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The last fortress fell: mass mortality of *Pinna nobilis* in the Sea of Marmara

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Abstract

A spring 2021 cruise to the south Marmara Islands revealed a mass mortality event of *Pinna nobilis*, which had been reported to be healthy just seven months ago. The cause of death might be associated with an epidemic disease or a catastrophic mucilage event seen after November 2020 in the Sea of Marmara. A total of 191 *Pinna nobilis* individuals were found at seven stations, of which 88% were dead. The population density (including dead and alive individuals) of *P. nobilis* was found to be between 0.3 ind.100 m^{-2} and 12 ind.100 m^{-2} in the area. A total of three live and four dead juvenile individuals were observed in the area, indicating low recent recruitment. The largest number of dead *Pinna nobilis* individuals was encountered in shallow waters (0-4 m depth). A total of 34 species, belonging to six taxonomic groups (Sipuncula, Oligochaeta, Polychaeta, Crustacea, Mollusca and Pisces), were found within the dead shells of four *P. nobilis* individuals. The mass mortality of *Pinna nobilis* in the Sea of Marmara, the last remaining disease-free sea, indicates the necessity of establishing and implementing emergency action plans for this species, including ex-situ conservation.

Keywords: Mass mortality; pandemic; pathogen; Sea of Marmara; Turkey.

Introduction

The pen shell *Pinna nobilis*, endemic to the Mediterranean Sea, has been suffering from a highly infective and lethal water-borne disease, caused primarily by the sporozoan parasite *Haplosporidium pinnae* and several pathogenic bacteria, since late 2016 (Vázquez-Luis *et al*., 2017; Catanese *et al*., 2018; Cabanellas-Reboredo *et al*., 2019; Katsanevakis *et al*., 2019; Panarese *et al*., 2019; Carella *et al*., 2020; Scarpa *et al*., 2020). The majority (>90%) of Mediterranean populations of *P. nobilis* have been affected by the disease, with mass mortality events reported in both the western and eastern parts of the Mediterranean Sea (Vázquez-Luis *et al*., 2017; Katsanevakis *et al*., 2019; 2021; Kersting *et al*., 2019; Öndes *et al*., 2020; Čižmek et *al*., 2020). This disease has devastated the *P. nobilis* populations in the region, leading IUCN to categorize it as a critically endangered species (Kersting *et al*., 2019). Recently, mass mortality of this species has been observed in the Çanakkale Strait (Özalp & Kersting, 2020; Acarli *et al*., 2021; Künili *et al*., 2021), the south gate of the Sea of Marmara, where several beds of healthy *P. nobilis* individuals had been reported in September 2020 (Çinar *et al*., 2021). It has previously been questioned whether the exceptionally healthy *P. nobilis* beds of the Sea of Marmara were due to some ecological conditions of the sea or if mass mortality was only a matter of time.

Unlike the Mediterranean Sea, the Sea of Marmara contains two stratified water bodies; the upper layer consists of Black Sea-originated water that is less saline (from north to south 18-25PSU) and the lower layer consists of Mediterranean-originated water that is high saline (almost homogenous across the basin 36-38 PSU) (Özsoy & Altıok, 2016). The disease outburst was proved to be very closely correlated to temperatures above 13.5 °C and a salinity range between 36.5 and 39.7 PSU (Cabanellas-Reboredo *et al*., 2019; García-March *et al*., 2020). Based on these data, it was thought that the brackish water character of the upper layer of the Sea of Marmara, where *P. nobilis* beds occur, might form an ecological barrier to the spread of the disease and the Sea of Marmara could thereby act as a reservoir area for the species (Çinar *et al*., 2021).

During the spring sampling campaign of the MarIAS project (Addressing Invasive Alien Species Threats at

Key Marine Biodiversity Areas Project), observations were made regarding the status of *Pinna nobilis* populations at 10 monitoring stations in the south Marmara Islands, as the Çanakkale populations of this species were already known to be significantly affected by the epidemic disease (Özalp & Kersting, 2020; Künili *et al*., 2021).

