Mediterranean Marine Science

early view

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doi: 10.12681/mms.39227

Short Communication

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
www.hcmr.gr
DOI: https://doi.org/10.12681/mms.39227

New and Long-Awaited Recruitment of the Critically Endangered Species *Pinna nobilis* in the Mar Menor Lagoon (Southeastern Spain, Murcia)

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Contributing Editor: Stelios SOMARAKIS

Received: 29 October 2024; Accepted: 04 March 2025; Published online: 23 April 2025

Abstract

The bivalve mollusk *Pinna nobilis* is critically endangered, with the Mar Menor coastal lagoon hosting one of only two remaining populations in Spain following the Mass Mortality Event in the Mediterranean. The lagoon's eutrophic conditions and broader environmental degradation have led to a sharp decline in the number of surviving individuals. In addition, these adverse conditions, combined with other stressors, have significantly hindered successful reproduction. This report presents the first recorded instance of new individual recruitment in the Mar Menor lagoon in several years, observed in May 2024. Although the entire detected cohort in the surveyed area did not survive the summer, their spatial distribution, size, and estimated age offer valuable insights for future conservation efforts targeting the species.

Keywords: *Pinna nobilis*; recruitment; juvenile; Mar Menor.

Introduction

The fan mussel, *Pinna nobilis* Linnaeus, 1758, a bivalve mollusk endemic to the Mediterranean Sea, is now critically endangered following a dramatic population collapse across the region in recent years (Kersting *et al*., 2019; Katsanevakis *et al*., 2021). A mass mortality event (MME) starting in 2016 was initially linked to infection by the protozoan *Haplosporidium pinnae* (Catanese *et al*., 2018). Additional pathogens have since been identified that act synergistically to accelerate mortality, including *Mycobacterium* bacteria and a newly discovered hemocyte-infecting picornavirus (Carella *et al*., 2019; Carella *et al*., 2023a). Currently, surviving populations of *P. nobilis* persist only in isolated refuges where environmental factors such as salinity and temperature appear to inhibit the spread and infectivity of *H. pinnae* (Katsanevakis *et al*., 2021; Carella *et al*., 2023b). In Spain, just two regions still support living populations: the Ebro Delta (Catalonia) and the Mar Menor coastal lagoon (Murcia) (Cortés-Melendreras *et al*., 2022; Prado *et al*., 2022). In both areas, elevated salinity levels are believed to hinder the pathogen, falling outside *H. pinnae*'s optimal range while remaining favorable for *P. nobilis* survival (Prado *et al*., 2022). Nevertheless, occasional salinity fluctuations have led to mortality among infected individuals even in these refuges (Cortés-Melendreras *et al*., 2022; Prado *et al*., 2022). Moreover, although largely shielded from the protozoan, individuals in both populations remain highly susceptible to adverse environmental changes (Prado *et al*., 2021).

In the Mar Menor, the population of *P. nobilis* has undergone a dramatic decline due to the lagoon's persistent eutrophic conditions (Giménez-Casalduero *et al*., 2020; Nebot-Colomer *et al*., 2021; Cortés-Melendreras *et al*., 2022). A major eutrophication event occurred in 2016, triggered by the continuous influx of nutrient-rich waters, organic matter, and suspended sediments, which led to hypoxic and anoxic conditions in both the water column and sediment layers (Ruiz *et al*., 2020; Ruiz *et al*., 2021). The lagoon has since experienced multiple similar episodes, occasionally reaching euxinic conditions where

water becomes rich in hydrogen sulfide and devoid of oxygen (Cortés-Melendreras *et al*., 2022). These extreme events have resulted in the widespread mortality of numerous aquatic plant and animal species. In 2014, the *P. nobilis* population in the lagoon was estimated at over 1.5 million individuals. However, continued eutrophication and ecological degradation have reduced this number to roughly one thousand, now limited to shallow zones (less than 3 m deep), where environmental conditions have been relatively less harmful (Giménez-Casalduero *et al*., 2020; Cortés-Melendreras *et al*., 2022). Recent research has aimed to monitor this remnant population, identify surviving individuals, assess their surrounding environmental conditions, and observe reproductive patterns. These efforts include deploying larval collectors at various lagoon sites and conducting targeted searches for juveniles settled in the sediment (Kersting & García-March, 2017; Kersting *et al*., 2020; Nebot-Colomer *et al*., 2024).

