of touristic areas. To address these threats, enhanced monitoring efforts and translocation actions for vulnerable individuals to safer areas were implemented. The findings of this study underscore the urgent need for reinforced protection measures and restoration actions to enhance the chances of *P. nobilis'* survival and recovery in the Greek seas.

Key words: *Pinna nobilis*; mass mortality; conservation; Greece; Mediterranean.

Acknowledgments: The present study was conducted in the framework of the European Union's LIFE PINNARCA project: "Protection and restoration of Pinna nobilis populations as a response to the catastrophic pandemic started in 2016" (Reference: LIFE20 NAT/ES/001265), which is focusing on the recovery of the fan mussel in the Mediterranean Sea and has received funding from the "LIFE Programme" of the European Union.

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MONITORING THE POPULATION OF *PINNA NOBILIS* SURVIVORS IN THE MAR MENOR LAGOON (SE SPAIN)

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Abstract: The fan mussel, *Pinna nobilis*, is a bivalve mollusk endemic to the Mediterranean that has been subjected to a severe mortality event, mainly associated with the parasitic infection of *Haplosporidium pinnae*. *P. nobilis* populations have virtually disappeared throughout the Mediterranean and have been confined to certain areas where the parasite has limited dispersal capacity. On the Spanish coast, one of the only two surviving populations is located in the coastal lagoon of the Mar Menor (Murcia). Unfortunately, this lagoon has experienced several episodes of eutrophication and environmental collapse since 2016, primarily due to the continuous input of water laden with organic matter and sediment. The lagoon's conditions have affected the fan mussel population, decreasing from over one and a half million individuals in 2014 to barely a thousand currently (Giménez-Casalduero et al., 202; Cortés-Melendreras et al., 2022). This study monitored specimen mortality from January 2022 until the end of 2023 and the beginning of 2024. Three locations on the eastern coast of the lagoon, characterized by a high density of survivors, were selected for this purpose. Periodic samplings at these locations revealed an important reduction in the density of surviving individuals, with mortality rates of 56%, 76%, and 31% in just two years. Furthermore, the remaining lifespan for each population locality has been estimated, concluding by the end of 2025, 2024, and 2028, respectively. These data highlight the serious situation facing *P. nobilis* in the lagoon and the urgency of implementing conservation actions.

Key words: *Pinna nobilis*, Mar Menor, mortality, monitoring, eutrophication.

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CONSERVATION STRATEGIES FOR THE CRITICALLY ENDANGERED *PINNA NOBILIS*: EVALUATING POTENTIAL SANCTUARY AREAS IN THE BALEARIC ISLANDS

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Abstract: Since a Mortality Mass Event (MME) started in 2016, the endemic bivalve *Pinna nobilis* from the Mediterranean Sea has become critically endangered. This MME driven by a multifactorial disease has devastated over 99% of the populations across the Mediterranean basin. Few populations remain unaffected within "sanctuary areas", where a natural barrier characterized by distinct environmental conditions from the open sea, shields the non-resistant population from the pathogen´s spread. Only two populations remain in Spanish waters, the Mar Menor lagoon and Ebro Delta, both of which are additionally subject to anthropogenic pressures. This study targets that natural barrier, exploring new potential optimal sites that could function as sanctuary areas for the implementation of conservation measures. Within the Balearic Islands, six locations have been considered regarding the criteria proposed for optimal sites within the frame of LIFE PINNARCA project. Thus, environmental data have been compiled and analyzed from those locations since 1982 complemented by monitoring of salinity and temperature throughout 2023 and 2024. Subsequently, five out of the six locations have been dismissed due to failure to meet minimal criteria. This data sheds light on the Estany des Ponts, located within the Albufera de Mallorca, which could play a key role in the reintroduction of this species. The exploration of new optimal sites, as proposed in this study, could be very useful to address the urgent situation of this critically endangered species.

Key words: Bivalve, *Pinna nobilis*, Mediterranean Sea, Pen shell, Sanctuary areas.

