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Pinna nobilis refugia breached: Ongoing Mass Mortality Event in the Gulf of Kalloni (Aegean Sea)

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Abstract

The endemic Mediterranean fan mussel *Pinna nobilis* is on the brink of extinction due to an infectious disease that has caused an ongoing Mediterranean Mass Mortality Event (MME) since 2016. Currently, healthy *P. nobilis* populations can only be found in a few refuges, mainly transitional waters such as coastal lagoons and estuaries. In the eastern Mediterranean, the only remaining fan mussel population is located in the Gulf of Kalloni (Lesvos Island, Greece). This study reports on a severe mortality event that occurred during summer 2023, leaving the population decimated and limited mainly to young individuals. The estimated density of live individuals dropped from 1.22 to 0.23 individuals per 100 m², indicating an 81% population decline within approximately three months. The significant reduction in the *P. nobilis* population in the Gulf of Kalloni highlights the ongoing threat of MMEs. Continuous monitoring of *P. nobilis* and environmental variables, such as temperature and salinity, enhanced protection from anthropogenic pressures, and research into disease resistance mechanisms are crucial for the conservation of this critically endangered species.

Keywords: *Pinna nobilis*; extinction; mass mortality event; *Haplosporidium*; distance sampling.

Introduction

Since 2016, Mass Mortality Events (MMEs) have been devastating populations of the Mediterranean fan mussel *Pinna nobilis* across the Mediterranean. The MMEs have led to numerous cases of local extinctions (Vázquez-Luis *et al.*, 2017; Çinar *et al.*, 2021; Pensa *et al.*, 2022; Papadakis *et al.*, 2023) and the species is now on the brink of extinction (Katsanevakis *et al.*, 2022).

The causes of the MMEs are not yet fully understood. Initially, the protozoan *Haplosporidium pinnae* was identified as the primary cause, along with multipathogen infections involving *Mycobacterium* and *Vibrio* bacteria (Grau *et al.*, 2022; Carella *et al.*, 2023a). However, recent studies have highlighted picornavirus infection as a potential major factor, as it leads to immunosuppression, making fan mussels more susceptible to opportunistic infections (Carella *et al.*, 2023b, 2024). Within four years (2016-2020), the MMEs have spread eastwards from the western Mediterranean, reaching the central and easternmost parts of the Mediterranean basin and decimating over 95% of the population (Kersting *et al.*, 2019; Katsanevakis *et al.*, 2022).

Populations of *P. nobilis* throughout the Mediterranean have been devastated, with only a few locations still harbouring live individuals (Kersting *et al.*, 2019; Katsanevakis *et al.*, 2022). These areas, mostly transitional waters such as coastal lagoons and estuaries, are considered potential refuges for *P. nobilis* protecting them from the causes of MMEs (Kersting *et al.*, 2019; Peyran *et al.*, 2022). Notably, these areas could serve as sources of larvae, potentially contributing to the natural recovery of the species (Kersting *et al.*, 2020). Salinity and temperature are thought to be the main abiotic factors contributing to the observed resistance to the disease, although the precise mechanisms are not well understood (Cabanellas-Reboredo *et al.*, 2019; Prado *et al.*, 2021).

In the eastern Mediterranean, the only remaining *P. nobilis* population is found in the Gulf of Kalloni on the island of Lesvos (North Aegean). Nevertheless, the fan mussel population of the Gulf of Kalloni has been substantially affected by the MME and is currently reduced to a small percentage of the initial population (Katsanevakis *et al.*, 2019; Zotou *et al.*, 2020; Papadakis *et al.*, 2023). The Gulf is a semi-enclosed shallow bay with high seasonal environmental fluctuations influenced significantly by river runoff, exhibiting a gradient from strong influence near the river mouths to less influence towards the strait connecting the Gulf with the open sea (Spatharis *et al.*, 2007, 2009; see also Fig. 1). Being the only area

