LIFE PINNARCA

LIFE NAT/ES/001265

DELIVERABLE DA.1

PLANNING CORRECTION MEASURES: GREECE

FECHA 31/01/2022

University of the Aegean
School of Environment
Department of Marine Sciences
UAEGEAN

University of the Aegean

School of Environment

Department of Marine Sciences

Technical Report:

A.1 Planning correction measures: Greece

Project: LIFE Pinnarca NAT/ES/001265
Protection and restoration of *Pinna nobilis* populations as a response to the catastrophic pandemic started in 2016

Mytilene, January 2022

UNIVERSITY OF THE AEGEAN
SCHOOL OF THE ENVIRONMENT
DEPARTMENT OF MARINE SCIENCES

Life

NATURA 2000

To be cited as:

Papadakis Orestis, Papadimitriou Evangelos, Katsanevakis Stelios, 2022. Technical Report A.1 - Planning correction measures: Greece, LIFE Pinnarca NAT/ES/001265 'Protection and restoration of *Pinna nobilis* populations as a response to the catastrophic pandemic started in 2016', Mytilene, January 2022, 19 p.

Contact person:

Stelios Katsanevakis

Professor

E-mail: katsanevakis@aegean.gr

Tel.: +30 22510 36821

Contents

Background

1 HISTORIC AND BIBLIOGRAPHIC REVIEW OF RECENT STUDIES ABOUT THE STATUS OF THE SPECIES IN GREECE

Since the first confirmed records of mass mortality events (MME) of *Pinna nobilis* populations in the Aegean sea in 2018 (Katsanevakis *et al.,* 2019), several studies have been conducted which show the MME escalation in Greek seas (Zotou *et al*., 2020; Zotou *et al*., 2021). Fan mussel populations have been severely affected in Greek seas, and hence the search and protection of surviving healthy populations or individuals, is crucial to increase the chances of recovery. The main results and conclusions of the recent studies about the status of the species in Greece are herein presented:

1.1 Katsanevakis et al. (2019)

Katsanevakis *et al.* (2019) attempted to assess the status of fan mussel populations in a case study area in the Aegean Sea by means of underwater visual surveys, and investigated through histological and molecular analyses of sampled fan mussels whether the parasite *Haplosporidium pinnae* had expanded its range in the eastern Mediterranean, infecting *P. nobilis* populations by the end of 2018. The study was conducted in Lesvos Island, northern Aegean Sea. Two elongated, shallow and semi-enclosed bays characterize the shape of the island, the Kalloni and Gera gulfs (Figure 1) which are highly productive marine ecosystems with rich biodiversity, and for this reason they are included in the Natura 2000 European network of protected areas (area code for Kalloni Gulf: SCI GR4110004, and for Gera Gulf SCI GR4110013). Both gulfs have supported large *P. nobilis* populations before the MME.

A total of thirteen sites were investigated through underwater visual surveys along the coastal waters of Lesvos Island between August and October 2018 (Figure 1, Table 1), to verify anecdotal reports of extensive mortalities of *P. nobilis* populations. Five of these sites were located in Kalloni and Gera Gulfs at locations where *P. nobilis* used to have very high population densities (Tsatiris *et al.,* 2018; *unpublished data*, for Kalloni). The surveys which lasted 45 to 60 minutes involved the counting of live and dead individuals. Live individuals were further assessed as "presumed healthy" (apparently unaffected) and "presumed moribund" (Catanese *et al.,* 2018; Vazquez-Luis *et al.,* 2017). Only freshly dead individuals were counted and shells that were not anchored were not counted as "dead" (Table 1). The selected depth range of the surveys was between 1–10 m at soft bottom habitats, seagrass meadows, or a mixture of these habitats.

Table 1. Number (N) of dead and live (presumed healthy or moribund) *Pinna nobilis* individuals, of the thirteen sites investigated in Lesvos Island. Sites where *P. nobilis* individuals were collected for further analyses are marked with an asterisk (*).

Site	Date	N Dead	N Live - healthy	N Live - moribund
1	26-08-18	20	0	0
2	28-08-18	4	0	0
3	30-08-18	4	0	0
4*	01-09-18	152	11	4

5	02-09-18	162	1	0
6*	06-09-18	24	17	26
7	11-09-18	2	0	0
8	16-09-18	171	2	0
9	23-09-18	38	0	0
10	07-10-18	200	0	0
11	08-10-18	2	0	0
12	20-10-18	35	0	3
13	27-10-18	72	0	0

Two sites (sites 4 and 6 in Table 1) were surveyed again three months after the initial sampling (i.e. in December 2018); these were the sites in which specimens were collected for histopathology and molecular analyses. Samples for histopathology were collected from the following tissues of both presumed healthy and infected specimens from both collection sites: muscle, byssus gland, digestive gland, gills, mantle and gonads. Molecular tests for *Haplosporidium pinnae* detection were conducted with PCR amplification and sequencing of a part of small subunit ribosomal DNA (SSU rDNA) gene, which has been used by Catanese *et al.* (2018) for molecular characterization of this new parasite species.