In the present paper, the mass mortality of this species in the southern part of the Sea of Marmara, particularly the south Marmara Islands, is documented and the other species established inside the shells and decaying tissues are reported.

Materials and Methods

Within the scope of the MarIAS project (www.istilacilar.org), a spring sampling campaign was conducted by the Research Vessel *K. Piri Reis* between the dates 02.04.2021 and 06.04.2021 to assess the population structures of alien species (especially *Rapana venosa* and *Asterias rubens*) at 10 monitoring stations in the south Marmara Islands and Kapıdağ Peninsula in the Sea of Marmara (Fig. 1). At each station, two 100 m belt transects, perpendicular to the coastline, were set on the seafloor, starting from a 0.5 m depth. Zones, 2 m wide on the right and left sides of the transect (for a total width of 4 m), were observed by two scientific divers each; the total surveyed area of each transect is 400 m^2. The initial plan was to place 4 transects at each station and for the left and right zones to be observed by a single researcher. However, due to the intense mucilage in the region in April 2021, the number of transects was reduced to two at each station and two divers were placed at the left and the right sides of each transect to perform a thorough observation.

At seven of the ten stations, *Pinna nobilis* individuals were observed and their status was noted (dead or alive). At station 1, where numerous dead *P. nobilis* individuals were encountered, tissues of four dead individuals were collected in separate nylon bags and fixed with 4% formalin on-board the ship to examine the species established on the inner part of the shells. In the laboratory, the material was first washed with tap water on 0.5 mm mesh, sorted according to taxonomic groups, and then preserved in 70% alcohol. This material was deposited at the museum ESFM (Ege University Su Ürünleri Fakültesi Müzesi).

Results

Mass Mortality of *Pinna nobilis*

The present study recorded a mass mortality of *P. nobilis* in the Sea of Marmara. Deaths in the region probably began in the mid- or late autumn of the year 2020, and appear to have continued during the winter months.