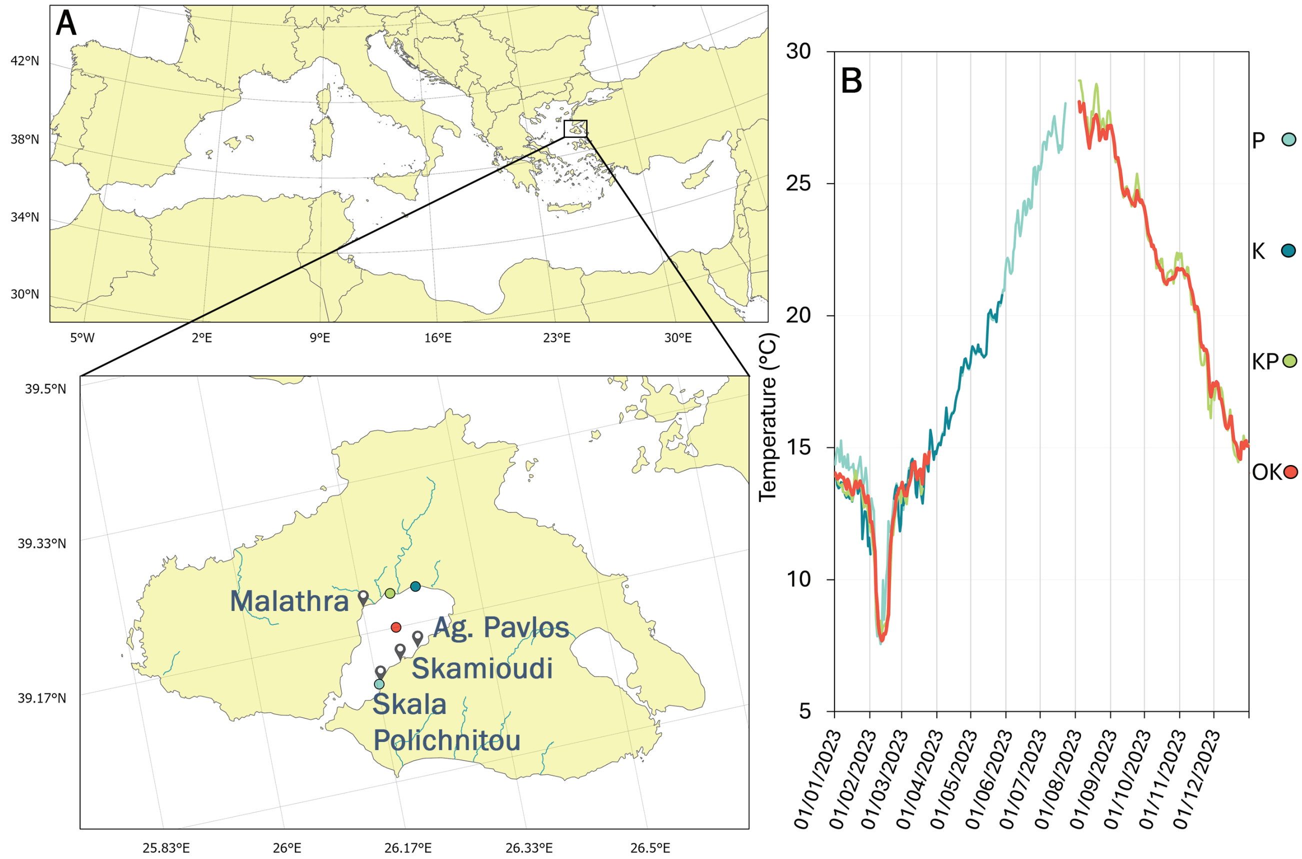

Fig. 1: (A) Figure of Lesvos Island and Kalloni Gulf with the four sampling sites. Coloured points depict the position of temperature loggers in the gulf. (B) Annual (2023) time-series of temperature data from the different temperature loggers. Abbreviations for the logger sites are: Polichnitos salt pans (P), Kalloni salt pans (K), Kalloni port (KP), and Oceanographic station (OK) of the Department of Marine Sciences, University of the Aegean. The map was created using ArcGIS software by ESRI, with data from "Rivers (e-per.gr)", available at https://geodata.gov.gr/el/dataset/potamoi-e-per-gr.