Like other bivalves, *P. nobilis* reproduces via external fertilization, involving the release of gametes followed by a vulnerable larval phase (Trigos *et al*., 2018; Cortés-Melendreras, 2023). Literature suggests a single annual spawning event, typically occurring in early summer when water temperatures reach 24ºC-25ºC (Prado *et al*., 2022). Following larval development, the settlement of juveniles marks another critical stage, dependent on optimal– though still not fully understood –environmental conditions. Specific substrate characteristics have been proposed as essential for successful settlement (Trigos *et al*., 2018; Cortés-Melendreras, 2023).

In natural ecosystems, the absence of juveniles often signals a failure in reproduction and/or recruitment, an unstable population, poor environmental conditions, or the presence of predators (Kersting & García-March, 2017; Karadurmuş *et al*., 2024). In well-preserved habitats, a mismatch between spawning and juvenile recruitment in *P. nobilis* has been noted, particularly in open waters where, prior to the MME, predation on juveniles was believed to be the cause (Kersting & García-March, 2017). Presently, this mismatch may stem from low individual density, poor connectivity, unfavorable environmental conditions, or stressors affecting the organisms (Kersting & García-March, 2017). Although recruitment has been observed in *P. nobilis* populations across the Mediterranean, certain regions report alarmingly low juvenile percentages (Prado *et al*., 2022). In the Mar Menor, Cortés-Melendreras (2023) documented gamete release in multiple years (2019, 2020, and 2022), but without successful recruitment. In 2021, for the first time since the 2016 eutrophication crisis, Cortés-Melendreras *et al*. (2021) recorded juvenile recruitment, but unfortunately, these juveniles died shortly after, likely due to predation by *Hexaplex trunculus*, a gastropod frequently identified as a predator of *P. nobilis* juveniles (Kersting & García-March, 2017; Cortés-Melendreras *et al*., 2021). Nebot-Colomer *et al*. (2024) highlight the alarming status of the species in the lagoon, noting the absence of recruitment in their monitoring efforts from 2019 to 2022.

In May 2024, a survey conducted by a collaborative team, including the Regional Ministry of Environment, Universities, Research, and Mar Menor (specifically the General Directorate of the Mar Menor and the General Directorate of Natural Heritage and Climate Action), along with the University of Murcia and the University of Alicante, resulted in the first detection of *P. nobilis* juveniles in the lagoon in several years. The current study aims to document this recruitment event, which occurred in the Mar Menor in May 2024.

Material and Methods

Study zone

The Mar Menor is a hypersaline coastal lagoon situated in the southeast of Spain, within the Region of Murcia (Fig. 1). Covering an area of about 135 km², it has a maximum depth of 7 m. Salinity fluctuates between 38 and 47 psu, with temperatures ranging from 10ºC in winter to 32ºC in summer (Conesa & Jiménez-Cárceles, 2007). Separated from the Mediterranean Sea by a 20 km stretch