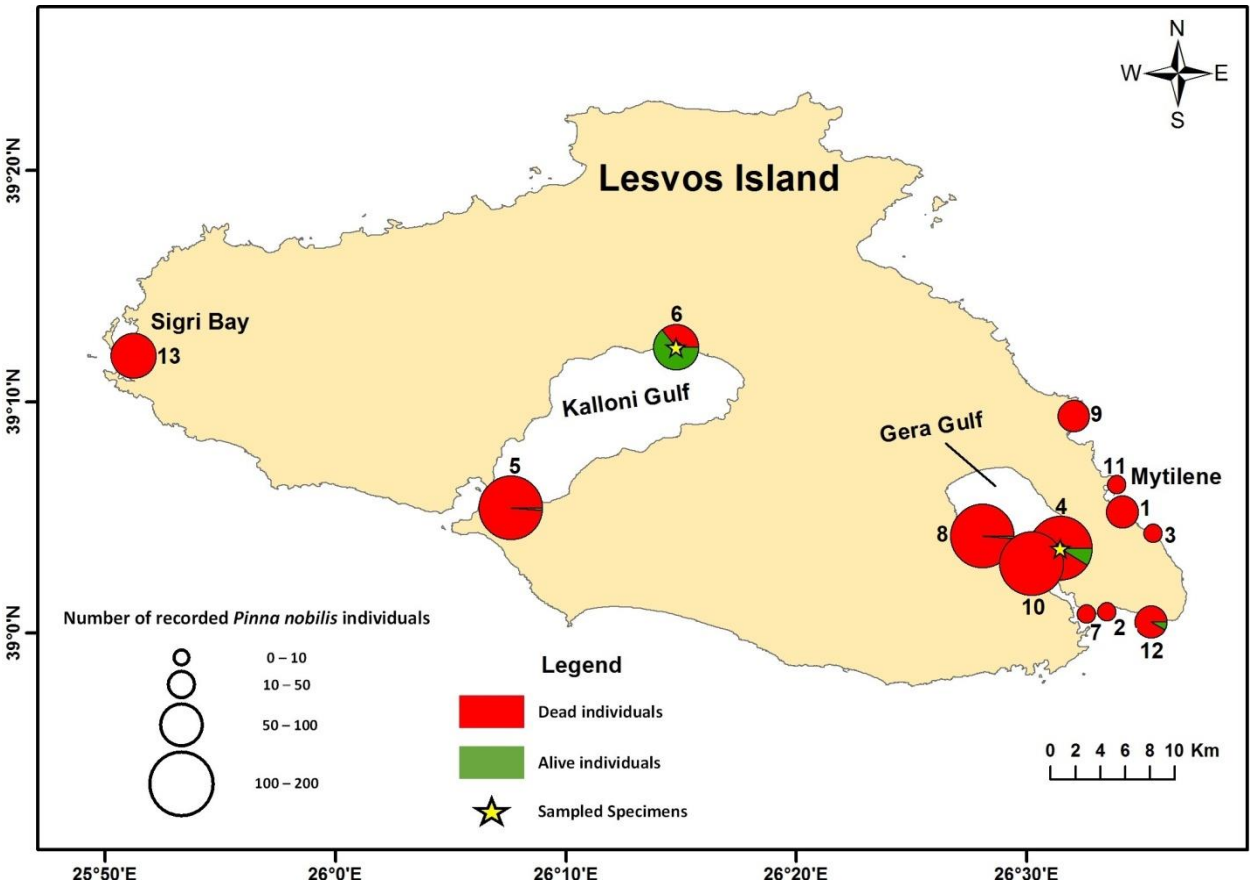

Figure 1. Mortality assessment of *Pinna nobilis* population, at thirteen sites at Lesvos Island (numbers 1-13). The size of the pie charts represents the number of recorded individuals of fan mussels. Red and green color of the pie charts, show the proportion of dead and live individuals, respectively. Yellow asterisks indicate sites where specimens were collected for histological and molecular analysis (from Katsanevakis *et al.,* 2019).