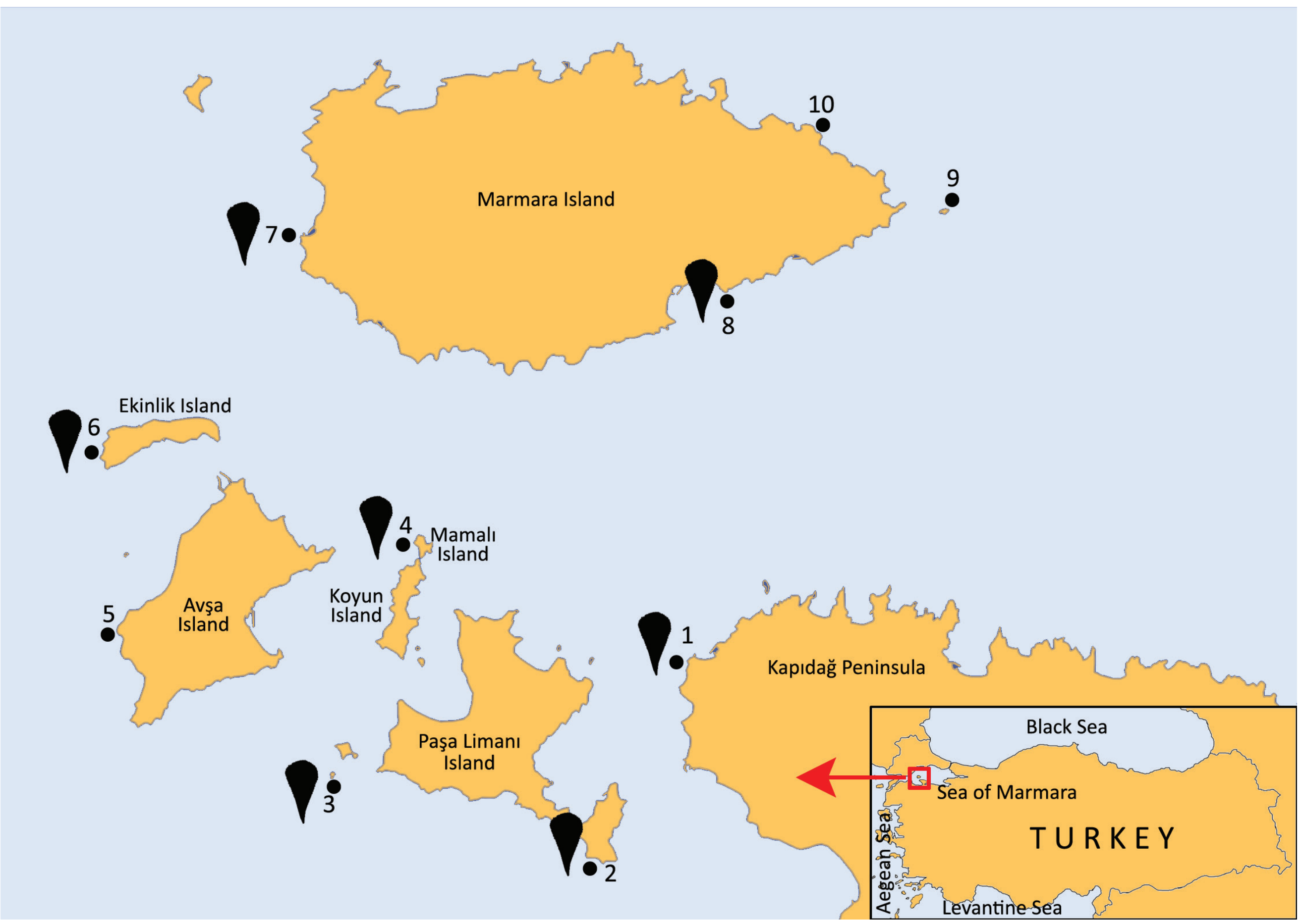

Fig. 1: Map of the investigated area (south Marmara Islands) with the locations of sampling sites. The shell icon indicates stations where *Pinna nobilis* individuals (live and dead) were found.

As of April 2021, when the present study was conducted, though most *P. nobilis* individuals had already decomposed into black sediment, the tissue of some dead individuals had not yet fully decomposed. The surface water temperatures were reported to be above 16°C from September 2020, when the last study was carried out in the region (Çinar *et al*., 2021), to December 2021; the recorded values were September: 25.2 °C, October: 21 °C, November: 18.5 °C and December: 16.2 °C (see https://www.mgm.gov.tr/FILES/resmi-istatistikler/denizSuyu/Marmara-Deniz-Suyu-Sicakligi-Analizi -2020.pdf).

Pinna nobilis population in the area

A total of 191 *Pinna nobilis* individuals were found along the belt transects at seven of the ten stations. Of these, 88% were dead; only 19 adults and 4 juveniles (<20 cm) were alive (Table 1). The population density (including dead and alive individuals) of *P. nobilis* at the stations ranged from 0.3 ind.100 m^{-2} (station 3) to 12 ind.100 m^{-2} (station 1). The population density of live individuals of *P. nobilis* were between 0.25 (at station 3) and 1.25 ind.100 m^{-2} (at stations 1 and 2); live individuals were entirely absent at two stations, 4 and 6) (Table 1). The majority of the individuals found at each station were dead, ranging from 0% (only one live individual, station 3) to 100% (stations 4, 6 and 7). Four live and four dead juvenile individuals were observed at stations 1, 4 and 7.