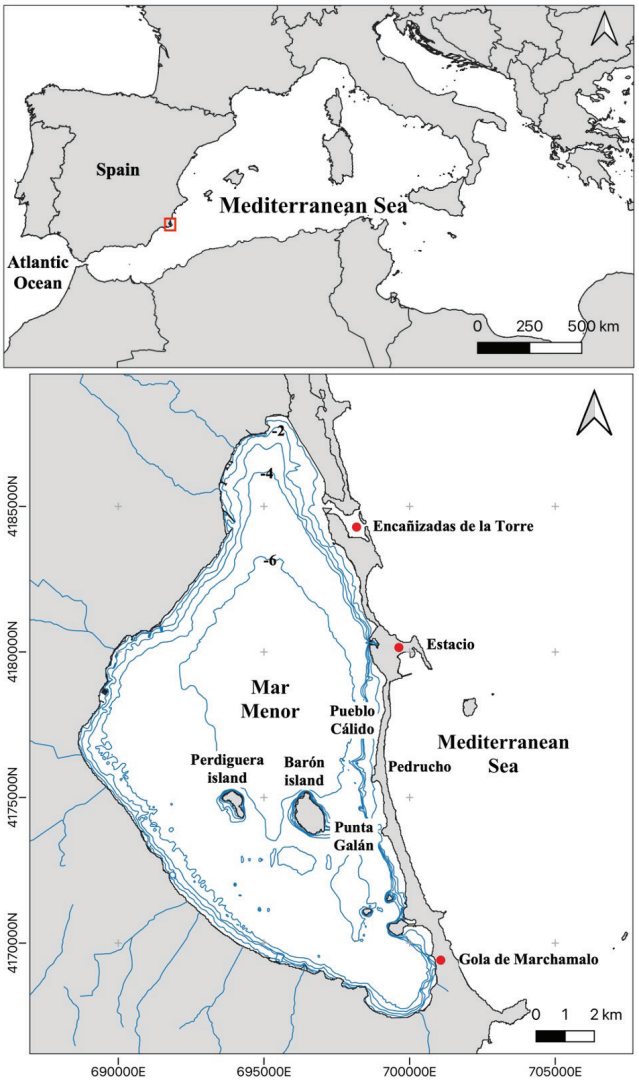

Fig. 1: Geographic location of the Mar Menor Lagoon in southeastern Spain, within the Western Mediterranean Sea. Source: gadm.org (administrative data); ign.es (bathymetry).

of land known as *La Manga* lagoon is connected to the sea through the several channels, most notably the *Encañizadas*, *Estacio*, and *Gola de Marchamalo* (Fig. 1). Water exchange between the lagoon and the Mediterranean is influenced by sea level differences (Fraile-Nuez *et al*., 2017).

The lagoon's bottom is predominantly covered by macrophyte meadows, primarily composed of *Caulerpa prolifera* and *Cymodocea nodosa*, which have coexisted for decades (Belando *et al*., 2021). The fauna of the lagoon is distinguished by its support for eurythermal and euryhaline species (Mas *et al*., 2017). Due to its significant ecological, historical, and scenic value, the Mar Menor is protected by several international conventions. It is recognized as a protected area under frameworks such as the RAMSAR Convention on Wetlands of International Importance, the Specially Protected Areas of Mediterranean Importance, the European Union Birds Directive (Council Directive 79/409/EEC), and the Natura 2000 Network (Conesa & Jiménez-Cárceles, 2007). Despite these protections, the lagoon has faced a severe environmental decline, primarily driven by worsening eutrophic conditions since 2016 (Ruiz *et al*., 2021).

Currently, the surviving fan mussel populations in the lagoon are restricted to shallow areas, mainly along the coastal strip of *La Manga* and around the islands (Cortés-Melendreras *et al*., 2022). These regions are less susceptible to hypoxia, anoxia, and euxinia during eutrophication events (Giménez-Casalduero *et al*., 2020; Cortés-Melendreras *et al*., 2022). However, they remain highly vulnerable to other threats, including boat anchoring, collisions or entanglement in fishing nets, and poaching.

Search for individuals and data collection

In May 2024, as part of underwater surveys in the lagoon aimed at locating *P. nobilis* specimens, a juvenile was spotted in the *La Manga* area (east coast; Fig. 1). This effort was a collaboration between the Regional Ministry of Environment, Universities, Research and Mar Menor (including the General Directorate of the Mar Menor and the General Directorate of Natural Heritage and Climate Action), the University of Murcia, and the University of Alicante. Following the sighting, an intensive search was launched in that area. Snorkel transects were conducted by a team of eight samplers, working in parallel to track juvenile *P. nobilis* individuals, defined as those measuring less than 20 cm in total length (Richardson *et al*., 1999).

A total of 2.5 hectares were surveyed. Juveniles found were tagged, and their locations were recorded using GPS coordinates. Additionally, the maximum shell width (MW) was measured. Given their small size and positioning, some individuals were deeply buried in sediment or tilted – maximum length measurements were not taken to avoid disturbing them. However, total length (Lt) was measured for two individuals (one alive and one dead) by carefully uncovering the valves, with particular care taken to avoid disturbing the live specimen.