in the eastern Mediterranean with live fan mussels, the Gulf of Kalloni has seen increased monitoring, research, and conservation efforts (Zotou *et al.*, 2020; Papadakis *et al.*, 2023).

In this context, the Gulf of Kalloni is subject to continuous monitoring of *P. nobilis* in order to track the progression of the MME. This paper presents the results of *P. nobilis* monitoring during 2023, and evaluates a summer mass mortality event that substantially reduced the remaining fan mussel population. Given the dynamic nature of the MME and the importance of the Gulf of Kalloni for the conservation of *P. nobilis* in the eastern Mediterranean, it is crucial to communicate the monitoring results and their implications to the scientific community rapidly.

Materials and Methods

To assess the state of the *P. nobilis* population in the Gulf of Kalloni, four sampling sites were monitored for both live and dead individuals between late spring - early summer (May-June) and autumn (September-October) 2023 (Fig. 1). Re-sampling the same sites in autumn was considered important given that an observed mass mortality episode occurred in late summer. Additionally, temperature loggers were installed at four different locations within the Gulf (Fig. 1), namely Polichnitos salt pans (P), Kalloni salt pans (K), Kalloni port (KP), and the Oceanographic station (OK) of the Department of Marine Sciences, University of the Aegean. These loggers were placed at a depth of 1 m and they recorded sea temperature every 30 minutes. At all locations, the HOBO 64K Pendant Temperature/Alarm (Waterproof) Data Logger was used; however, after early August, two stations (KP and OK) were replaced with an EnvLogger T2.4 due to the loss of the original loggers.

The abundance of *P. nobilis* was assessed through distance sampling using line transects (Buckland *et al.*, 1993). Transect lines of 200 m in length were deployed at regular intervals on the seabed at each site. A SCUBA diver swimming at constant speed along each transect line recorded each individual encountered as well as their perpendicular distance (y) from the transect line. In total, 61 line transects were deployed; 23 line transects were deployed at Malathra, 10 at Agios Pavlos, 10 at Skamioudi, and 18 at Skala Polichnitou. Additionally, for each living individual recorded, visibility, habitat type (either soft/mixed substrate or seagrass), shell width, and depth were recorded. Detailed methodology and concepts of distance sampling are provided as Supplementary Material.

To model the *detection function* and thus make an estimate of population density, the Distance 7.5 release 2 software (Thomas *et al.*, 2010) was used. A set of candidate models were fitted to the data for the two seasons to estimate the *detection function* for live individuals for the two sampling periods. In total, 22 models were fitted using both conventional distance sampling (CDS) and multiple covariate distance sampling (MCDS) models (see Table S1 for the set of candidate models). Candidate models were fitted using two 'key' functions, Hazard rate and Half-normal, with various adjustment terms and covariates (habitat, visibility, and the shell width).

Model selection was based on Akaike's information criterion (AIC; Akaike, 1974). Additionally, the overall goodness-of-fit was examined using Kolmogorov-Smirnov and Cramer-von Mises tests (Burnham *et al.*, 2004).

Results and Discussion

During the first sampling period (May-June), a total of 244 live and 295 dead individuals were recorded, whereas during the second period (September-October) 37 live and 340 dead individuals were counted. Most of the live individuals were found at Malathra, where most of the sampling effort was concentrated (Table 1). Malathra had the lowest dead/total ratio for both periods (47% and 87%, respectively) in contrast to the other sampling stations (Table 1 and Fig. S1). Malathra, is located in the innermost part of the Gulf, near most river mouths (Fig. 1). As demonstrated by other studies, river influence is higher in the inner Gulf and decreases towards the open sea (Spatharis *et al.*, 2007, 2009). Therefore, the Malathra population may exhibit higher resistance due to stronger riverine influence and higher variability in temperature and salinity compared to the other sites. However, a long time series and a denser network of environmental monitoring stations is required to reach solid conclusions.