The majority of dead *P. nobilis* individuals (92) were found in shallow waters (1-4 m depth), but dead individuals were also observed at depths greater than 10 m, even at the lower depth limit of its distribution in the area (max depth 14.9 m; station 2) (Fig. 2). Living *P. nobilis* indi-

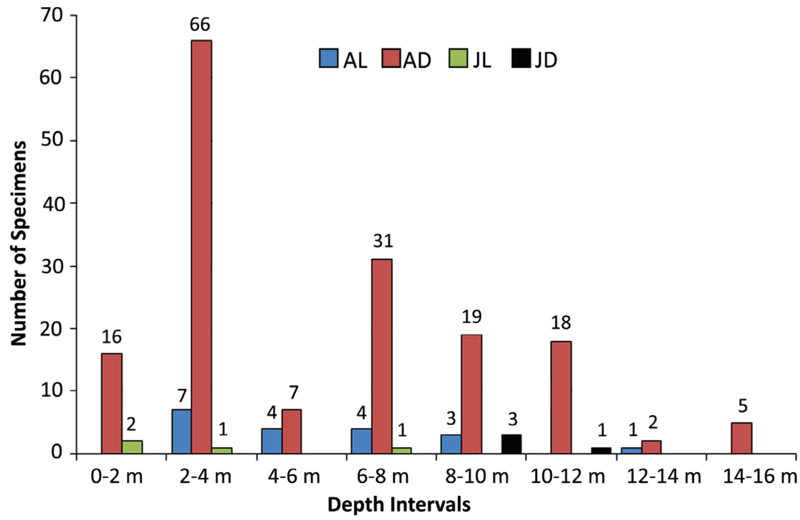

Fig. 2: The number of live and dead adults and juvenile individuals of *Pinna nobilis* in relation to depth in the Sea of Marmara. AL: adult alive, AD: adult dead, JL: juvenile alive, JD: juvenile dead.

Table 1. The number and density of live and dead adults and juvenile individuals of *Pinna nobilis* at stations in the Sea of Marmara. DR=Depth Range.

Stations	**1**		**2**		**3**	**4**	**6**		**7**		**8**
Transects	**T1**	**T2**	**T1**	**T2**	**T1**	**T1**	**T1**	**T2**	**T1**	**T2**	**T1**
DR (m)	1-4	1-4	5-15	4-14	13	10	3-14	3-14	8-13	6-11	8-10
Live Adult	3	4	2	5	1				1		3
Dead Adult	40	42	22	22		4	15	4	6	6	3
Live Juvenile	2	1							1		
Dead Juvenile						3				1	
Total	**45**	**47**	**24**	**27**	**1**	**7**	**15**	**4**	**8**	**7**	**6**
Density (ind.100 m^{-2}) (Dead + Live)	11.3	11.8	6	6.8	0.3	1.8	3.8	1	2	1.8	1.5
Density (ind.100 m^{-2}) (only Live)	1.25	1.25	0.5	1.25	0.25	-	-	-	0.5	-	0.75
Dead%	**89**	**89**	**92**	**81**	**0**	**100**	**100**	**100**	**75**	**100**	**50**

viduals were encountered at depths between 2.3 m and 13 m, but the largest number (14 individuals) were found at depths of 2.3-6 m (Fig. 2). Living juvenile individuals were observed at depths ranging from 0.8 m to 8 m. The largest cluster of dead individuals (82) was found in a *Cymodocea nodosa* bed at station 1 (Fig. 3); other dead individuals were encountered on gravelly coarse sand at other stations.

During the sampling period, a mass mucilage event happened in the Sea of Marmara, occurring in all depths at all stations studied, significantly diminishing underwater visibility and resulting in a dense accumulation of mucilage on *P. nobilis* individuals and *C. nodosa* meadows (Fig. 4A).