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ENGAGING CITIZENS IN LIFE PINNA PROJECT: THE STRATEGIC ROLE OF COMMUNICATION ACTIONS FOR THE CRITICALLY ENDANGERED SPECIES CONSERVATION

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Abstract: In 2021, an even worse outbreak status respect to the project conceptualization occurred: increased and widespread massive decline stroke the population of *Pinna nobilis*, the largest bivalve in the Mediterranean (Vázquez-Luis et al. 2017; Scarpa et al. 2020). The consequent data paucity on the occurrence of alive specimens, was promptly addressed by the launch of "Report the Pinna" citizen science campaign in search for survivors. Citizen science activities, in charge of ARPAL coordinating beneficiary of LIFE PINNA, were carried out with the support of IMC, Shoreline and Triton Research, which oversaw the communication and public involvment activities. For this purpose, ARPAL periodically updated an online interface allowing to collect and display data received. Most of reports have been validated by IMC and Shoreline researchers. On Project channels (website, social networks and general press) the campaign has been regularly highlighted, mostly during spring-summer, through press office work and *ad hoc* content creation (e.g. news and videoclips) by Triton Research. One year of insights data revealed that ~6,000 different users visited lifepinna.eu website and 1,200 specific citizen science pages; 22 contents on citizen science campaign were published and reaching +95,000 user (cover data) and obtained over 14,000 interactions (Facebook); 18 more contents (Instagram) reached +170,000 users (cover data) and the publication of 4 video clips totalized +22,000 plays. A series of public live and online training initiatives, also with PADI collaboration, attracted over a thousand people engaging and educating with LIFE PINNA gadget awards assignment. This approach led to the collection of +40 reports in one year, most of which were of undoubted interest to researchers, accounting for +50 specimens discovered. Among the most relevant, three surviving populations were discovered in middle Adriatic and Sardinia Seas. Citizens have so proven to be key players in *P. nobilis* conservation. These discoveries contributed to avoid a drastic revision of the project aims, which can thus count on new potential donor sites

(Grech et al. 2023) hopefully to be preserved as sanctuaries for the conservation of this species.

Key words: Pinna nobilis, communication, conservation, citizen science, LIFE program

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LIVING ON THE EDGE: CURRENT SCENARIO OF THE LAST POPULATIONS OF *PINNA NOBILIS* IN THE SANCTUARY AREAS OF SPAIN

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Abstract: The critically endangered bivalve *Pinna nobilis*, endemic to the Mediterranean Sea, faces a drastic population decline throughout the Mediterranean Sea due to a Mass Mortality Event (MME) caused by a multifactorial disease detected in 2016, which has brought the species to the border of extinction. Nevertheless, isolated populations confined to coastal lagoons and estuaries are found across the Mediterranean, where environmental conditions differ from the open sea allow survival due to the presence of a natural barrier against the pathogens. There are only two remaining populations (sanctuary areas) in Spanish waters, the Ebro Delta and the Mar Menor lagoon, which hosts *P. nobilis* non-affected individuals; however, many pressures and impacts affects the resident *P. nobilis* populations (fishing, anchoring, water sports, agricultural discharges, etc...). Monitor key demographic processes, population fitness and vital rates is crucial for the effective conservation of this threatened species, which may provide the scientific basis of its population dynamics needed to implement successful recovery programmes. Between 2021 and 2023, the population dynamics of *P. nobilis* were monitored in both areas with similar results. In both cases the trends are negative, with high mortality rates that have led to the loss of more than a third of the specimens in both areas, with the Ebro Delta being the area with the worst scenario, having suffered the same proportion of losses in half of the time. Moreover, the lack of recruitment recorded, if maintained, will lead to the disappearance of the populations in the medium term, unless there is a drastic change. It should also be noted that part of the mortality recorded in both areas is due to physical impacts suffered by the specimens, being more important in the Ebro Delta, where mortality due to impact has been recorded in more than a third of the dead specimens. Effective protection measures for *P. nobilis* individuals inhabiting these areas are extremely urgent.

Key words: *Pinna nobilis*, population dynamics, monitoring, conservation, human impacts

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CHASING *PINNA NOBILIS* SURVIVORS IN OPEN-SEA: CURRENT STATUS IN SPAIN