The best models of the *detection function* during the two sampling periods were the simple Hazard Rate and the Half-Normal one with shell width as a covariate, respectively (Table S2). Both selected models demonstrated good fit to the data, as indicated by the Kolmogorov-Smirnov tests and both cosine-weighted and unweighted Cramer-von Mises tests (all p values > 0.05).

When pooling data from all sampling sites, the estimated density of live individuals in May/June was 1.22 [0.96, 1.54] individuals per 100 m^2 (95% confidence intervals in brackets), with an estimated abundance of 15572 [12282, 19743] individuals. In September-October, the estimated density was 0.23 [0.13, 0.41] individuals per 100 m^2, with an abundance of 2897 [1608, 5219] individuals. Hence, within approximately three months, the fan mussel population in the Gulf of Kalloni decreased by 81% during a marked MME.

The highest densities during the first sampling period were recorded at Malathra and Agios Pavlos (1.74 and 1.59 individuals/100 m^2, respectively), while the lowest were recorded at Skala Polichnitou and Skamioudi (0.53 and 0.51 individuals/100 m^2, respectively). A similar pattern was noted for the second period with the highest densities at Malathra followed by the other sites (Table 1), although no live individuals were found at Skamioudi. Most of the observed live individuals were young, with only few adults with shells larger than 8 cm in width (Fig. 2).

Relationships between total shell length and maximum shell width, as described by Tempesta *et al*. (2013) and Sarafidou *et al*. (2023), were used to estimate the length of live individuals and infer the population's age structure by inspecting various shell length and age relationships (Rabaoui *et al*., 2007; Kersting & García-March *et al*., 2017; García-March *et al*., 2020). The finding suggests that most individuals were less than 3 years old, indicating that the majority are post-MME recruits (Katsanevakis *et al*., 2019), including successful recruits from 2022 (Papadakis *et al*., 2023). The reason for the higher sur-

Table 1. Density (D) and abundance (N) estimates from the best fitted models. 95% confidence intervals of D and N estimates are provided in brackets.

Site	Observations and results of best model for live individuals in May-June 2023			
	Alive	Dead	D (individuals/100 m^2)	N (individuals)
Agios Pavlos	57	72	1.59 [1.30, 1.94]	2386 [1953, 2914]
Malathra	138	123	1.74 [1.24, 2.42]	10410 [7455, 14535]
Skala Polichnitou	32	51	0.53 [0.30, 0.95]	1815 [1025, 3213]
Skamioudi	17	49	0.51 [0.22, 1.14]	961 [427, 2167]
	Observations and results of best model for live individuals in September-October 2023			
	Alive	Dead	D (individuals/ 100 m^2)	N (individuals)
Agios Pavlos	5	53	0.044 [0.0062, 0.32]	66 [9, 473]
Malathra	29	191	0.44 [0.24, 0.82]	2646 [1426, 4910]
Skala Polichnitou	3	48	0.054 [0.014, 0.21]	185 [48, 717]
Skamioudi	0	48	0	0

