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***Pinna nobilis* in the Greek seas (NE Mediterranean): on the brink of extinction?**

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Abstract

The Mediterranean endemic fan mussel *Pinna nobilis* is suffering an ongoing basin-scale mass mortality event (MME) since 2016. As most Mediterranean populations have collapsed, the species has been declared as Critically Endangered in the IUCN Red List of threatened species. In an effort to track the progress of the MME and provide updated information on the status of the species in the Greek seas, data collected through dedicated surveys and opportunistic assessments during 2019 and 2020 have been compiled. During surveys conducted at 258 sites, a total of 14,589 fan mussels were recorded, of which 81.1% were dead. Of the remaining 2,762 live individuals, 256 were juveniles. Two marine areas that still sustain living populations were identified, namely Kalloni Gulf (Lesvos Island), and Laganas Bay (Zakynthos Island). The inner part of Kalloni Gulf appears to maintain the largest surviving population of the species in the eastern Mediterranean, with an abundance estimate of 684,000 individuals (95% confidence interval: 322,000-1,453,000). Solitary, potentially resistant, scattered individuals were recorded at several sites. Other previously abundant populations that had been assessed in the past, specifically those of Lake Vouliagmeni (Korinthiakos Gulf), Souda Bay (Crete) and Gera Gulf (Lesvos Island) with a total of ~350,000 individuals, have now been wiped out. Our results document the collapse of most *P. nobilis* populations throughout the Greek seas. The MME has progressed substantially between early 2019 and mid-2020, as indicated by the increase in mortality at sites consecutively monitored multiple times. This work highlights the urgent need for continuous monitoring of surviving populations and calls for immediate implementation of an effective protection and management strategy that will ensure the persistence of surviving individuals and the production of resistant offspring.

Keywords: Pinnidae; pen shell; population assessment; abundance; critically endangered; *Haplosporidium pinnae*; conservation status; mass mortality events.

Introduction

The Mediterranean endemic fan mussel *Pinna nobilis* Linnaeus, 1758 is the largest bivalve in the Mediterranean Sea and one of the largest worldwide. Its elongated triangular shell can reach a length of up to 120 cm (Schultz & Huber, 2013), and its lifespan can exceed 45 years (Rouanet *et al.*, 2015). Until recently, the species was well-distributed at a variety of habitats throughout all Mediterranean ecoregions, at depths between 0.5 m and 60 m (Zavodnik *et al.*, 1991; Butler *et al.*, 1993).

Part of the shell is buried and anchored by byssus threads in soft sediments usually covered with seagrass meadows, mainly *Posidonia oceanica* (Rabaoui *et al.*, 2010; Vázquez-Luis *et al.*, 2014), but also *Cymodocea nodosa* (Russo, 2012; Kersting & García-March, 2017), *Zostera* spp. (Russo, 2012; Prado *et al.*, 2014; Kersting & García-March, 2017) and the long-established alien *Halophila stipulacea* (Katsanevakis & Thessalou-Legaki, 2009). The species may also occur on unvegetated sandy bottoms (Richardson *et al.*, 1999; Katsanevakis, 2006), estuaries (Addis *et al.*, 2009), pebbly bottoms (Zavodnik *et al.*, 1991; Richardson *et al.*, 1999) or sandy patches among hard substrates (Kersting & García-March, 2017; Tsatiris *et al.*, 2018). It is a habitat-forming species, as the surface of its shell provides a hard substrate for many epibionts on a soft bottom surrounding (Giacobbe, 2002; Addis *et al.*, 2009; Rabaoui *et al.*, 2009). It can thus function as an ecosystem engineer, and very dense populations can create biogenic reefs (Katsanevakis, 2016). Being a filter feeding mollusc, *P. nobilis* plays a key ecological role, retaining large amounts of organic matter and reducing turbidity (Trigos *et al.*, 2014), while enhancing benthic-pelagic coupling (Basso *et al.*, 2015).

Over the years, human stressors have caused the decline of fan mussel populations across the Mediterranean basin (De Gaulejac, 1995; Richardson *et al.*, 1999; García-March, 2005; Katsanevakis, 2007; Deudero *et al.*, 2015; Basso *et al.*, 2015). Exploitation of the species can be traced back to antiquity; in addition to its exploitation for human consumption, its byssus threads were used to produce a precious textile, “sea silk” (Maeder, 2008). Furthermore, the degradation of *P. oceanica* meadows – a vital fan mussel habitat – as a result of several human activities (e.g. trawling, illegal fishing methods, uncontrolled anchoring) is increasingly threatening the survival of the species (Katsanevakis *et al.*, 2008; Vázquez-Luis *et al.*, 2015; Basso *et al.*, 2015). Additional stressors affecting fan mussel populations include climate change, marine pollution, and invasive species (Basso *et al.*, 2015; Kersting *et al.*, 2019).

Consequently, *P. nobilis* has been listed as an endangered species and is protected under the EU Habitats Directive (92/43/EEC, Annex IV), the Protocol for Specially Protected Areas and Biological Diversity in the Mediterranean of the Barcelona Convention (Annex II), and the national legislation of most Mediterranean countries. However, despite its protection status, commercial exploitation of the species continues illegally in some countries, with collected individuals being destined either

for consumption as a delicacy (Katsanevakis *et al.*, 2011), or to fulfil decorative purposes (Kersting *et al.*, 2019).

Until the autumn of 2016, no parasitic or bacterial infection had been reported as constituting a threat for the survival of fan mussel populations (Darriba, 2017). A mass mortality event (MME) of *P. nobilis* along the SE coasts of the Iberian Peninsula, with mortality rates exceeding 90% within a few weeks, alarmed the scientific community. By June 2017, the MME had expanded northwards (Balearic Islands), affecting all age classes of fan mussel populations at all depths and habitat types, with mortalities reaching up to 100% at some locations (Vázquez-Luis *et al.*, 2017). Histological examination of affected individuals showed heavy inflammatory host response and severe general dysfunction (Darriba, 2017). Thereafter, severe mortalities of *P. nobilis* populations were reported from additional locations along the Spanish coastline, as well as France and Italy (Catanese *et al.*, 2018; Carella *et al.*, 2019; Panarese *et al.*, 2019). Soon after the first reports, new MME records were reported in Greece, Cyprus, Turkey, Algeria, Tunisia, Morocco and Croatia, with mortality rates often reaching 100% (Katsanevakis *et al.*, 2019; Kersting *et al.*, 2019; IUCN, 2019; Čížmek *et al.*, 2020). Due to the severe decline of its global population and its high risk of extinction, in 2019, *P. nobilis* was classified as Critically Endangered in the IUCN Red List of Threatened Species (Kersting *et al.*, 2019).

The search for the cause of this large-scale MME that has affected most Mediterranean populations has puzzled the scientific community. Two pathogens have been identified and associated with the disease affecting *P. nobilis* populations; a newly described, highly species-specific pathogenic protozoon of unknown origin, named *Haplosporidium pinnae* (Catanese *et al.*, 2018), and a *Mycobacterium* sp. (Carella *et al.*, 2019). The coexistence of both pathogens in some affected populations has been reported (García-March *et al.*, 2020; Lattos *et al.*, 2020; Carella *et al.*, 2020; Čížmek *et al.*, 2020). Furthermore, in some cases, increased sea temperature seems to favour the proliferation of another opportunistic pathogen in *P. nobilis*, namely *Vibrio mediterranei* (Rodríguez *et al.*, 2018), which was recently found to intensify pathogenicity and cause increased mortality of stabled fan mussels at temperatures of about 25–26°C (Prado *et al.*, 2020).

Despite the dramatic population declines in impacted areas, there are still some surviving populations across the Mediterranean [e.g. Fangar Bay (Spain), Mar Menor (Spain), Rhone Delta (France), Etang de Thau (France), Diana and Urbino (Corsica), the inner part of Kalloni Gulf, Lesvos (Greece) and the Venice lagoon (Italy)], possibly due to local environmental conditions that delay the expansion of the disease or are unfavourable for the parasites (Catanese *et al.*, 2018; Katsanevakis *et al.*, 2019; Kersting *et al.*, 2019; Cabanellas-Reboredo *et al.*, 2019).

In Greece, no unusual mortality of the species had been recorded before the summer of 2018, when anecdotal information about an ongoing MME in the Aegean Sea triggered the first mortality assessments. In early autumn

2018, a survey conducted along the coasts of Lesvos Island provided a mean mortality estimate of 93%, thus confirming the spread of the disease in the Aegean Sea. The *P. nobilis* population in Gera Gulf had an estimated mortality between 91% and 100%, while in Kalloni Gulf mortalities varied from 36% (northern, inner part of the gulf) to 99% (southern, outer part of the gulf). Respective molecular analyses confirmed the presence of *H. pinnae* in infected individuals from Gera Gulf (Katsanevakis *et al.*, 2019). Later on, Lattos *et al.* (2020) confirmed the presence of both *H. pinnae* and *Mycobacterium* sp. in specimens collected from Thermaikos Gulf and the north Aegean (Kalloni Gulf and Limnos island) during 2018-2019. Currently, it is clear that the Mediterranean MME has affected previously thriving *P. nobilis* populations in Greek waters. However, the lack of past information on the population status of the species in the Aegean and Ionian seas, with the exception of a few localities (Katsanevakis, 2006; Katsanevakis & Thessalou-Legaki, 2009; Tsatiris *et al.*, 2018), constitutes a challenge for the assessment of its status at national level (Katsanevakis *et al.*, 2019).