Data treatment

From the surveyed area, the density of live juvenile *P. nobilis* individuals per 100 m² was calculated. Additionally, the size distribution of all individuals was analyzed based on their maximum shell width (MW). Using the total length (Lt) measurements from two individuals, their ages were estimated using the equation proposed by García-March *et al*. (2020) for coastal lagoons:

$$Lt = 56.5 \times (1 - e^{-0.30(t + 0.05)}),$$

where Lt represents total length in centimeters and t is age in years.

To examine the spatial distribution of the recruitment, the geopositioning data of both live and dead juveniles were used to generate a distance matrix map. This was done using the raster analysis tool in QGIS v.3.22.2, an open-source software.

Results and Discussion

With nearly all open water *P. nobilis* populations depleted, reservoir areas have become critical for the species' survival. As noted by Kersting *et al.* (2020), these populations act as a source of larvae for unaffected populations, potentially aiding the species' recovery. Unfortunately, mortality has been recorded in most reservoirs, primarily due to pathogenic infections or other persistent threats, such as poaching, boat anchoring, and pollution (Conesa & Jiménez-Cárceles, 2007; Hendriks *et al.*, 2013; Foulquie *et al*., 2023; Papadakis *et al*., 2023). In the Mar Menor, nearly 99% of the population has vanished (Giménez-Casalduero *et al*., 2020; Cortés-Melendreras *et al*., 2022). Moreover, the absence of juveniles is an alarming issue for the lagoon's population.

In the surveyed area of this study, a total of 24 juveniles were detected (one of which was dead) (Fig. 2), all at depths greater than 1 m. The cause of death for the single deceased juvenile remains unclear. Its intact valves and vertical anchoring to the sediment suggest it was not impacted by nets or anchors, indicating it may have been part of the natural mortality rate for *P. nobilis* juveniles.

The density of live specimens is 0.1 ind./100 m², which falls within the range of adult density estimated for the lagoon in 2020 by Cortés-Melendreras *et al.* (2022), although it is lower than their average value of 1.005 ± 0.006 ind./100 m^2. Regarding spatial distribution, Figure 3 shows how the juveniles are concentrated in the northwest and central parts of the prospected polygon. The minimum and maximum distances between them are 4.7 m and 125.5 m, respectively, with an average distance of 59.6 ± 32.3 m. This juvenile clustering corresponds with existing literature, which suggests that individuals are distributed in patches, creating areas of high density surrounded by areas with no individuals (Cortés-Melendreras *et al*., 2022; Prado *et al*., 2022).

The average MW of all juveniles is 3.7 ± 0.6 cm. More than half of the juveniles had an MW between 3 and 3.5

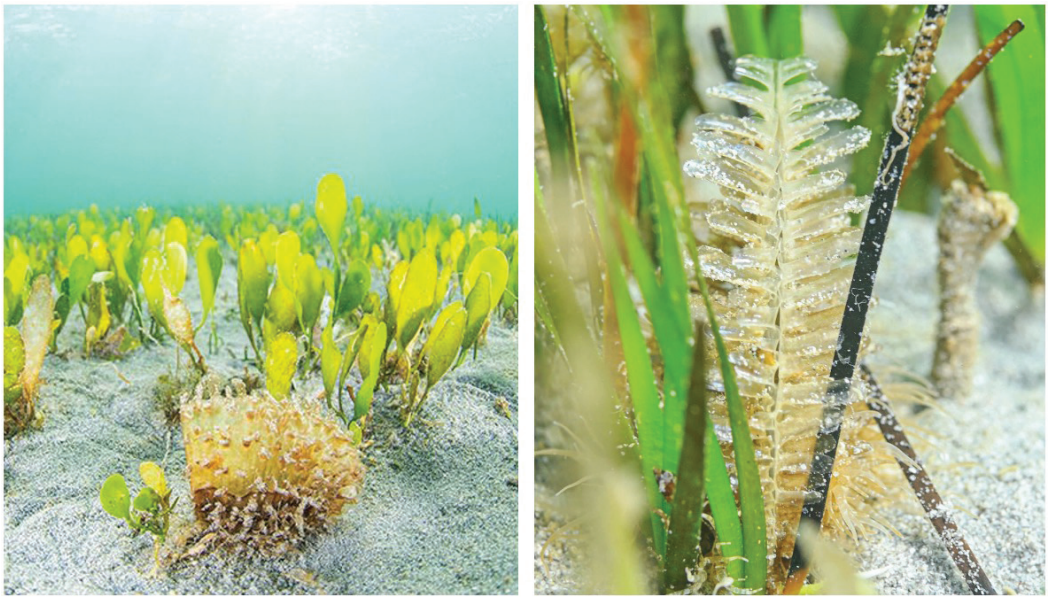

Fig. 2: Juveniles of *Pinna nobilis*.