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Abstract: Marine biodiversity faces a series of threats producing a variety of impacts, among which species extinction is one of the most dramatic scenarios. In this situation is the largest and endemic bivalve to the Mediterranean Sea, the critically endangered species *Pinna nobilis*. At the present times, the species faces an unprecedented challenge after the Mass Mortality Event (MME) that has affected its populations since autumn 2016. The present study aims to perform a comprehensive study on the survivors in the open sea after the MME. The main results of the monitoring in the Spanish Mediterranean coast indicate that, throughout a 7-year period after the start of the MME (2017-2023), the geographical distribution of the survivors is currently concentrated in the Autonomous Regions of Catalonia, the Balearic Islands and, to a lesser extent, the Valencian Community, with focal points of density of specimens in Cap de Creus (Catalonia) and Menorca (Balearic Islands). During the exhaustive monitoring of individuals of *Pinna nobilis*, the active participation of citizen science became decisive, locating almost half of the survivors. Most individuals were found in marine protected areas, mainly in *Posidonia oceanica* meadows at a depth of 1-10 m. Taxonomic analyses, both morphological and molecular, allowed the identification of some hybrids among the survivors. These hybrids seem to be resistant to the MME and have been found scattered during the census of individuals. Finally, as a conservation management measure, several survivors were translocated to safer areas and the impact of

the translocation was evaluated without finding significant negative effects. The implications of these results emphasized the necessity for collaborative monitoring, especially to understand the current critical situation of *P. nobilis* and to implement effective conservation measures of these valuable individuals.

Key words: critically endangered, *Pinna nobilis*, monitoring, bivalve, mass mortality event.

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CONCRETE ACTIONS FOR THE RESTORATION OF *PINNA NOBILIS* WITHIN THE EUROPEAN LIFE PINNA PROJECT: TRANSPORT, CAPTIVE BREEDING AND TRANSPLANTATION

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Abstract: In the context of the European LIFE PINNA project, following the intensification of the mortality event, which also affected *Pinna nobilis* in the Gulf of Trieste, activities were initiated in the lagoon of Venice with the objective of collecting adult individuals for artificial reproduction. Here, the high number of specimens still alive allowed collection without damaging the population. In June 2023, a total of eleven specimens of *P. nobilis* were therefore collected from the Venice lagoon and transported to a facility located in Camogli (Genoa, Italy). A closed system has been setted with specimens placed horizontally in three different tanks to facilitate all the procedures. A system for changing, cleaning and disposing of the water was also set up to minimise the potential impact of the water used for rearing.
It was not necessary to trigger spawning by a temperature shock as the gamete release occurred spontaneously shortly after collection and transport (as in Hernandis et al., 2023). The released eggs were collected, counted and the rapid development of the zygotes was monitored up to the early larval stages. Three times a week, the larvae were fed with a mixture of three microalgae. Filters from the three tanks in which *Pinna nobilis* individuals were kept in captivity were analysed for pathogens using molecular diagnostic analyses.

These analyses focused on the presence of protozoa of the genus *Haplosporidium*, in particular the species *Haplosporidium pinnae*. The same analyses were also carried out on *Pinna nobilis* individuals in the tanks and on their larvae. Four of the eleven individuals survived 6 months after transport to the laboratory(survival rate of 36.4 %). These survived specimens, tested negative for *H. pinnae,* were successfully transplanted to the marine protected area of Capo Mortola (Liguria, Italy) on 14th December 2023 to ensure gonad maturation in the natural environment. Monitoring measures are carried out monthly. The transport, maintenance and transplantation protocols for *P. nobilis* have proven to be appropriate and are now ready for upscale with the next foreseen activities by the project.

Key words: Pen shell, Transport, Reproduction, *Haplosporidium pinnae*, transplantation

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LONG PERSISTENT PICORNAVIRUS INFECTIONS IN *PINNA NOBILIS* POPULATION OF THE EBRO DELTA (SPAIN)

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Abstract: Persistent viral infection is a condition of metastability in which the pathogen and the host coexist, where viral replication is held in check, but not cleared. With a clear benefit for the virus at the population level, persistent infections can act as ecosystem modulators (Villareal et al., 2009). Along with the recurring infection goes cell damage, further contributing to animal immunosuppression. Since 2021, an infection due to a Picornavirus affecting the Pen shell hemocytes was discovered affecting estuarine population of Alfacs Bay in Ebro Delta (Spain); in Fangar Bay (northern hemidelta) study started in 2022, and since then observed permanently (Carella et al., 2023). Mortality trends of individuals monitored (that include all causes) in central and inner parts of Alfacs Bay, has reached average annual values (2022-2023) of 38.96%. In contrast, annual mortalities in Fangar Bay in the same period are slightly lower, (30.56%) and the population features a shorter natural life expectancy compared to Alfacs Bay due to environmental factors (4-7 vs. 5-15 year; Prado et al., 2020, 2021). In the 2 areas of Spain, samples of hemocytes from Pen shell individuals (55) were collected for TEM (Transmission Electron Microscopy) associated to evaluation of the Total Hemocyte Counts (THC) and qPCR of *P. nobilis* PV. The THC values varied among individuals and sites over the years, decreasing over time from 2021 to 2024. In Alfacs Bay, in all the individuals, hemocyte ultrastructure showed advanced alterations of cytoskeleton, while in Fangar Bay the extent and degree of cytoskeletal reorganization presented a more conserved structure, possibly still preserving its function. This could be related to local environmental variables acting on viral dynamic. Retrieved samples from Alfacs Bay, date back the virus presence to 2016. Further studies are needed