Approximately one year after the onset of the MME in Greece, several research teams across the country initiated visual surveys to assess fan mussel mortalities and investigate the expansion of the MME. By compiling information collected through such dedicated surveys, conducted in 2019 and 2020, as well as other opportunistic records, this work aims to provide the most comprehensive and updated information on the health status of *P. nobilis* populations in the Greek seas.

Materials and Methods

General approach for data collection

This study presents the state of *P. nobilis* populations as a mosaic of individual case studies, conducted in different marine areas, in order to assess the mortality rates of impacted populations in the Greek seas, and estimate the current extent of the MME. A number of independent surveys were conducted between February 2019 and June 2020. In exceptional cases, certain populations have been monitored occasionally over the past years (Katsanevakis, 2009; Katsanevakis & Thessalou-Legaki, 2009; Tsatiris *et al.*, 2018), thus allowing a ‘Before & After MME’ comparison of their population status. However, since long term monitoring data of marine benthic communities is particularly scarce in Greece, most of the information provided herein comes from one-off assessments, carried out after the onset of the MME. Aiming to cover a large geographical scale, the dataset consists of eight dedicated case studies focusing on *P. nobilis* MMEs and opportunistic assessments from 70 additional sites carried out within the framework of various projects (Table 1).

In order to assess the mortality of *P. nobilis*, both dead and live individuals were counted during visual surveys, lasting 20-120 minutes. Only recently dead individuals were counted, excluding individuals presumed to be dead for longer periods (i.e. according to the type and degree of fouling), or having died due to mechanical damage (e.g. due to anchoring) (Katsanevakis *et al.*, 2019).

Table 1. Sampling methodology followed by the research groups in each marine area. “45 min” refers to 45 minutes respectively of visual observations applying the protocol described in Case Study 1, “30 min” refers to the same protocol but less survey time, “Opportunistic” refers to opportunistic records without applying a strict protocol, “Transect” refers to observations along strip transects and “Distance” when Distance sampling methodology was applied.

| Geographic Area | Sampling methodology | |
|------------------------------|-----------------------|---------------|
| | 2019 | 2020 |
| Saronikos Gulf | 45 min | - |
| Petalion Gulf | 45 min; Transect | - |
| South Evoikos Gulf | 45 min; Opportunistic | Opportunistic |
| North Evoikos Gulf | 45 min | Opportunistic |
| Maliakos Gulf | - | Distance |
| Kalloni Gulf (Lesvos Island) | 45 min; Distance | 45 min |
| Gera Gulf (Lesvos Island) | 45 min | 45 min |
| inner Thermaikos Gulf | Opportunistic | Distance |
| outer Thermaikos Gulf | 45 min | Opportunistic |
| Toronean Gulf | - | Opportunistic |
| Korinthiakos Gulf | 30 min; Opportunistic | - |
| Elounda Bay (Crete) | 45 min; Opportunistic | - |
| N.M.P.A.N.S. | 45 min; Opportunistic | - |
| N.M.P.Z. | Transects | - |
| Other Areas | Opportunistic; 45 min | Opportunistic |

Case study 1: Saronikos Gulf, South and North Evoikos Gulfs, Petalion Gulf, Maliakos Gulf

In September 2019, four marine areas in Greece (Saronikos Gulf, Petalion Gulf, South and North Evoikos Gulfs) were investigated by conducting a dedicated survey targeting the mortality of *P. nobilis*. These gulfs are adjacent and interconnected. The narrow Evripus Strait links the South and North Evoikos Gulfs. Several of the surveyed locations are included in the Natura 2000 European network of protected areas (Site Codes: GR3000005, GR3000004, GR2420016, GR2440002). Published data from previous surveys (e.g. Sini *et al.*, 2017), anecdotal information (including communication with local dive centres, divers, fishermen, and research scientists), combined with Google Earth images, were used to select the 32 sampling sites, targeting shallow waters covered with seagrass (Fig. 1 CS 1). The surveys were conducted over hard, soft or mixed substrates, with the dominant vegetation being *P. oceanica*, *C. nodosa* and *Cystoseira* spp., at a depth range of 0.5 and 20 m (Supplementary file). Each visual survey lasted 45 minutes and was conducted using either SCUBA equipment or free diving, depending on the depth and the visibility of each location. Live individuals were characterized as “presumed healthy” (clearly unaffected populations) or “presumed moribund”, based on the delayed valve-closing reflex, as described in Vázquez-Luis *et al.* (2017). Juvenile individuals were recorded separately.

Additional records from two independent research groups that investigated parts of the aforementioned ma-

rine areas were also included, since the data recorded in March 2019 and between March and June 2020 allow comparison of the species status in three different periods. In March 2019, 9 sites were investigated at a depth range between 0 and 14 m, while in early 2020, 7 sites were visited at a depth range between 0 and 27 m.

Case study 2: Kalloni Gulf – Lesvos Island

Kalloni Gulf is the larger of the two shallow, semi-enclosed embayments of Lesvos Island (NE Aegean Sea), with an approximate surface area of 130 km² (Kefalas *et al.*, 2016). Kalloni Gulf is a productive marine ecosystem that supports a variety of ecologically valuable habitats and communities (Evangelopoulos & Koutsoubas, 2008; Kefalas *et al.*, 2016; Sini *et al.*, 2019), and is included in the Natura 2000 European network of protected areas (Site Code: SCI GR4110004). Before the spread of the MMEs in the eastern Mediterranean, Kalloni Gulf hosted a large *P. nobilis* population that, however, had never been quantitatively assessed (Katsanevakis *et al.*, 2019).

In a dedicated study conducted in June 2019, a total of 20 sites were surveyed in Kalloni Gulf, following the same protocol as the one described in Case Study 1. The sampling sites were systematically positioned every ~3 km along the coastline (Fig. 1 CS 2&3), at a depth range of 0-9 m, and were investigated by free diving. Furthermore, additional surveys, following the same protocol as the one described in Case Study 1, provided data for different periods (4 sites visited in August-September 2019

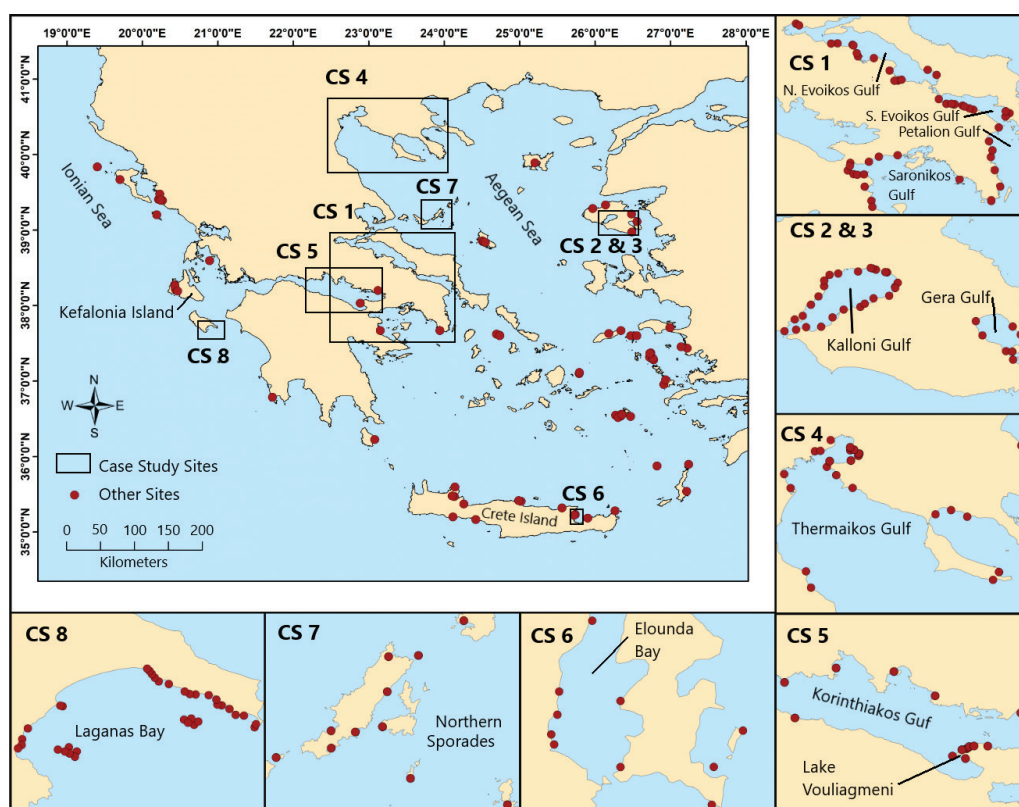


Fig. 1: Map of Greece depicting the sampling sites selected for the assessment of the status of *Pinna nobilis* populations. Inset maps indicate the sampling sites used during the dedicated case studies (CS 1-8), whereas the main map indicates other scattered sampling sites.

and 3 sites in May-June 2020), at a depth range between 0.5 and 3 m.