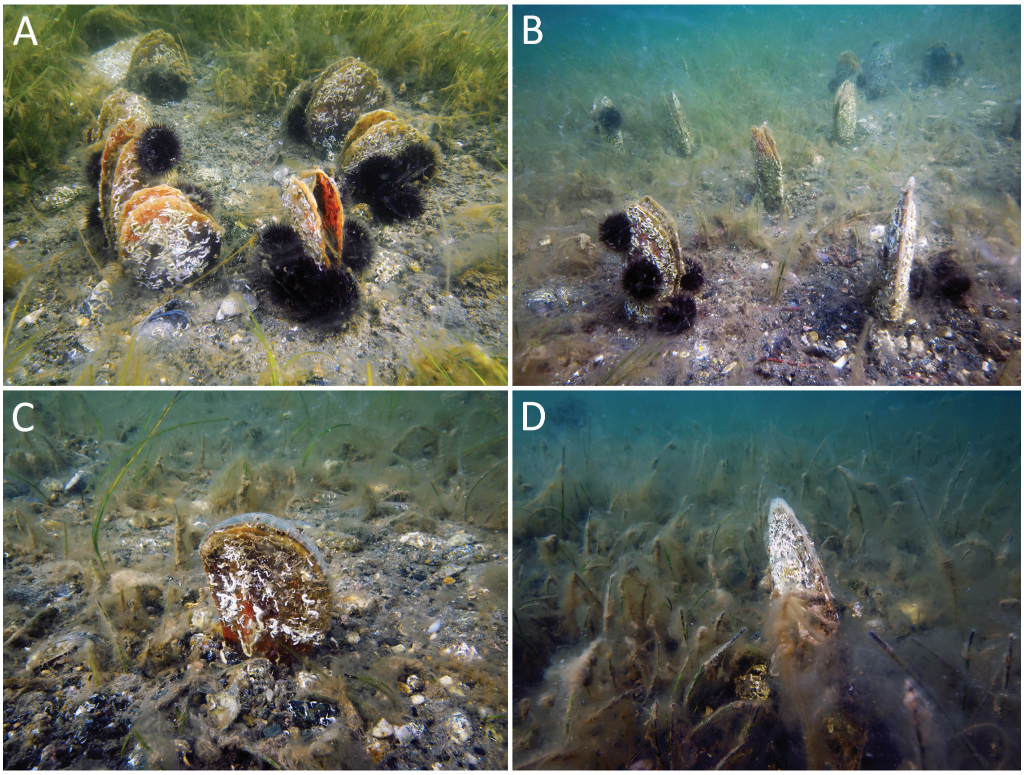

Fig. 3: *Pinna nobilis* population at station 1. A and B) dead individuals; C) live juvenile, D) live adult.