to better define the pathogenetic mechanism behind the viral replication and host-pathogen interaction.

Key words: *Pinna nobilis*, Mass mortality, Picornavirus, critically endangered species, invertebrate

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HEALING ATTEMPTS OF *PINNA NOBILIS* INDIVIDUALS USING PHYTOTHERAPY AND TEMPERATURE CONTROL

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Abstract: The pen shell *Pinna nobilis* is an endemic bivalve of the Mediterranean Sea. Since 2016, the species has suffered a mass mortality event, leading to the collapse of most Mediterranean populations and ultimately being declared Critically Endangered by the IUCN (Basso *et al.*, 2015). The simultaneous presence of several pathogens, such as *Haplosporidium pinnae* and *Mycobacterium* spp. have been been repeatedly reported, accompained by the last recent discovery of a Picornavirus affecting the animal hemocytes leading to its immunosuppression (Carella *et al.*, 2023). In the context of the LIFE PINNARCA Project, the ecological role of *P. nobilis* has prompted significant investigations on captive breeding. One key aspect for conservation and recovery is to find an effective disease treatment for the animals maintained in captivity. For this purpose, low temperature (8°C-14°C) and salinity (34psu) have proven efficient for *H. pinnae*, reducing its proliferation in infected animals. This study proposes a new preliminary therapeutic approach, coupling low temperature (14°C) with phytotherapy. Herbal medicine products contain bioactive compounds with different biological effects, such as immunostimulants (Plaul *et al.*, 2022), growth promoters, curative for several pathogens such as protozoa, bacteria and virus (Abarike *et al.*, 2022; Prieto *et al.*, 2005). The treatment was applied to four adult of *Pinna nobilis* all affected by the picornavirus; moreover, one was also infected by *H. pinnae*, and another by both *H. pinnae* and *Mycobacterium* spp. Additionally, the same treatment was used for juveniles (0.5 - 4.0 cm length). In order to evaluate the effect of the treatment, daily filtering activity and diagnostic of bacterial and parasite infection of the adult pen shells, along with variations in growth rates of juvenile individuals were evaluated. Optimizing this treatment could aid infected individual recovery, preserving the original genetic and ecological traits, and enhancing the phenotypical plasticity of the species.

Key words: *Pinna nobilis*, curation, fitomedication, temperature, immune system

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PEOPLE LOOKING FOR *PINNA NOBILIS*: CITIZEN SCIENCE IN MAR MENOR COASTAL LAGOON

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Abstract: The Mar Menor coastal lagoon has become one of the last refuges and could become one of Spain's coastal reservoirs of *Pinna nobilis*. Since the beginning of the eutrophic crisis, several volunteer and citizen science activities have been carried out to monitor this population. These activities have been supported by different sources such as the RECUPERA PINNA project funded by the Biodiversity Foundation or the TERRITORIO PINNA project funded by CARM. Currently LIFE PINNARCA is a European project dedicated to the protection and recovery of the *Pinna nobilis* population in the Mediterranean. As part of the project, volunteer campaigns from 2019-2023 are being developed to provide information about the lagoon's specimens through citizen science.
In this way, sampling was carried out by diving, snorkelling or kayaking. Prior to this, a theoretical introduction to the problems associated with the disappearance of *Pinna nobilis* specimens and an explanation of the sampling to be carried out were given. These activities involved at least 150 participants. A total area of 155,34 ha was surveyed, where 214 were live and 22 dead fan mussel individuals were found. The use of citizen science is a very powerful tool for research projects. It can help to increase the information available about the marine species and habitats they observe.