The June 2019 assessment indicated a low mortality population in the northern, inner part of Kalloni. To estimate the abundance of the presumably unaffected (or less affected) population in the inner part of Kalloni Gulf, 10 sampling sites were chosen in a systematically random way along the coastline (i.e. one site was randomly chosen, and the others were sequentially positioned at a constant distance of 1 km) (Fig. 2). At each site, a 200-m transect, perpendicular to the coastline, was set and surveyed following the line transect distance sampling method (Buckland *et al.*, 2001) using SCUBA diving. Distance sampling has been widely used for the estimation of *P. nobilis* abundance in the Aegean Sea (Katsanevakis, 2006; Katsanevakis & Thessalou-Legaki, 2009; Tsatiris *et al.*, 2018). This method properly accounts for imperfect detection (Buckland *et al.* 2001), given that fan mussel detectability can be very low, especially for small individuals (Katsanevakis & Thessalou-Legaki, 2009), and if ignored can lead to substantially biased estimates. For each detected fan mussel, one observer recorded its size (shell width in cm) and perpendicular distance, y (cm), from the line transect. A hazard-rate function, $g(y) = 1 - \exp\left[-\left(\frac{y}{\sigma}\right)^{-b}\right]$, was used to model detectability (Buckland *et al.*, 2001). The total area sampled (Fig. 2) was 1.7 km², estimated using ArcMap 10.1.

Case study 3: Gera Gulf – Lesvos Island

Gera Gulf is a semi-enclosed mesotrophic embayment in the south-eastern part of Lesvos Island (Fig. 1 CS 2&3) with a total surface of 40.099 km² (Tsatiris *et al.*, 2018). Close to the coastline there is a narrow zone (with a total area of 0.10 km²) of mixed bottoms, while further offshore there are extensive areas of sandy (13.7 km²) and muddy (24.8 km²) sediments, the latter covering the central part of the gulf. Scattered *P. oceanica* meadows (1.21 km²) can be found along the coastline of the Gulf, with the largest detected meadow located in the south-western part (Tsatiris *et al.*, 2018). Gera Gulf is included in the Natura 2000 European network of protected areas (Site Code: GR4110005). According to the assessment carried out by Tsatiris *et al.* (2018) in 2016 (i.e. two years before the beginning of the MME in the area), the estimated total fan mussel population was ~213,000 individuals (95% CI: 97,600-466,000), with a population density peak at a depth range of 1.5-8 m (practically zero at depths >15 m), and the highest densities in *P. oceanica* meadows, followed by mixed bottoms. Dedicated follow-up monitoring surveys at three sites of high population density during August-October 2018, provided mortality estimates of between 91% and 100%, while the total collapse of the Gera fan mussel population was confirmed in December 2018 when no live individual was recorded (Katsanevakis *et al.*, 2019).

During July-August 2019, 7 sites in Gera Gulf (Fig. 1 CS 2&3) were investigated through 45-min standardized free-diving surveys, by applying the same protocol

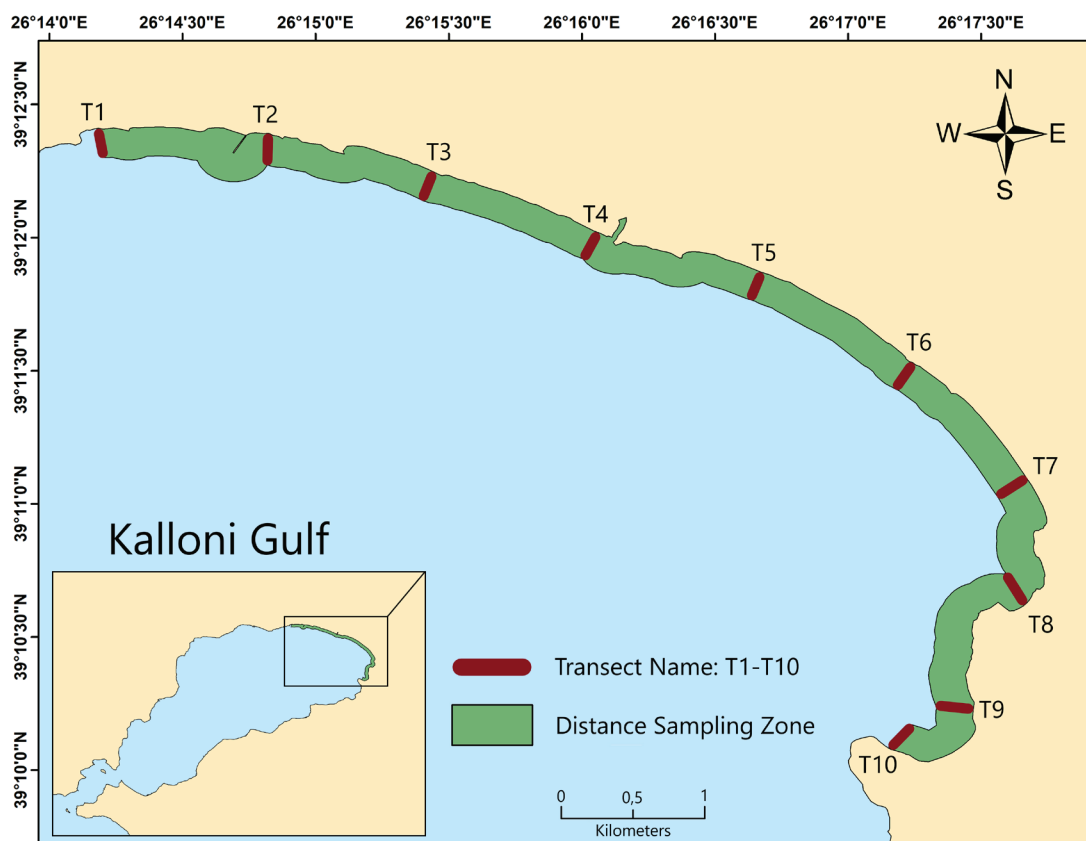


Fig. 2: Map of the inner part of Kalloni Gulf (Lesvos Island) showing the ten transects positioned perpendicular to the coastline used to estimate the abundance of live fan mussels.

as the one described in Case Study 1. The depths in the surveyed sites varied between 0.5 and 5 m. An additional site in Gera Gulf was also visited in June 2020, at a depth range of between 0.5 and 4 m, using the same protocol.

Case study 4: Thermaikos Gulf

Thermaikos Gulf is located at the north-western Aegean Sea, and its inner part is considered a heavily polluted area due to urban, industrial, and agricultural wastes (Arditsoglou & Voutsas, 2012). It receives the outflow of five rivers (Aliakmon, Axios, Loudias, Gallikos, Pineios), with part of the extensive wetland and estuarine system of the former four designated as a Natura 2000 site (Site Code: GR1220010). It is characterized by high nutrient content, particularly favouring a high biodiversity of filter feeders, such as bivalves. Among the various bivalve species of the Gulf, a large *P. nobilis* population had been reported in the past (Katsanevakis *et al.*, 2008), with densities locally exceeding one individual per m² (Galinou-Mitsoudi *et al.*, 2006).

Fan mussel population mortality in Thermaikos Gulf was assessed by two independent research groups. The first group focused on the inner part of the Gulf, while the second one on the outer part. In the inner Thermaikos, a total of 8 sites were investigated (Fig. 1 CS 4). Initially, 2 sites were sampled during May 2019. However, following the reports of local fishermen relating to unusual mortalities of the species, these 2 sites were revisited and assessed during July 2019, along with 6 additional sites. The depth range surveyed was between 3 and 9 meters, and both live and dead individuals were recorded. The dedicated survey in the outer Thermaikos Gulf, conducted between July and October 2019, included 13 sampling sites that were investigated following the methodology described in Case Study 1 (Fig. 1 CS 4). The depth range investigated was between 0 and 18 m, on soft and mixed substrates, covered with *Cymodocea nodosa*, *Zostera noltei*, *Cystoseira* spp. or *P. oceanica*. Furthermore, opportunistic records obtained from different research groups that investigated the area during different periods were also included. A total of 8 additional sites were visited in March and June 2020, at a depth range between 0 and 10 m.

Case study 5: Korinthiakos Gulf

Korinthiakos Gulf is a narrow embayment providing a link between the Aegean and Ionian seas through the Corinth Canal and the Rion-Antirion Strait, respectively (Perissoratis *et al.*, 2000). It is 115 km long and its width ranges from 5 to 30 km, with a maximum depth of more than 900 m in the centre of the basin (Lykousis *et al.*, 2007; Papageorgiou *et al.*, 2017). This semi-enclosed basin is of significant geologic interest due to its peculiar morphological characteristics that resemble a small-scale ocean basin (with shelf, slope and deep basin) (Perissoratis *et al.*, 2000) and is included in the Natura

2000 European network of protected areas (Site Code: GR2530007). On the northern side, the substrate is mainly rocky, in contrast to the sandy and muddy southern coasts (Papageorgiou *et al.*, 2017), with mud deposits being dominant at the deeper parts of the basin (Poulos *et al.*, 1996). Korinthiakos Gulf supports a rich biodiversity of marine flora and fauna (Issaris *et al.*, 2012), with significant populations of marine mammals (Papageorgiou *et al.*, 2017; Frantzis & Herzing, 2002).