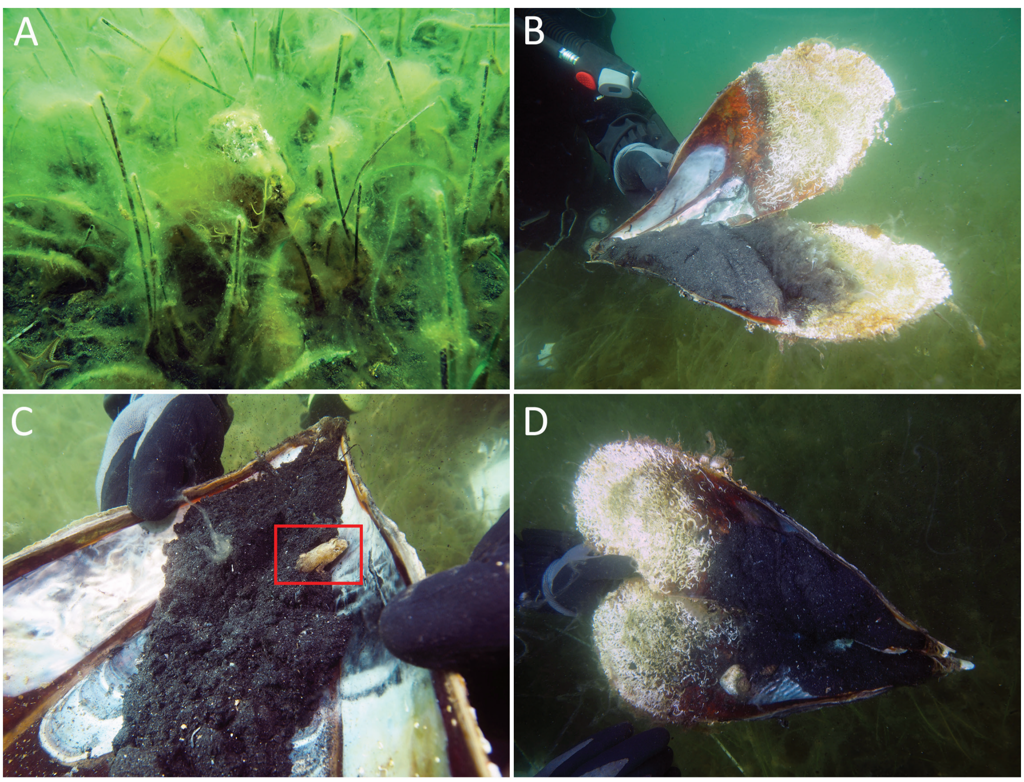

Fig. 4: A. Dense mucilage aggregations on dead *Pinna nobilis* individuals and *Cymodocea nodosa* meadows, station 1, B-D. Examples of decaying tissue and associated fauna, station 1. Note the presence of *Parablennius tentacularis* in Figure 4C.

Associated fauna within shells of dead *Pinna nobilis*

A total of 34 species belonging to six taxonomic groups (Sipuncula, Oligochaeta, Polychaeta, Crustacea, Mollusca and Pisces) were found inside the dead shells of four *P. nobilis* individuals collected at station 1 (Table 2, Fig. 4B-D). The unburied lengths of these shells were broadly similar, ranging from 15 cm to 20 cm. The most dominant species on the shells was the serpulid *Spirobranchus triqueter*, which densely covered the upper side of the inner

Table 2. Species list found within dead tissue (WT), on tissue (OT) and on the inner surface of shells (S) of four dead *Pinna nobilis* individuals collected at station 1.

Individual	**N1**	**N2**	**N3**	**N4**	**Substrate**
Unburied length (cm)	**20**	**18**	**15**	**17**	
SIPUNCULA					
Aspidosiphon muelleri (Diesing, 1851)	1		1		WT
OLIGOCHAETA					
Oligochaeta (sp.)	1	3		2	WT
POLYCHAETA					
Sigambra tentaculata (Treadwell, 1941)		1			WT
Oxydromus pallidus Claparède, 1864		1			WT
Mysta picta (Quatrefages, 1866)		1			WT
Neanthes acuminata (Ehlers, 1868)	1	1	1	2	WT
Glycera fallax Quatrefages, 1850	1				WT
Schistomeringos rudolphi (Delle Chiaje, 1828)				1	WT
Prionospio maciolekae Dagli & Çinar, 2011	1	1			WT
Capitella teleta Blake, Grassle & Eckelbarger, 2009	6	25	1	1	WT
Notomastus sp.	1				WT
Heteromastus filiformis (Claparède, 1864)				5	WT
Caulleriella bioculata (Keferstein, 1862)		1			WT
Spirobranchus triqueter (Linnaeus, 1758)	>100	>100	>100	>100	S
CRUSTACEA					
Xantho poressa (Olivi, 1792)		1		1	OT
Pagurus sp.		1			OT
MOLLUSCA					
Mytilaster lineatus (Gmelin, 1791)			1		S
Rhyssoplax olivacea (Spengler, 1797)	1				S
Bittium reticulatum (da Costa, 1778)	25	10	25	12	S
Steromphala adansonii (Payraudeau, 1826)	1	2	3	8	S
Steromphala albida (Gmelin, 1791)				1	S
Steromphala rarilineata (Michaud, 1829)				1	S
Clanculus cruciatus (Linnaeus, 1758)	2				S
Mangelia unifasciata (Deshayes, 1835)				1	S
Tritia incrassata (Strøm, 1768)				1	S
Tricolia pullus (Linnaeus, 1758)			1		WT
Alvania mamillata Risso, 1826	1				S
Vitreolina antiflexa (Monterosato, 1884)	1				S
Weinkauffia turgidula (Forbes, 1844)	1				S
Pusillina sp.	1	1			S
Tritia reticulata (Linnaeus, 1758)		2			S
Mangelia costulata Risso, 1826		1	1		S
Retusa truncatula (Bruguière, 1792)		1			S
PISCES					
Parablennius tentacularis (Brünnich, 1768)	1		1		OT