Key words: *Pinna nobilis,* Mar Menor coastal lagoon, citizen science, volunteering

Acknowledgments: This study is supported by the EU LIFE Programme Project "Protection and restoration of *Pinna nobilis* populations as a response to the catastrophic pandemic started in 2016" (LIFE PINNARCA) [grant number LIFE20 NAT/ES/001265]. We would like to thank those responsible at the Consejería de Agua, Agricultura, Ganadería, Pesca y Medio Ambiente (Regional Government of Murcia) for the management of *Pinna nobilis* in the Mar Menor and for facilitating our work. And thanks to all the participants in the

volunteering activities carried out. The authors are also grateful to the Biodiversity Foundation of the Ministry for Ecological Transition and the Demographic Challenge for supporting the Recupera Pinna project in the 2020 call for proposals. Thanks to scuba diving centers and Associations: Posidonia Ecoesports, Club Gris, Planeta Azul and Hipocampus Association.

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ECOLOGICAL INSIGHTS INTO *PINNA RUDIS*: SPATIAL DISTRIBUTION PATTERNS IN MARINE RESERVES OF COLUMBRETES ISLANDS AND CABO DE PALOS, SPAIN

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Abstract: Species inhabiting benthic environments, characterized by slow growth rates and long lifespans, are particularly susceptible to environmental impacts (Deudero et al., 2015). Implementing the correct measures in marine protected areas could mitigate biodiversity loss by safeguarding critical habitats (Garcia-Charton et al., 2008). In the case of the pen shell *Pinna rudis*, despite its protected status, there remains a significant knowledge gap regarding its ecology. The aim of this study was to investigate the population parameters (size structure, abundance, and presence of life and dead individuals) and spatial distribution of *P. rudis* within the marine reserves of the Columbretes Islands (Castellón) and Cabo de Palos (Murcia), Spain. Visual census by SCUBA diving were conducted to collect data on size structure, abundance and presence of live and dead individuals across various locations in the study area at depths ranging from 10 to 30 meters (for methodology see: Vázquez-Luis et al., 2014; Nebot-Colomer et al., 2016). This study elucidates spatial distribution patterns and preferences of *P. rudis* specimens, highlighting similitudes and disparities in density, size structure and alive/dead percentage across locations, depths, and substrates. Notably, it represents the first study of its kind in Cabo de Palos, with limited existing information available for the Columbretes Islands (Kersting & Ballesteros, 2021). Given the observed proliferation of *P. rudis* along the Spanish coastline after the mass mortality incident involving *P. nobilis* (Vázquez-Luis et al., 2017) and its designation as a vulnerable species, comprehensive information on *P. rudis* is crucial to understand how this species may evolve. This investigation offers key insights into *P. rudis* ecological dynamics, aiding conservation efforts for this vulnerable bivalve species.

Key words: *vulnerable species, Marine Protected Areas, bivalve, benthic invertebrate, Ecology*

Acknowledgments: We thank the Regional Governments of local authorities, and national authorities (MITECO) for permits, specially the Secretaria General de Pesca (MAPA) for the permits to work in the marine reserves. We are very grateful so several colleagues for their valuable assistance during the fieldwork.

Funding: This research has been performed in the scope of BIODIV project: "Scientific and technical advice for the monitoring of marine biodiversity: protected marine areas and species of state competence (2022-2025)". Project funded by the European Union - NextGenerationEU through the Recovery, Transformation and Resilience Plan; and promoted by the Directorate General for Biodiversity, Forests and Desertification of the Ministry for Ecological Transition and the Demographic Challenge and CSIC, through the Spanish Institute of Oceanography (IEO-CSIC); and EsMarEs project (order IEO by Ministerio para la Transición Ecológica, Spanish Government).

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SPATIAL DISTRIBUTION OF THE FAN MUSSEL (*PINNA NOBILIS*) IN THE EBRO DELTA SANCTUARY: TO KNOW, TO PROTECT