In Korinthiakos Gulf, a total of 12 sites were investigated, between May and August 2019, at a depth range of 0 to 30 m (Fig. 1 CS 5). Visual surveys lasting 30 minutes were carried out either with SCUBA or free diving, on hard or mixed substrates. Both live and dead individuals were counted. Juvenile individuals were considered separately. Additionally, opportunistic records from two other research groups were obtained from 5 sites that were surveyed in October 2019, at a depth range of 1 to 35 m (Fig. 1 CS 5).

Case study 6: Elounda – Crete

Elounda Bay is a semi-enclosed embayment located on the NE coast of the island of Crete (Fig. 1 CS 6). It covers a surface area of 6.5 km², and at its northern part is connected to the open sea via an elongated canal, 1400 m long and 700 m wide. At its southern side, the bay is also connected to the open sea through an artificially created narrow strait, 10 m wide and 140 m long. The western part of Elounda Bay is generally characterized by a sandy coast, while the eastern part is characterized by a rocky coastline. The bay's depth reaches a maximum of 8.8 m and the substrate consists of fine sand and silt, with rare small rocky reefs. The vegetation consists mainly of the seagrass *C. nodosa* at shallow depths and the green macroalga *Caulerpa prolifera* at depths greater than 5 m. *P. nobilis* has been one of the dominant sessile invertebrates found in Elounda Bay, while the area is also considered an important habitat for the growth of juvenile fish species, contributing to the conservation and maintenance of the local marine biological resources (Koulouri *et al.*, 2016).

The 10 sampling sites for the assessment of *P. nobilis* population covered a geographically representative area in the bay as well as deeper locations outside the bay (Fig. 1 CS 6). The surveys were conducted in June, August and October 2019. Sampling was performed with SCUBA diving, at a depth range of 1 to 7 m within the bay, and 4 to 20 m outside the bay, according to the protocol described in Case Study 1.

Case study 7: National Marine Park of Alonissos - Northern Sporades - N.M.P.A.N.S.

Established in 1992, the National Marine Park of Alonissos and Northern Sporades (NMPANS), is Greece's first marine park and one of Mediterranean's largest marine protected areas covering an area of approximately

2,200 km² (Natura 2000 Site Code: GR1430004). The shallow coastal seascape is predominantly covered by rocky reefs and seagrass meadows. The NMPANS is important for marine mammals, such as cetaceans and the Mediterranean Monk Seal.

Between June and October 2019, sampling was carried out at 11 sites (Fig. 1 CS 7). At each site, free roaming SCUBA diving surveys were performed for 45 minutes at depths ranging from 2 to 45 m. Furthermore, an additional site was surveyed as an opportunistic record by another research group, in April 2019, at a depth range of 26 to 30 m.

Case study 8: Laganas Bay – NMPZ – Zakynthos Island

This case study was conducted in the National Marine Park of Zakynthos (NMPZ), which is located in the southernmost part of Zakynthos Island, eastern Ionian Sea (Fig. 1 CS 8), and hosts one of the most important rookeries of the threatened marine turtle *Caretta caretta* in the Mediterranean (Margaritoulis, 2005). Acknowledging the prime ecological importance of this area, the NMPZ was formally established in 1999 (Presidential Decree 906D/1999) and covers an area of 83.3 km² in Laganas Bay.

In August 2019, 35 sampling sites were selected in a systematic way along the rocky part of the NMPZ coastline, to estimate the population of *P. nobilis* along the shallow habitats of the NMPZ (with consecutive sites having a 200 m distance between them) (Fig. 1 CS 8). At each sampling site, a 200-m transect was set parallel to the coastline using a diving reel. Fan mussel individuals were recorded (number of live and dead individuals, number of juveniles) along each transect by snorkelling, during 20-min long surveys at depths between 0 and 10 m.

Data from other areas

Apart from case studies focusing on *P. nobilis* MME, additional opportunistic records of fan mussel populations were also included herein, in order to cover as much of the Greek coastline as possible, and to depict the status of the species in different marine areas (Fig. 1).

Other data originates from different sites located along the coasts of mainland Greece, 28 Aegean Sea islands, 6 Ionian Sea islands, and the island of Crete (Supplementary file). Among these opportunistic records of *P. nobilis* populations, Souda Bay in Crete and Lake Vouliagmeni have been assessed in the past, since both locations used to host large *P. nobilis* populations (Katsanevakis & Thessalou-Legaki, 2009; Katsanevakis, 2009).

Results

General description of the dataset

Overall, 258 visual surveys were carried out between February 2019 and June 2020, by 9 research groups. In total, 14,589 (dead or live) *P. nobilis* individuals were recorded at a depth range of 0-55 m. Among the recorded individuals, 18.9% were alive and 81.1% dead. Of the 2,762 live individuals, 9.3% were juveniles, most of which were found in Kalloni Gulf, indicating successful recruitment in specific areas despite the ongoing MME.

Case study 1: Saronikos Gulf, Evoikos Gulfs, Petalion Gulf, Maliakos Gulf

From the data collected in March 2019, a total of 1,949 individuals were recorded, 101 of which were found alive, resulting in a mortality rate estimate of 94.8%. However, these records refer to two marine areas; Petalion Gulf where no live individuals were found, thus the recorded mortality rate was 100%, and South Evoikos Gulf where out of 133 individuals recorded, only 32 were found dead, resulting in a mortality rate estimate of 24.1% (Fig. 3a).

During the dedicated Case Study carried out in September 2019, 895 *P. nobilis* individuals were recorded, 670 of which were found dead, resulting in a total mortality rate estimate of 74.9% in the study area. By September 2019, *P. nobilis* populations had totally collapsed in Saronikos Gulf, Petalion Gulf, and S. Evoikos Gulf, where no *P. nobilis* individuals were found alive (Fig. 3b). Live individuals were only found along the coasts of N. Evoikos Gulf, where the population appeared to be in relatively good state, with an estimated mortality rate of 31.4%. Among the 225 live individuals, 2.7% were noted as moribund and 6.7% were juveniles. Dead juveniles were also present in most marine areas visited.

In many locations around Saronikos Gulf, where no individuals of *P. nobilis* were recorded, old and broken shells of individuals presumed to be dead for a long time, indicated the past presence of the species in the area. Similarly, at the sampling sites of Petalion Gulf, numerous ‘old’ shells were noted but not included in the assessment, as they did not match the assessment criteria of the current survey. Overall, 73.3% of all dead individuals originated from the S. Evoikos Gulf where not a single individual was found alive at the two locations visited that hosted dense populations of large fan mussels. The N. Evoikos Gulf was the only Case Study 1 area with recorded live fan mussels. However, the mortality rates at different locations in the Gulf varied. In September 2019, most of the sites located along the western coastline of the N. Evoikos Gulf sustained surviving individuals, and populations displayed a relatively low mortality rate (22.5%).

However, the situation deteriorated in 2020. Two hundred and eighty four (284) dead individuals were reported from the Evoikos and Maliakos gulfs and not a single live

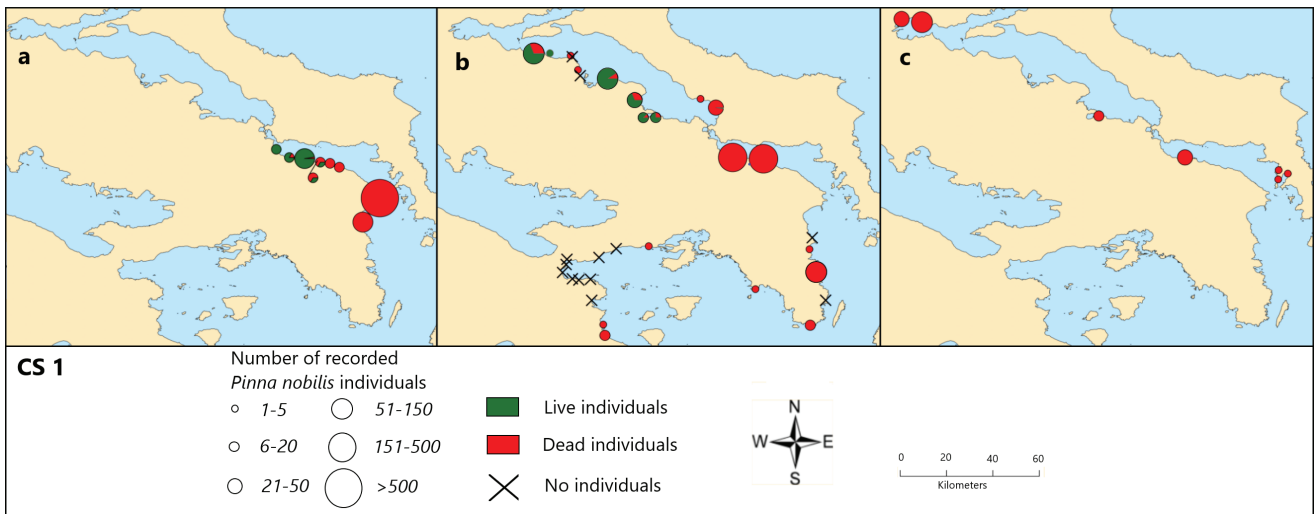


Fig. 3: Mortality assessment of *Pinna nobilis* populations at the sites of Case Study 1 (Saronikos Gulf, Evoikos Gulfs, Petalion Gulf, Maliakos Gulf), in March 2019 (a), September 2019 (b), and March-June 2020 (c). The size of the pie charts represents the number of recorded individuals. The red and green colour in the pie charts show the proportion of dead and live individuals, respectively.

fan mussel (mortality rate: 100%). This highlighted the collapse of the last surviving populations in the area (Fig. 3c).