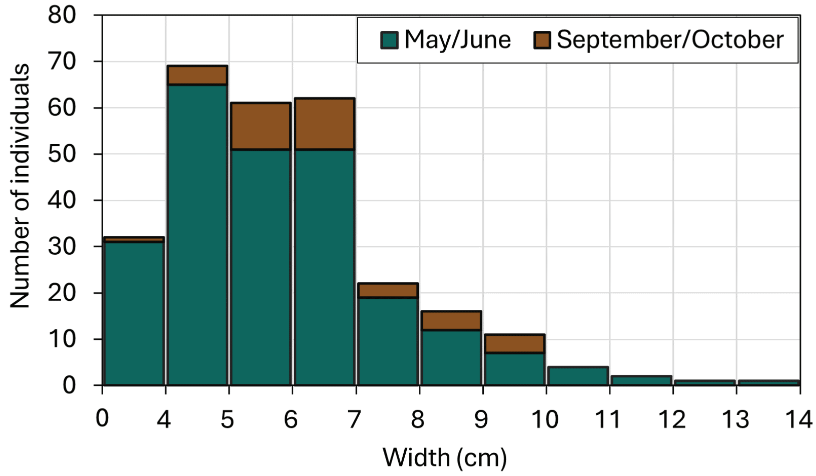

Fig. 2: Frequency of live individuals from all sites in different shell width classes in May/June and September-October.

vival rates among younger individuals compared to older ones remains unclear, although similar trends have been reported by Prado *et al*. (2021).

In line with other studies, our results indicate significant population losses for *P. nobilis* in refuge areas for various reasons, which is a cause of concern (Prado *et al*., 2021; Cortés-Melendreras *et al*., 2022; Donato *et al*., 2023; Papadakis *et al*., 2023; Karadurmuş *et al*., 2024). For instance, certain areas in the Sea of Marmara experienced a notable population decline in 2021 (Çinar *et al*., 2021), affected by an intense mucilaginous outbreak. Nevertheless, juveniles (post-MME recruits) are still observed in many of these areas, as noted in the Gulf of Kalloni and the Sea of Marmara (Papadakis *et al*., 2023; Karadurmuş *et al*., 2024), thus highlighting their importance as potential larval sources (Kersting *et al*., 2020; Papadakis *et al*., 2023).

The remaining fan mussel populations in the Mediterranean Sea, mainly in lagoons, embayments and estuaries, are usually already subject to significant human pressures. Fishing, including illegal and destructive practices, and eutrophication are common threats, leading to additional mortalities of *P. nobilis* (Katsanevakis, 2016; Papadakis *et al*., 2023; Cortés-Melendreras *et al*., 2022; see Supplementary Video for destructive shellfish dredging in Kalloni Gulf). Furthermore, natural phenomena can also impact *P. nobilis* populations in these areas. For instance, fluctuations in rainfall affecting salinity levels can influence the resistance against pathogen infections (Cortés-Melendreras *et al.*, 2022), while severe storms can disrupt the optimal environmental conditions for these populations (Prado *et al*., 2021).

Climate change must also be integrated into *P. nobilis* conservation planning as ocean warming and extreme events are increasing (IPCC, 2023). Recently, it has been demonstrated that pathogen infections are exacerbated by higher temperatures (Lattos *et al.*, 2023), thus rendering the remaining *P. nobilis* populations increasingly vulnerable in the context of climate change.

Given these challenges for fan mussel conservation, continuous monitoring, including extensive measurements of salinity and temperature, and enhanced protection of the remaining populations from anthropogenic pressures are essential to diminish losses due to human-induced causes. Additionally, if *P. nobilis* populations in refuge areas continue to decline, the potential for natural recovery through larval dispersal to other Mediterranean areas will be severely hindered. Future research should focus on obtaining a better understanding of the mechanisms of resistance against MMEs and implementing effective conservation actions for protecting existing populations and repopulating extinct ones.

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Supplementary Material

The following supplementary material is available for this article:

Detailed distance sampling methodology.
Table S1. Set of candidate models.
Table S2. Best models selected based on AIC and results of goodness of fit tests.
Fig. S1: Pie charts for each study site showing the number of live and dead individuals between May-June (left) and September-October (right). The size of each pie chart is proportional to the total number of individuals.
Video of destructive shellfish dredging in the Gulf of Kalloni.