surfaces of the shells. Mollusks (mostly gastropods) were found crawling on *S. triqueter* tubes, but *Tritia reticulata* individuals were found feeding on the decaying tissue of the shells. High numbers of organic-pollution indicator species such as the annelids *Neanthes acuminata* (previously known as *N. caudata*) and *Capitella teleta*, which was particularly common, were present in the decaying tissues of all *P. nobilis* individuals. Three motile species, i.e., the crab *Xantho poressa*, the pagurid *Pagurus* sp. and the fish *Parablennius tentacularis*, were observed on decaying tissues (Fig. 4C).

Discussion

It was known that the Sea of Marmara, especially the southern Marmara Sea, had dense beds of *P. nobilis* (Öndes *et al*., 2020; Çinar *et al*., 2021). In a study conducted on these beds in September 2020, it was reported that 90% of the population *of P. nobilis* was healthy and that the epidemic disease that has caused mass mortality of this species in the Mediterranean had not occurred in the region (Çinar *et al*., 2021). It was stated that this promising situation was presumably due to an ecological feature of the Sea of Marmara, the brackish water. However, it was also suggested that transmission of the disease to the Sea of Marmara's *P. nobilis* individuals might be inevitable. Unfortunately, the present study, which was carried out just seven months after the aforementioned survey, indicated that the majority of *P. nobilis* individuals (>85%) found in the south Marmara Islands and Kapıdağ Peninsula had died off. In a recent study conducted in the Çanakkale Strait (Künili *et al*., 2021), *Haplosporidum pinnae* and the bacteria *Vibrio coralliilyticus*, *V. tubiashii*, *V. mediterranei* and *V. hispanicus* were identified; these were presumably responsible for the death of *P. nobilis* individuals in that region. It is plausible that the same pathogens are affecting the *P. nobilis* population of the southern Marmara Sea; but this remains to be investigated by molecular and histological studies. Another plausible cause of the mass mortality event experienced by fan mussels in the Sea of Marmara is the mass mucilage event that was observed during the same period causing mass benthos mortality of sponges such as *Aplysina aerophoba*, *Sarcotragus* spp., *Ircinia* spp. and *Haliclona* spp., and the mussel *Mytilus galloprovincialis*. The existence of live adult and juvenile individuals recorded in this study might be a beacon of hope for the species' survival in the region. However, whether the survivors will remain unaffected can only be revealed by further surveys that monitor the health status of *P. nobilis* in the area.

As nearly all *P. nobilis* individuals appeared to be healthy in September 2020 and a mass mortality event was detected in April 2021, the onset of deaths in the region might have occurred in mid or late autumn, when the surface sea water temperatures were still above 20 °C. It is estimated that deaths of *P. nobilis* continued until April, when the sampling was conducted, as the surface water temperature remained above 13.5 °C (at least in December), the threshold under which disease-causing parasite and bacteria become inactive (García-March *et al*., 2020).

The percentage of mortality varied among the stations, but it was 100% in the vast majority of the stations in the investigated area. Similar high mortalities have been reported in the western and eastern Mediterranean (90%-100%) (Vázquez-Luis *et al*., 2017; Katsanevakis *et al*., 2021), in the Adriatic Sea (36%-100%) (Čižmek *et al*., 2020), in the Aegean Sea (75%-100%) (Katsanevakis *et al*., 2019; Öndeş *et al*., 2020), on the southern coast of the Çanakkale Strait (99%) (Özalp & Kersting, 2020), and along the Çanakkale Strait (9%-100%) (Acarli *et al*., 2021; Künili *et al*., 2021).