Case study 2: Kalloni Gulf

During the dedicated visual surveys conducted in Kalloni Gulf (June 2019), a total of 2,675 *P. nobilis* individuals were recorded, of which 1,021 live and 1,654 freshly dead, resulting in an average mortality of 61.8%. Based on the spatial pattern of mortalities, the area of Kalloni Gulf can be divided into three distinct subareas (Fig. 4a), namely, i) the southern seaward part of the Gulf where no fan mussel was recorded, ii) the central part of the Gulf, where the assessment revealed a higher than 70% mortality, and an increased number of old dead shells that were severely damaged or stacked in the upper swash zone, and iii) the north-eastern inner part of the Gulf (Fig. 4a), where mortality rates were relatively low

ranging between 14.1% and 23.4%, apart from one site where dead individuals reached 50.8%. Throughout the Gulf, 94.4% of the total recorded individuals were adults, of which 1592 (63%) were dead. Juveniles (149 individuals or 5.6% of the total records), were present at only half of the assessment sites, and displayed a mortality rate of 41.6%.

Distance sampling was applied in June 2019 in the north-eastern part of Kalloni Gulf, where a total of 815 live individuals were recorded along 10 line transects (Fig. 2). The shell width of recorded individuals ranged between 4 and 18 cm, with a mean value (\pm SD) of 11.2 ± 2.3 cm. Mean visibility across all transects was estimated at 2.1 ± 1.5 m. The probability of *P. nobilis* detection was estimated at 0.19 ± 0.08 for 5-m wide transects. The total abundance of the species in the surveyed zone was estimated at 684,000 individuals (95% CI: 322,000-1,453,000).

Subsequently, four sites in the inner part of the Gulf

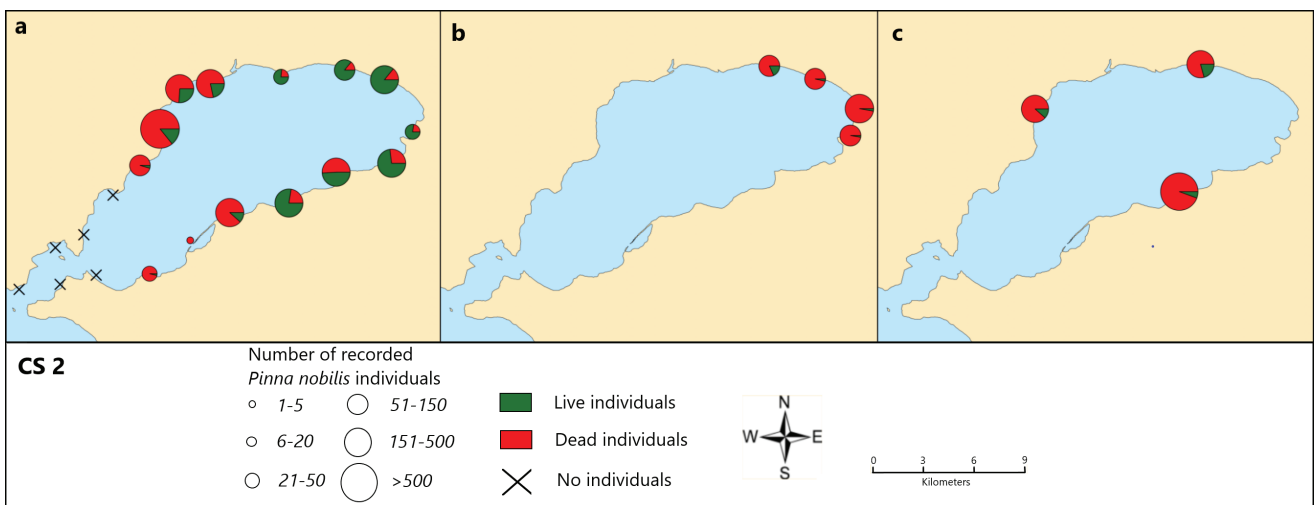


Fig. 4: Mortality assessment of *Pinna nobilis* populations at the sites of Case Study 2 (Kalloni Gulf), in June 2019 (a), August-September 2019 (b), and May-June 2020 (c). The size of the pie charts represents the number of recorded individuals. The red and green colour in the pie charts show the proportion of dead and live individuals, respectively.

were revisited in August-September 2019. A total of 526 individuals were recorded, of which only 33 were alive; the estimated mortality rate was 93.7% (Fig. 4b). Finally, three sites were surveyed during late May-June 2020. A total of 1,233 individuals were recorded, of which only 133 were found alive (estimated mortality rate: 89.2%) (Fig. 4c); 30.8% of the surviving individuals were juveniles.

Case study 3: Gera Gulf

In 2019, 469 dead individuals and only one live were recorded in Gera Gulf (Fig. 5 CS 3). The live individual was a juvenile found at 0.8 m on mixed bottom. Hence, the estimated average mortality rate in Gera Gulf was 99.8%. In June 2020, no live individual was recorded during a revisit to one of the sites.

Case study 4: Thermaikos Gulf

During the first investigation in the inner Thermaikos Gulf (May 2019), two sites were visited and a total of 150 live individuals were counted (0% mortality), of which

23.3% were juveniles. Two months later (July 2019), the same two sites were revisited, along with six additional sites. A total of 1,092 individuals were recorded, of which 227 were dead (mortality rate estimate: 20.1%) (Fig. 6a). Among the 865 live individuals, 42 were juveniles (4.9%). However, opportunistic records obtained from visual surveys conducted in June 2020 in the inner Thermaikos Gulf indicated the collapse of the aforementioned surviving populations, since 254 individuals were counted, and all of them were found dead (Fig. 6c).

During the dedicated survey in the outer Thermaikos Gulf (late July-October 2019), a total of 2,221 *P. nobilis* individuals were recorded, of which only 2 were alive (mortality rate estimate: 99.9%) (Fig. 6b). No *P. nobilis* individuals were detected at several locations around the Thermaikos Gulf. However, in some cases broken shells washed ashore indicated the past presence of the species in the area.

In 2020, five more sites were investigated in the outer Thermaikos Gulf and the Toronean Gulf (Chalkidiki peninsula). The survey revealed 543 dead and no live ones thus indicating 100% mortality in the area (Fig. 6c).