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Abstract: Since 2016, the Mediterranean endemic fan mussel *Pinna nobilis* is severely threatened by a Mass Mortality Event (MME), caused by immunosuppressive virus and the protozoan *Haplosporidium pinnae*, among other factors, which have brought this species on the brink of extinction. Nevertheless, few populations of *P. nobilis* still exist in isolated coastal areas, where environmental conditions limit the spread of the MME, giving room for hope for the recovery of this species. In the Spanish Western Mediterranean two populations of *P. nobilis* are still surviving in the Mar Menor lagoon and in the Ebro Delta, and surveys are being carried out in order to manage effectively this species. In this regard, despite this species being listed as "critically endangered", *P. nobilis* should be granted with strict protection, since many anthropogenic pressures (eg. anchoring, water sports, contamination and fishing) still affect the last surviving individuals. Besides, in order to protect the species from anthropogenic pressures, policy makers need distribution maps that allow the identification of areas with high population abundance, so that appropriate decisions can be made. In the Alfacs bay (Ebro Delta) the distribution of the *P. nobilis* was modelled between 2022 – 2023, using the "distance sampling" methodology. Results indicate that *P. nobilis* are still present throughout the northeastern and the southern parts of the bay; the latter hosting the highest densities of the individuals. However, in areas closer to the mouth of the bay that are more affected by open sea conditions, very few

isolated individuals were found alive, coupled with higher densities of empty shells. Besides, transects that were repeated the two years showed a 20.3% increase in the percentage of dead over living individuals, suggesting a decrease in the freshwater discharge into the bay due to agricultural water restrictions during summer drought in 2023 could have weakened the salinity barrier against the epizootic disease. Given the urgency of conservation measures, these distribution maps will help to promptly guide conservation efforts and policy decisions in the area.

Key words: *Pinna nobilis,* population dynamics, monitoring, conservation, human impacts

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DEMOGRAPHIC REGULATION PROCESSES IN ENVIRONMENTAL CONTRASTING POPULATIONS OF *PINNA NOBILIS*: IMPLICATIONS FOR RESTOCKING

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Abstract: A mass mortality event (MME) affected the endemic bivalve *Pinna nobilis* across the Mediterranean Sea in autumn 2016. This event affected specimens of all shell sizes, depth ranges and habitat types, resulting in a drastic decline in the species population reaching almost 100% of individuals. Indeed, the consequences of a potential recovery, and therefore the viability of the populations of the endangered endemic species *Pinna nobilis* are unknown. In order to determine optimal areas for future reintroduction programmes, the population dynamics is essential knowledge to develop a demographic model that inform of the likelihood of success when designing the reintroduction of the species. Here we compare two population subunits of *Pinna nobilis* from two areas with different environmental conditions, located in Cabrera National Park (protected area with low exposure to hydrodynamic activity) and San Telmo (unprotected area exposed to hydrodynamic activity), both in the Balearic Islands. More than 250 individuals were tagged and monitored annually between 2011 and 2014, prior to the MME. Matrix population models were used to reduce the complexity of the age-structured population dynamics and assessing the degree of elasticity; a populations metric that reports on when and where the population growth is most sensitive to changes in its vital rates of survival or recruitment in the two population subunits. Results showed that the *Pinna nobilis* subunit of San Telmo showed highly variable values of demographic rates, higher stochasticity and higher sensitivity to environmental variability. The study has demonstrated the importance of the buffering effect of a large demography in the population subunit of Cabrera National Park, as its age structure helps to cope with highly variable reproductive success. Therefore, these population subunits are more stable regardless of depth and would be a more suitable site for future reintroduction programs.

Key words: *Pinna nobilis*, Conservation, Population dynamics, endangered species, marine invertebrates, Mediterranean Sea.

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IDENTIFICATION OF KEY FACTORS AND THRESHOLDS FOR THE SURVIVAL OF *PINNA NOBILIS* IN THE MAR MENOR LAGOON (SE SPAIN)

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Abstract: *Pinna nobilis*, an endemic bivalve species in the Mediterranean Sea, is critically endangered, mainly due to the devastating impact of the protozoan parasite *Haplosporidium pinnae*. The population within the Mar Menor lagoon (Murcia, Spain) represents one of only two surviving populations along the Spanish coast. This relict population has experienced a significant decline since 2016, from more than one and a half million of individuals to barely a thousand, primarily due to eutrophication episodes and other anthropogenic impacts affecting the lagoon (Giménez-Casalduero et al., 2020; Cortés-Melendreras et al., 2022). Given the high mortality rates observed in certain areas of the lagoon, it is imperative to identify the most suitable zones for the survival of this species, where, if necessary, adult individuals could be translocated from affected areas to enhance their chances of survival. In the present study, we identify the key factors that pose a threat to *P. nobilis* in the lagoon, and determine the thresholds for the survival. A total of 18 factors were identified through an extensive bibliographic review and an *ad hoc* methodology based on the knowledge of the authors. The key thresholds for each factor have been determined, and additionally, baseline cartography for each factor has been obtained using Geographic Information Systems (GIS). This can be useful for future studies searching for optimal survival zones in the lagoon.