In this study, the highest population density (including dead and live individuals) of *Pinna nobilis* was estimated as 11.8 ind.100 m^{-2} at station 1. In a previous study performed in the region by Çinar *et al*. (2021), the density reported was as high as 400 ind.100 m^{-2}. This discrepancy in the density scores is mainly related to differences in the methodology applied. In the study conducted by Çinar *et al*. (2021), sampling was carried out only at the depth where the *P. nobilis* beds were present. However, in the present study, observations were made along transects placed perpendicular to the shore starting from a 0.5 m depth. In this case, not only the depths and habitats where *P. nobilis* beds occurred, but also other depths and habitats (including rocky areas) in the region were included in the calculation.

The Sea of Marmara is affected by eutrophication (Ediger *et al*., 2016). The region is densely populated and industrialised, with more than 20% of Turkey's population and 50% of industry located in its drainage basin. The municipal and industrial inputs to its drainage basin, together with nutrients and organic inputs from the Black Sea, have polluted the Marmara Sea since the 1970s (Polat & Tuğrul, 1995). Increased nutrient inputs and global warming have led to mucilage events in recent years (Aktan *et al*., 2008). Mucilage aggregations have become more intensive and frequent than ever before in the region, including the south Marmara Islands (pers. obs.), from October 2020 until the time of writing (May 2021). Many habitats and species have been affected by the coverage of extensive mucilage accumulations and the resulting oxygen depletion from bacterial biodegradation has caused the mass mortality of many species (including fish). During our research, dense mucilage in the water column considerably decreased underwater visibility (to ca. 3-4 m) and was observed smothering mussel beds, sponges, *Cymodocea nodosa* meadows and *P. nobilis* individuals. It has previously been reported that eutrophication events caused high mortalities of *P. nobilis* in refuge sites like the Mar Menor in Spain (Giménez-Casalduero *et al*., 2020). If the mortality was somehow related to the mucilage event, there is still the possibility of the Sea of Marmara being a refuge for the species. It will therefore be crucial to monitor survivors and to have information about other *P. nobilis* populations in the area. In the Ebro's Delta (Spain), it was reported that some populations have been only partially affected by the disease following a salinity gradient (Prado *et al*., 2021).

Numerous macrozoobenthic species have been identified within the decaying tissues of the *P. nobilis* individuals. Some (e.g., *Spirobranchus triqueter*) used dead shells as a substratum, while others (especially gastropods) were observed feeding on filamentous algae that had settled on dead shells. Some species (*Parablennius tentacularis* and *Xantho poressa*) used the dead shells as a shelter. Opportunistic polychaete species found in organic polluted sediments in the Mediterranean Sea (Ergen *et al*., 2006; Çinar *et al*., 2009) were found living within the rotting tissues of *P. nobilis*. Some of these species were previously reported on the outer surface of the shells of living *P. nobilis* individuals (Çinar *et al*., 2021).

The Sea of Marmara seems to be no longer a viable hope for the future of *P. nobilis*. What to do next? Surveys to monitor the health status, identify the cause of the observed mortality and track the mortality outbreaks of the species in the region are essential for guiding actions to protect the species and its habitats, particularly where the *P. nobilis* populations are still unaffected by the epidemic. Currently, healthy individuals of *P. nobilis* in the Mediterranean Sea have been confined at some localities: Thau Lagoon (France, Foulquié *et al*., 2020), Ebro's Delta and Mar Menor Lagoon (Spain, Cabanellas-Reboredo *et al*., 2019; García-March *et al*., 2020), Venice Lagoon (Italy, Katsanevakis *et al*., 2021), Kalloni Gulf (Greece, Katsanevakis *et al*., 2021) and some localities in the Çanakkale Strait (Acarli *et al*., 2021). These areas not only act as refuges but are also potential larval exporting sites (Kerstling *et al*., 2020). Surveys to determine the disease mechanism of the pathogens and why some individuals are more resistant to pathogens should be carried out continuously. If the current trend continues, we might face the regional extinction of this species. The ex-situ conservation of *P. nobilis* should be taken into consideration, though no attempts to complete its full life cycle in captivity have been successful (Katsanevakis *et al*., 2021).

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