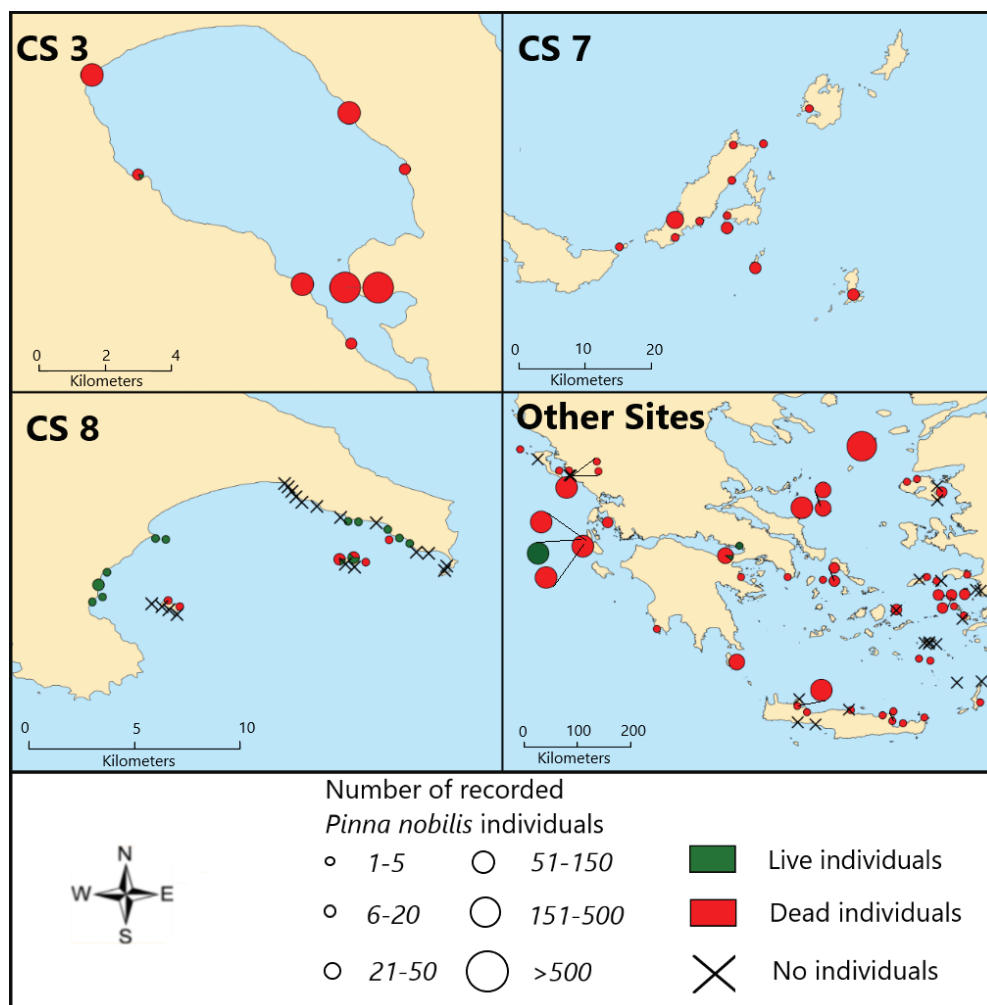


Fig. 5: Mortality assessment of *Pinna nobilis* populations at the sites of Case Studies 3 – Gera Gulf (July-August 2019, June 2020), 7 – NMPANS (June-October 2019), and 8 – Laganas Bay (August 2019), as well as other sites not included in a specific case study. The size of the pie charts represents the number of recorded individuals. The red and green colour in the pie charts show the proportion of dead and live individuals, respectively.

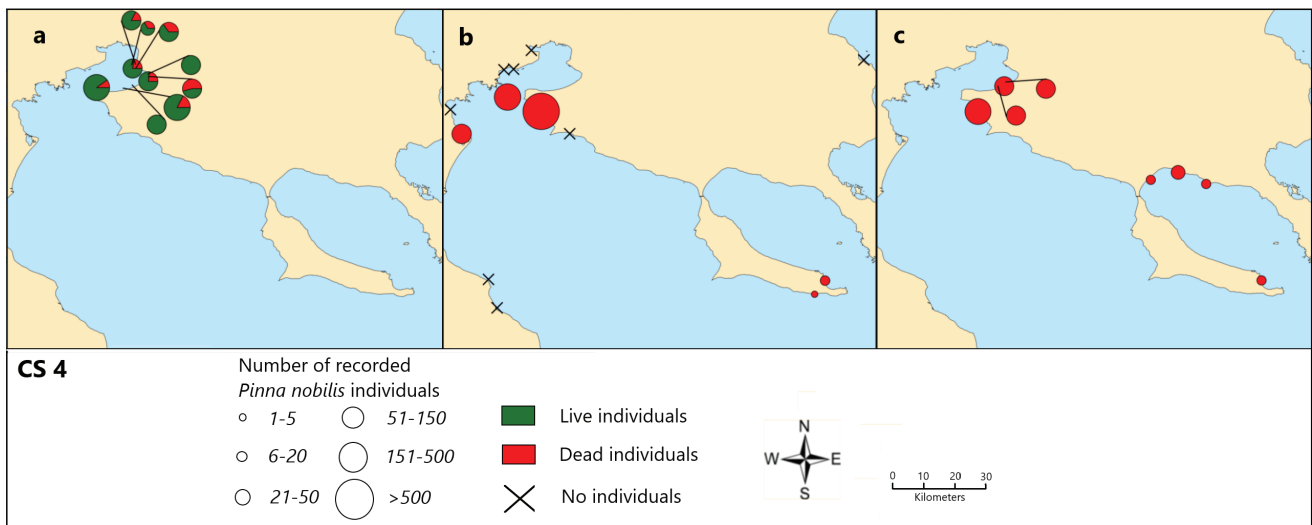


Fig. 6: Mortality assessment of *Pinna nobilis* populations at the sites of Case Study 4 (Thermaikos Gulf), in May-July 2019 (a), July-October 2019 (b), and June 2020 (c). The size of the pie charts represents the number of recorded individuals. The red and green colour in the pie charts show the proportion of dead and live individuals, respectively.

Case study 5: Korinthiakos Gulf

During May-August 2019, 155 dead and only 21 live individuals were recorded in the Korinthiakos Gulf, resulting in an overall mortality rate estimate of 88% (Fig. 7a). Of the remaining live individuals, 5 were juveniles. Resampling of certain sites in October 2019 indicated 100% mortality at the investigated sites, as all 50 recorded individuals were dead (Fig. 7b).

Case study 6: Elounda Bay

Overall, 498 *P. nobilis* individuals were counted at the four sites surveyed in Elounda Bay in June 2019, of which

385 (77.3%) were dead (Fig. 8a). At two sites, all individuals were dead (Fig. 8a), whereas at the other two sites, both live and dead individuals were recorded. Among the counted individuals, 13 were juveniles, of which 4 were dead. Moribund individuals ($n = 20$) were encountered among the live ones, indicating that the MME was in progress at the time of the survey.

In August and October 2019, six additional sites were surveyed both within Elounda Bay and in the marine area just outside the bay. During these revisits, all 125 recorded individuals were dead, indicating that between June and August/October all fan mussels were infected and died (Fig. 8b).

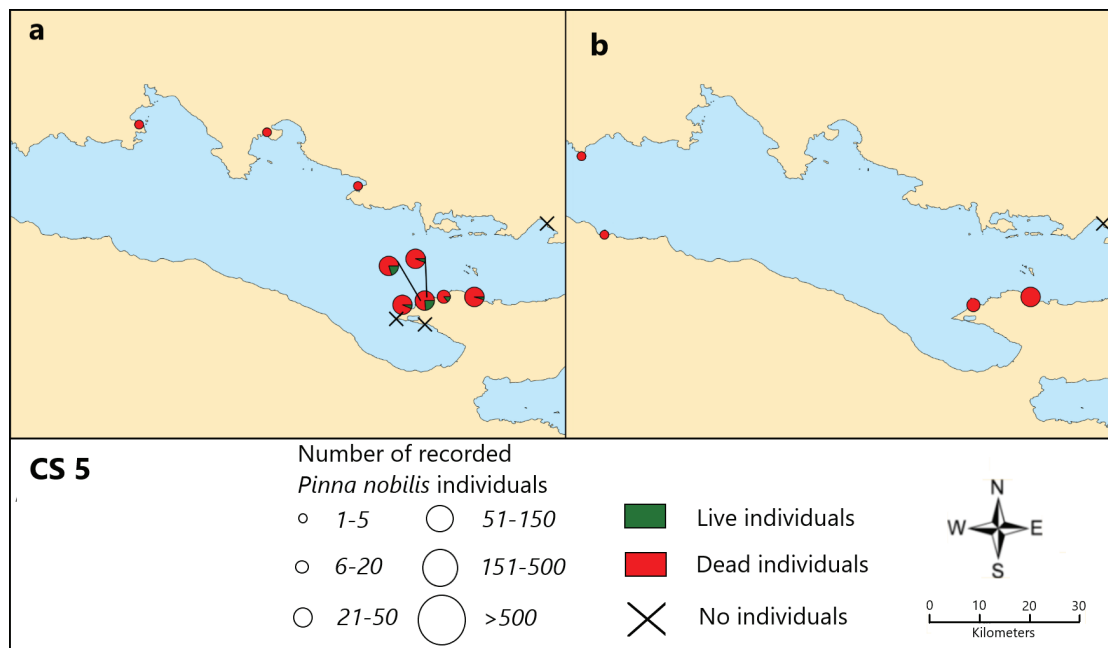


Fig. 7: Mortality assessment of *Pinna nobilis* populations at the sites of Case Study 5 (Korinthiakos Gulf), in May-August 2019 (a) and October 2019 (b). The size of the pie charts represents the number of recorded individuals. The red and green colour in the pie charts show the proportion of dead and live individuals, respectively.