During the visual surveys in Lesvos Island, between August and October 2018, a total of 950 *P. nobilis* individuals were recorded, of which 886 were dead, and 64 alive (Figure 1; Table 1). These numbers

indicate an average mortality rate of 93% in the surveyed sites of Lesvos Island. The assessment revealed the collapse of the previously thriving population of Gera Gulf (estimated at ~ 215,000 individuals in 2016; Tsatiris *et al.,* 2018), with mortality rates ranging between 91% and 100%. The northern (inner) site of Kalloni Gulf (site 6; Table 1) was the only site where the ratio between live and dead *P. nobilis* individuals appeared to be not very far from normal, with a 36% mortality rate. However, at the southern part of Kalloni, a dense population thriving in shallow waters up to 1.5 m deep had a 99% mortality rate. At the remaining sites, which were situated outside the two semi-enclosed gulfs, 100% mortality was recorded in seven of them, while in one site three moribund specimens were found showing an extremely slow valve-closing reflex. In early December 2018, mortality rates in site 6 were estimated at 20% (94 live and 23 dead individuals were detected), substantially lower than three months earlier. A prominent number of juvenile individuals (ten individuals of age class 0+) was recorded among the live fan mussels, with only one recorded dead juvenile. At site 4 in Gera Gulf no live individuals were detected in the December survey (among 130 detected dead individuals), indicating that the MME was still in progress in early September when the samples were collected.

The histopathological study revealed the presence of a haplosporidan-like protozoon in different life cycle stages within the digestive gland of the two infected *Pinna nobilis* individuals from Gera Gulf, whereas no visual observation of parasites was evident in the samples from Kalloni Gulf. Moreover, few sporocysts were found in intramuscular connective tissue of an individual *Pinna nobilis* with a very heavy infection in its digestive gland. All presumed healthy *Pinna nobilis* specimens (controls) had a normal histological structure of their organs and tissues without any visual identification of protozoan or metazoan parasites. The molecular analysis of all three individuals from Gera Gulf gave positive PCR results which provided a direct evidence that the protozoon which had been observed in histological analyses was actually *H. pinnae.* In Kalloni Gulf, no PCR amplification was achieved in any of the tissue samples, neither from the healthy nor from the presumed moribund individuals.

1.2 Zotou et al. (2020)

In an effort to track the progress of the MME of the fan mussel in the Greek seas by 2020, Zotou et al. (2020) provided updated information on the status of their populations by compiling information collected through dedicated surveys conducted in 2019 and 2020, as well as other opportunistic records. Aimed to cover a large geographical scale, the dataset consisted of eight dedicated case studies conducted in different marine areas, focusing on *P. nobilis* MMEs, and opportunistic assessments from 70 additional sites carried out within the framework of various projects (Figure 2; Table 2). A number of independent visual surveys which lasted between 20-120 minutes were conducted between February 2019 and June 2020 in order to assess the mortality of *P. nobilis*. During the surveys live and recently dead individuals were counted, excluding individuals presumed to be dead for longer periods.

Overall, 258 visual surveys were carried out between February 2019 and June 2020, by 9 research groups. In total, 14,589 (dead or alive) *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. All data collected within the framework of the study conducted by Zotou *et al.,* (2020) were combined to depict the status of *P. nobilis* in the Greek seas (Figure 3). 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 (Figure 4). Despite the inherent representativeness bias of their dataset (as different sites were surveyed during each time interval), this result indicated that the *P. nobilis* MME had been progressing rapidly during 2019 and 2020, devastating many fan mussel

populations in several coastal areas of Greece. By June 2020, healthy or less affected populations were restricted only to the inner parts of Kalloni Gulf and Laganas Bay (Figure 3).

Laganas Bay (CS-8) is a marine protected area, and although not dense, the fan mussel population in 2019 appeared to maintain a relatively healthy state, possibly benefited by the management measures of National Marine Park of Zakynthos (N.M.P.Z.). In August 2019 the mortality rate was estimated at 40% and the maximum density was estimated at 11 individuals per 200 m transect length. The presence of dead individuals was restricted mainly to 6 sites whereas healthy populations of *P. nobilis* with no dead individuals were recorded at 11 sites. The presence of juveniles was estimated at 27.3% of the live individuals.

In Kalloni Gulf (CS-2), the visual surveys conducted in June 2019 resulted in an average mortality rate estimation of 61.8%. The total abundance (Distance sampling) of the species in the surveyed zone was estimated at 684,000 individuals and was the highest that had ever been recorded in the eastern Mediterranean. Moreover, as this survey covered only part of the Gulf, it was quite likely that the number of live individuals in the Gulf exceeded one million at the time. Despite the high mortality rates estimated from opportunistic records in August-September 2019 at 4 sites (93.7%) and May-June 2020 at 3 sites (89.2%) which translated into hundreds of thousands of dead individuals, juvenile *P. nobilis* were still