Key words: *Pinna nobilis*, Mar Menor, key factor, survival, eutrophication.

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management of *Pinna nobilis* in the Mar Menor and for facilitating the work carried out. This study is supported by the EU LIFE Programme Project "Protection and restoration of *Pinna nobilis* populations as a response to the catastrophic pandemic started in 2016" (LIFE PINNARCA) [grant number LIFE20 NAT/ES/001265]**.**

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UNRAVELING THE TREND: ANALYSIS OF *PINNA NOBILIS* MORTALITY RATE IN THE ALFACS BAY, EBRO DELTA

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Abstract: Over the past decade, *Pinna nobilis* has experienced a dramatic population decline, attributed to a multifactorial disease outbreak caused by various pathogens. This mass mortality event (MME) is pushing the species to the brink of extinction, underscoring the substantial impact of pathogens on its survival throughout the Mediterranean region. As of current understanding, the main remaining populations of *P. nobilis* are confined to the Mar Menor lagoon and the Ebro Delta bays (Spanish Western Mediterranean coast). Moreover, recent data linking MME in *P. nobilis* to a newly identified hemocyte picornavirus infection have shed light on the cause of this ecological catastrophe, particularly in the Alfacs Bay, where it was discovered in samples from 2016. Temporal patterns of mortality in four translocated areas have been monthly monitored over a three-year period, establishing relations with environmental factors, and the presence of the recently identified virus. In the areas most significantly affected by exposure to open Mediterranean waters, notably the mouth of the bay and the Banya peninsula, higher mortality rates were observed (87.5 % and 79.3 %, with an initial population (Ni) of 15, and 527, respectively). Inner areas of the bay exhibited comparatively lower but still significant mortality rates, with Trabucador showing values of 68.5 % (Ni = 286) and Torre St. Joan at 72.7 % (Ni = 22). Furthermore, the reduction in freshwater discharge last year from drought conditions in the Ebro Delta, particularly in the northern areas, has altered environmental conditions in the area and may have facilitated the dispersal of infections, thereby exacerbating the severity of the situation. Although the transmission dynamics are not yet fully understood, observed mortality pattern was related to environmental parameters, such as increases in water temperature and changes in salinity. This situation highlights the critical need for ongoing research into these populations to determine whether this trend will persist in other populations such as those in Fangar Bay,

and to implement conservation measures to improve the management of declining populations.

Key words: Noble pen shell, mortality patterns, conservation, picornavirus infection

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RESPONSE TO DIET CONCENTRATION OF *PINNA* SPP IN CAPTIVITY

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Abstract: In a few years, populations of the fan mussel *Pinna nobilis* have been erased from the Mediterranean Sea affected by a disease caused by a new discovered species of protozoa, *Haplosporidium pinnae*, in combination with other pathogens (Carella et al., 2023). The fan mussel only resists on a few populations unaffected by this mortality located in isolated areas such as coastal lagoons and estuaries (Katsanevakis et al., 2022), apparently due to differences in salinity, and on very few scattered individuals in open sea, which seem to be resistant to the disease.

In addition, the condition of some of this surviving populations is critical. The Mar Menor lagoon population has severely decline over the last decade, and the shorter life span of the populations inhabiting this type of environment and the lack of recruitment point out to a gradual disappearance of some of these populations (García-March et al., 2019; Nebot-Colomer et al., 2024).

Achieving captive breeding could avoid the extinction of the species, but for now all attempts on this matter have been unsuccessful (Hernandis et al., 2023). Food quality and quantity is one of the main factors affecting reproduction success for bivalves in captivity. To improve the knowledge on this matter, an experiment has been performed using *Pinna rudis* as a proxy to *P. nobilis*. For this, the physiological rates clearance rate, ingestion rate, absorption efficiency, absorption rate, and respiration rate were measured and all integrated in the scope for growth (SFG). A standard FAO diet consisting of *Tisochrysis lutea* and *Chaetoceros neogracilis* was provided at different concentrations to test for the optimal diet concentration supply (25.000, 50.000, 75.000, 100.000 and 125.000 cel ml^{-1}).

The obtained results allowed the development of models of the different physiological measurements depending on the microalgae concentration to adopt better feeding protocols for the species.

Key words: Physiology, endangered species, captivity, diet

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