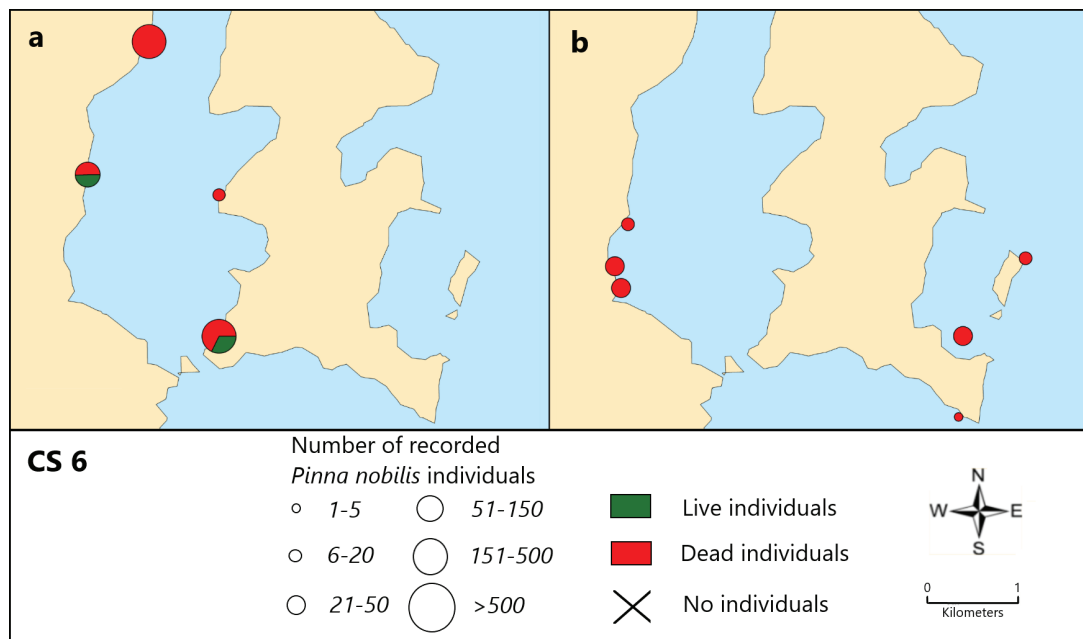


Fig. 8: Mortality assessment of *Pinna nobilis* populations at the sites of Case Study 6 (Elounda), in June 2019 (a) and October 2019 (b). The size of the pie charts represents the number of recorded individuals. The red and green colour in the pie charts show the proportion of dead and live individuals, respectively.

Case study 7: N.M.P.A.N.S.

Overall, 87 *P. nobilis* individuals were counted at the 11 sites surveyed in N.M.P.A.N.S. between June and October 2019, and all were found dead (100% mortality estimate) (Fig. 5 CS 7). More than half of the counted individuals were observed at Tsoukalia Bay (G8 in the Supplementary file), where 40 freshly dead individuals were found uprooted, lying on the seabed. No live individuals were recorded during a previous opportunistic survey conducted in April 2019 thus indicating that the collapse of the fan mussel population in the area, might have occurred before summer 2019.

Case study 8: Laganas Bay

During August 2019, a total of 55 *P. nobilis* individuals were recorded along transects of 7 km total length, of which 33 were alive and 22 dead (mortality rate estimate: 40%) (Fig. 5 CS 8). The maximum density was estimated at 11 individuals per 200 m transect length. The presence of dead individuals was restricted mainly to 6 sites. Healthy populations of *P. nobilis* with no dead individuals were recorded at 11 sites, whereas the presence of juveniles (27.3% of the live individuals) was evident at 4 sites.

Data from other sites

Overall, 1,134 *P. nobilis* individuals were recorded at 70 sites in several marine areas of the Greek seas (see Supplementary File), of which only 64 (5.6 %) were found alive. At most sites, no live specimens were found (Fig. 5 'Other Sites'). The Gulf of Argostoli (Kefalonia

Island, Ionian Sea) was surveyed by two research groups, during two different periods (July and November 2019). During the first visit (July 2019), all 56 recorded individuals were alive. Five months later (November 2019) only one live juvenile was found among 310 dead individuals, indicating that the specific population was severely affected during the summer of 2019. Similarly, only one surviving fan mussel was reported from Limnos Island (NE Aegean Sea), among a dense population of 299 dead individuals. In Kalamaki Viotias (Korinthiakos Gulf), two live individuals were recorded in April 2019; however, in July 2019 all specimens recorded in the area were dead. In the marine Lake Vouliagmeni (Korinthiakos Gulf), where *P. nobilis* populations had been thriving before the onset of the MME (Katsanevakis, 2006), the estimated mortality rate reached 89.7%, while the few live individuals recorded were considered moribund. In Souda Bay (NW Crete), only dead individuals were encountered in July 2019, indicating that a previously recorded dense population (Katsanevakis & Thessalou-Legaki, 2009) had been wiped off.

Integrated mapping of *P. nobilis* status in Greece

All data collected within the framework of this study were combined to depict the status of *P. nobilis* in the Greek seas by June 2020 (Fig. 9). Healthy or less affected populations are restricted to the inner Kalloni Gulf and Laganas Bay. Pooling together all records, most live individuals were recorded before June 2019, but mortality rates gradually increased, reaching 95% in 2020, at the surveyed sites (Fig. 10). Despite the inherent representativeness bias of our dataset (as different sites were surveyed during each time interval), this result indicates that the *P. nobilis* MME has been progressing rapidly during

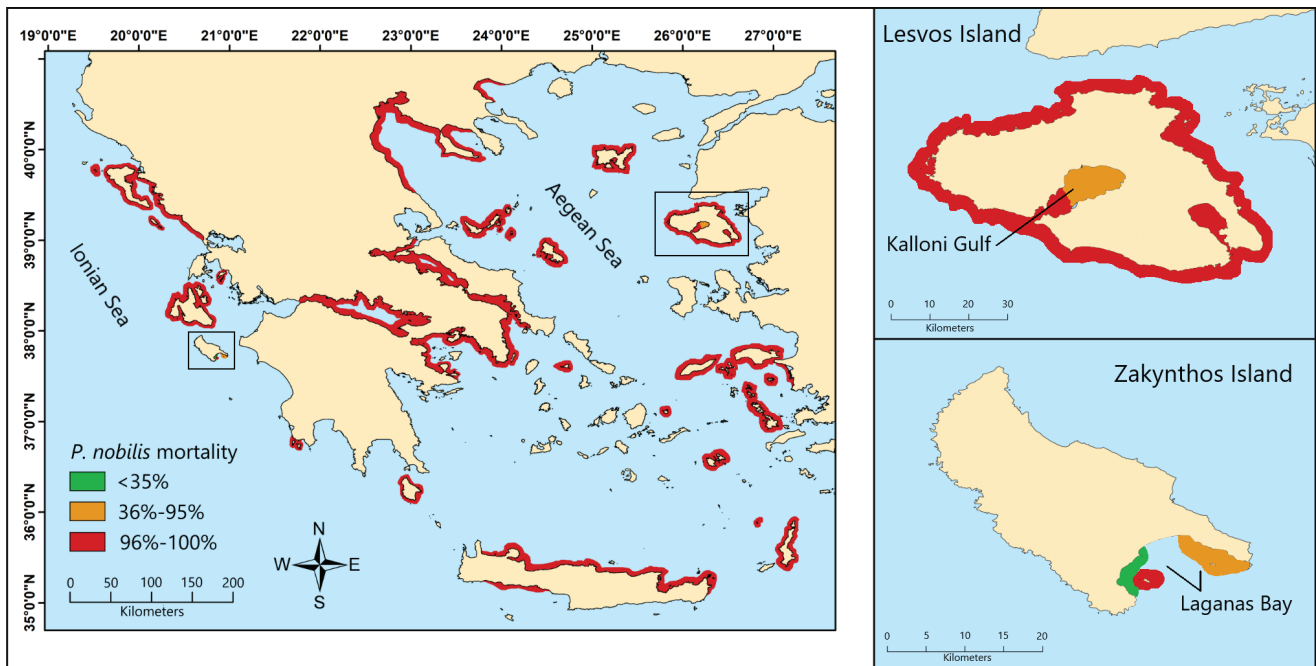


Fig. 9: Integrated map of *P. nobilis* status in Greece. Red coastlines represent the sites where fan mussel populations have collapsed. Inner Kalloni Gulf, and Laganas Bay (right panels) are the only known areas with surviving populations. Coastlines with no colour represent fan mussel populations that have not been assessed.

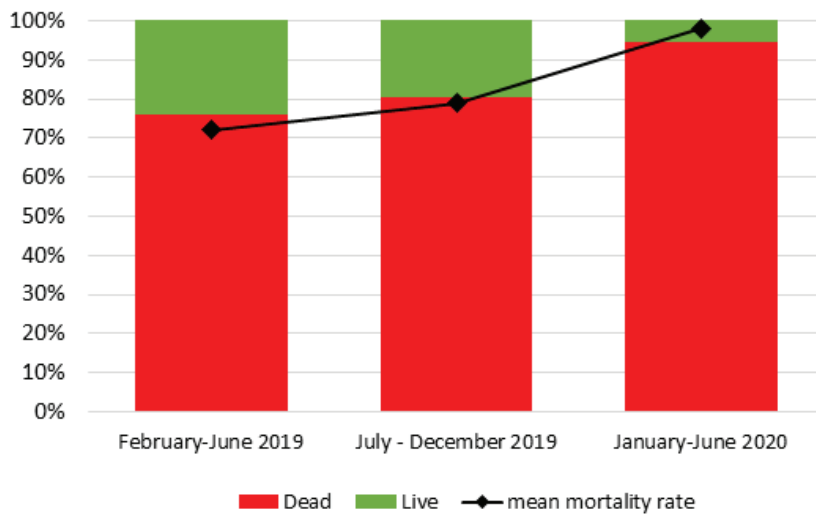


Fig. 10: Temporal progress of the MME in the Aegean Sea in 2019, compiling data obtained from all case studies and opportunistic records. The stacked bars were created by including all dead and live individuals recorded during each period, while the line refers to the average mortality rate among surveyed sites during each period (treating all sites equally in the estimation of the mean, ignoring differences in abundance).