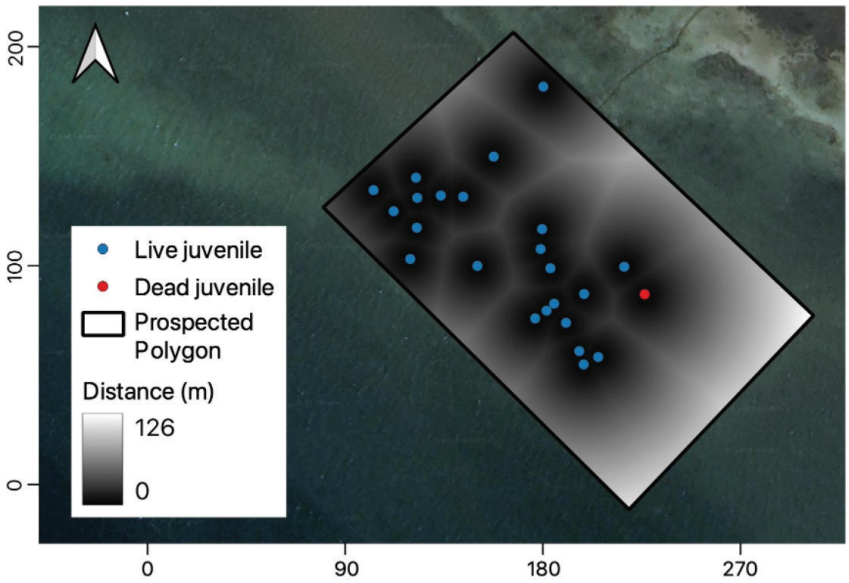

Fig. 3: Prospected polygon and distance matrix illustrating the distances between juveniles (alive or dead) within the area. The axes represent distance in meters.

cm (Fig. 4). The Lt of the deceased individual measured 9.5 cm (3.5 cm MW), while the living juvenile measured 13 cm (5 cm MW), corresponding to ages of 0.8 and 1 year, respectively. This size difference could reflect the response of individuals from a single cohort to external factors, although the possibility of two reproductive pulses since around May 2023 cannot be excluded. Historical temperature data indicate that May 2023 coincided with water temperatures of 23°C-24°C (Canal Mar Menor, 2024). Previous studies, such as that by Nebot-Colomer *et al*. (2024), found no individuals in larval collectors installed between April and June. It is important to note that installing collectors in the lagoon in June is likely too late; they should be installed in April or early May, when the water temperature begins to rise. Even when installed at the correct time, their effectiveness has primarily been shown in open water at greater depths (greater than 5.5 m in Kersting & García-March, 2017), while the average depth in the lagoon is 3.5 m. It remains unclear whether larvae move closer to the bottom in the lagoon's bathymetry, suggesting that collectors in Mar Menor should be placed at shallower depths or attached to the substrate.

All the juveniles found were located on a seabed covered by a mixed, sparsely dense meadow of the seagrass *C. nodosa* and the algae *C. prolifera*. *P. nobilis* is known to prefer settling on substrates covered by meadows, as the network of roots and rhizomes provides an ideal anchoring area for the individuals' byssus (García-March, 2005; Guallart & Templado, 2012). This location may represent an optimal area for recruitment, although it might not be the same as the ideal conditions for adult survival (Martínez-Martínez *et al*., 2024).