2019 and 2020 and devastating many fan mussel populations in several coastal areas of the Greek seas (in some cases only within a couple of months).

Discussion

This study documents the large-scale progression of the *P. nobilis* MME throughout the Greek seas during 2019 and 2020 and the collapse of fan mussel populations in most of the marine areas investigated. Infection

by pathogens seems to have affected populations in both protected and unprotected areas, in the Aegean and Ionian seas, at all longitudes and latitudes. The spread of the disease in Greek waters, similarly to the ongoing spatial expansion of mortality events in other Mediterranean ecoregions (Čižmek *et al.*, 2020), indicates that the fan mussel MME that started in 2016 is still in progress, and no local population in the Mediterranean may be considered safe.

Few past assessments of *P. nobilis* populations in Greece allowed the estimation of local loss due to the on-

going MME. In Vouliagmeni Lake (Korinthiakos Gulf) the fan mussel population was monitored between 2004 and 2007, and its abundance varied between ~4,200 and 8,300 individuals (Katsanevakis, 2009). During May 2019, the survey of a single site in Vouliagmeni Lake indicated a mortality rate of more than 89%, whereas live individuals were clearly affected. This translates into at least 3,700-7,400 dead individuals (assuming the abundance of the species in 2019 remained within the 2004-2007 range) by May 2019. Similarly, in Souda Bay (NW Crete) in 2007, the estimated total abundance of the species was approximately 130,900 individuals (with a 95% CI of 100,600-170,400) (Katsanevakis & Thessalou-Legaki, 2009). According to our results, this dense population has greatly been affected, since at the sites surveyed during June and July 2019 only dead individuals were encountered. A local professional diver (Nikos Giannoulakis interviewed by D. Poursanidis) also reported full mortality of the species up to 60 m depth. Although further surveys would be needed to confirm that all fan mussels in the entire gulf are dead, it is highly likely that the entire population of ~130,900 individuals is lost. Finally, a study conducted in Gera Gulf (Lesvos Island) in 2016, revealed the greatest (until then) fan mussel population recorded in Greece, with an estimated abundance of 213,300 (CI: 97,600-466,000) individuals (Tsatis et al., 2018). Visual surveys conducted during August-October and December 2018 provided mortality rate estimates greater than 90% and 100% respectively, indicating the collapse of the previously thriving population (Katsanevakis et al., 2019). Revisiting the area during the summers of 2019 and 2020 (Case Study 3) confirmed the great loss of this population, since the estimated mortality of fan mussels in the gulf reached 99.8%, with only a single live individual recorded. Hence, in these three areas alone, it is estimated that ~350,000 *P. nobilis* individuals were lost due to the MME. Obviously, this is only a small fraction of the total number of lost individuals in the Greek seas.

It is promising for the survival of the species that surviving populations were found in two areas (Kalloni Gulf, Laganas Bay). Laganas Bay is a protected area, and although not dense, the fan mussel population there appeared to maintain a relatively healthy state in 2019, also benefiting from management measures that are in place, such as prohibition of anchoring and trawling, regulation of maritime traffic, and seasonal fishing closures.

The fan mussel population in Kalloni Gulf should be considered as the largest currently living population in the eastern Mediterranean. The abundance of live individuals estimated by the present study (~684,000 individuals) is the highest ever recorded in the eastern Mediterranean. Moreover, as this survey (Case Study 2) covered only part of the Gulf, it is quite likely that the number of live individuals in the Gulf exceeded one million by June 2019. Despite the high mortality rates estimated after June 2019 from opportunistic records (August-September 2019: 93.7%, May-June 2020: 89.2%), translated into hundreds of thousands of dead individuals, juvenile *P. nobilis* were also present in the studied area (30.8% of the live individuals recorded in May-June 2020), indicat-

ing successful reproduction in 2019.

In North Evoikos Gulf, consistently strong currents prevailing in the Evripus Strait presumably delayed the arrival of the pathogen from the South Evoikos Gulf, and as a result, the populations surveyed in September 2019 appeared to be unaffected. However, 2020 records have confirmed the expansion of the MME, and consequently the collapse of these populations.

The two areas with important live fan mussel populations in 2019-2020 (Kalloni Gulf and Laganas Bay) should be regularly monitored, also adopting targeted research activities aiming to shed light on the role of environmental conditions for the resilience of the species. Proper management aimed at minimizing accidental mortality due to human interactions (e.g. anchoring and fishing activities) or poaching should be pursued, as local anthropogenic stressors can further threaten the viability of the surviving populations. Experience gained since 2016 from the conservation of the remaining fan mussel populations (García-March et al., 2020), will allow effective management of the remaining Greek populations. These populations may potentially act as sources of recolonization for other areas where fan mussels are now locally extinct. For example, the remaining population in Kalloni Gulf could be the source of the identified single juvenile in the neighbouring Gera Gulf where the population was entirely lost in 2018. Recolonization of Gera Gulf and other coastal areas around Lesvos Island will benefit from safeguarding the offspring production of Kalloni Gulf. The fact that juveniles have been observed in Kalloni Gulf (and in other areas) indicates successful reproduction.

Areas with surviving populations are very important for the conservation of the species, and systematic regular monitoring is highly recommended. Field studies in different areas and depths might reveal more locations with live populations. Deep sites are under-sampled (the vast majority of surveys reported herein are at depths <30 m), and thus the status of *P. nobilis* populations in the deeper part of their depth range (i.e. 30-60 m) needs further investigation. The resistance of surviving populations might be due to local abiotic conditions (e.g. salinity or temperature) that either control or delay the possible expansion of the pathogen, resulting in natural refugia against the pathogen(s), or improving the host's health and immune system (Cabanellas-Reboredo et al. 2019). Assessment of genetic variation of surviving populations is therefore considered essential to track potential recolonization events of affected areas in the near future and support sound management decisions.

Even in heavily affected populations, a small number of surviving individuals, potentially resistant to the disease, were found. Such likely resistant strains may produce resistant offspring, but being isolated decreases the probability of successful reproduction, as reproductive success depends on the proximity of other individuals spawning synchronously. Hence, a proper strategy is needed to assist viability and increase the chances of reproduction of these resistant strains, e.g. by transplanting them in groups in well-protected areas (Katsanevakis,

2016). Nevertheless, it should be confirmed that these individuals did not survive due to reasons other than being resistant (e.g. due to local hydrodynamics, physicochemical conditions or just chance), otherwise transplantation might have a negative outcome.

Excluding the eight case studies, data from other areas used for this study consisted of reports and observations made by scientists, assessing the ongoing fan mussel MME or gathering this information as a side project. As a result, the geographical range of available data does not fully cover the entire Greek coastline and is not representative of the species bathymetric distribution. Anecdotal information provided by citizens (divers, fishermen etc.) has been published in social media, reporting mortalities at other locations (not included herein). The participation of citizen scientists through a dedicated structured online platform and a proper verification strategy may prove useful for filling the gap of knowledge and contribute valuable information, helping scientists to understand the spatiotemporal progress of the disease (Vázquez-Luis *et al.* 2017; Cabanellas-Reboredo *et al.*, 2019).

Apart from *Haplosporidium pinnae* (Vázquez-Luis *et al.*, 2017; Catanese *et al.*, 2018; Cabanellas-Reboredo *et al.*, 2019), and *Mycobacterium* sp. (Carella *et al.*, 2019, 2020), which have been identified as the primary suspects of this Mediterranean pandemic, the actual spread of the pathogenic organism/s may be reinforced by the wider deterioration of the coastal marine environment and the effects of climate change. For example, the increase in frequency and extent of mucilaginous events (Goffredo & Dubinsky, 2014) has been linked to sea warming, and these aggregates can act as carriers of microorganisms, facilitating the spread of pathogenic bacteria (Danovaro *et al.*, 2009). Increased temperature can facilitate pathogen outbreaks and intensify the pathogenicity of specific species, such as in the case of *Vibrio mediterranei* infecting *P. nobilis* (Prado *et al.*, 2020). The observed multitude of pathogens associated with the *P. nobilis* MMEs suggests that the immune status of the animals could be involved (Carella *et al.*, 2020), and it is likely that the spreading of the pathogens associated with the disease is favoured by a, yet unknown, common driver (Carella *et al.*, 2019). Whether the latter may be an increase in sea water temperature, linked *per se* to episodes of mass mortality of sessile organisms both at Mediterranean (Garrabou *et al.*, 2019) and at global scale (Smale *et al.*, 2019), or its positive impact on other causes/events, such as the formation of marine mucilage aggregates or the transformation of a previous symbiont into a pathogen due to changes in the inner conditions of the host (Darriba, 2017), it seems that potential cascading impacts of climate change may play an important role in driving biological changes, thus altering the status of vulnerable marine ecosystem components. More research is needed to understand the real cause of the *P. nobilis* MME, but time is limited as the fan mussel populations are collapsing and the species is on the brink of extinction.

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Supplementary data

The following supplementary information is available online for the article:

Detailed information on all 258 surveyed sites (date, location, coordinates, depth, predominant habitats, number of live and dead fan mussels).