Regarding the threats to which they are exposed, juveniles found in 2021 were likely preyed upon by the gastropod *H. trunculus* (Cortés-Melendreras *et al*., 2021). However, the 2024 recruitment shows some spine development on the valves, potentially providing protection from predation (pers. obs.). Despite this, they face

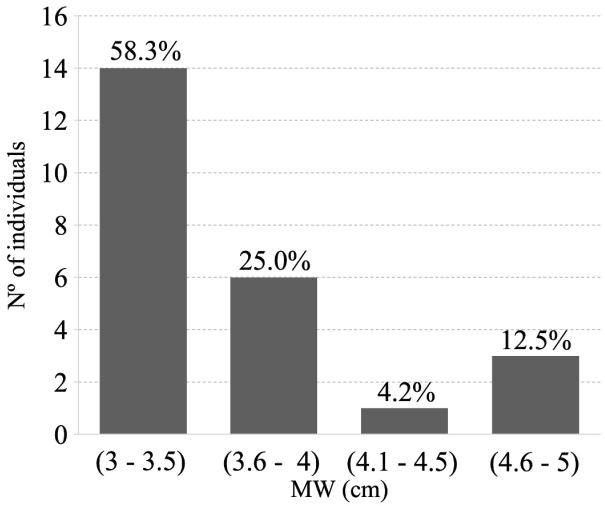

Fig. 4: Distribution of juveniles categorized by their maximum width (MW), showing both the number and percentage of each category.

significant risks beyond the lagoon's environmental instability. The presence of fishing nets very close to the individuals has been detected, along with marks and bare patches in the meadows resulting from the past presence and dragging of other nets. Additionally, the parasite *H. pinnae* remains a serious concern. Areas connected to the Mediterranean, like *Encañizadas* or *Estacio* (Fig. 1), exhibit lower salinity due to water exchange (Fraile-Nuez *et al*., 2017), making individuals there more susceptible to infection. Furthermore, meteorological events causing temporary drops in salinity (such as the DANA in 2019) also increase the risk of pathogen entry. In fact, a group of individuals in the *Encañizadas* area died from *H. pinnae* infection in 2017 (Cortés-Melendreras *et al*., 2022).

Monitoring juveniles, studying their growth and health, and assessing area-specific threats are critical for effective conservation efforts (Kersting *et al*., 2019). This comprehensive evaluation will help determine whether relocating some individuals to lower-risk areas is necessary to ensure their survival. It is highly probable that additional juveniles exist in the area and other parts of the lagoon with similar conditions, such as shallow depths and abundant macrophyte cover. Detecting settled juveniles is challenging, particularly due to the dense meadow cover. Furthermore, many sections of the lagoon remain unsurveyed, which may contain both adult and juvenile individuals. As such, expanding the search to other adjacent, similar areas is essential to get a more accurate assessment of their distribution. Additionally, studying larval transport throughout the lagoon to better trace their origins would provide valuable insights. The closest adult nucleus is in *La Manga*, a few kilometers south, though juveniles could also originate from other high density areas such as Barón Island (Fig. 1).

Unfortunately, during the monitoring of juvenile individuals in September 2024 (post-summer), no juveniles were detected. The significant anthropogenic pressure in the area, compounded by the lack of timely and effective conservation measures, has likely led to the destruction or disappearance of the entire identified cohort. However, the confirmed potential for reproduction and recruitment provides a hopeful outlook, contingent upon the implementation of more proactive conservation management strategies. The recruitment observed in 2024, as described in this report, is of great significance for the conservation and management of the species both in the lagoon and throughout the Mediterranean. This development also serves as an encouraging indicator, suggesting the presence of healthy individuals and favorable conditions for the reproduction of *P. nobilis* in the Mar Menor.

Acknowledgements

We would like to thank those responsible at the Regional Ministry of Environment, Universities, Research and Mar Menor (Regional Government of Murcia) for the management of *Pinna nobilis* in the Mar Menor and for facilitating the work carried out.

This study has been funded by the LIFE PINNARCA (LIFE20-NAT/ES/001265) (supported by the LIFE Programme of the European Union) and by the project "Proyecto para la cría ex situ de *Pinna nobilis* y creación del Banco de Especies protegidas y singulares del Mar Menor," supported by Dirección General del Mar Menor. Consejería de Medio Ambiente, Universidades, Investigación y Mar Menor (CARM).

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