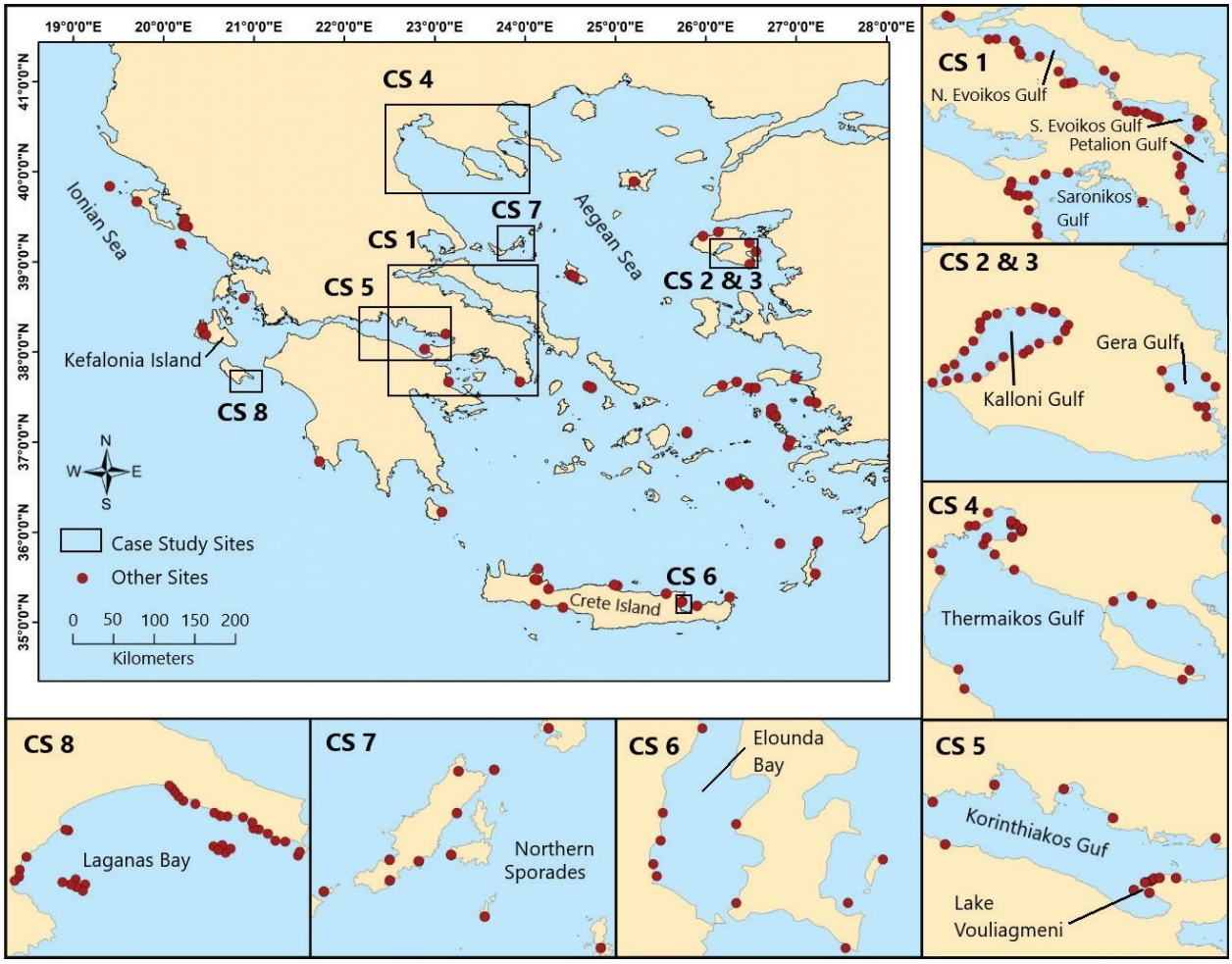

Figure 2. Map of Greece depicting the sampling sites selected for the assessment of the status of *Pinna nobilis* populations. Inset maps indicate the sampling sites used during the dedicated case studies (CS 1-8), whereas the main map indicates other scattered sampling sites (from Zotou *et al.,* 2020).

present (30.8% of the live individuals recorded in May-June 2020), indicating successful reproduction and recruitment in 2019.

Table 2. Research groups in each marine area and case studies (CS 1-8) followed by the sampling methodology. "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 (from Zotou *et al.,* 2020).

Marine area	Case Study	Sampling methodology	
		2019	2020
Saronikos Gulf	CS 1	45 min	-
Petalion Gulf		45 min; Transects	-
South Evoikos Gulf		45 min; Opportunistic	Opportunistic
North Evoikos Gulf		45 min	Opportunistic
Maliakos Gulf		-	Distance
Kalloni Gulf	CS 2	45 min; Distance	45 min
Gera Gulf	CS 3	45 min	45 min
Inner Thermaikos Gulf	CS 4	Opportunistic	Distance
Outer Thermaikos Gulf		45 min	Opportunistic
Toronean Gulf		-	Opportunistic
Korinthiakos Gulf	CS 5	30 min; Opportunistic	-
Eloounda Bay (Crete)	CS 6	45 min; Opportunistic	-
N.M.P.A.N.S.	CS 7	45 min; Opportunistic	-
N.M.P.Z.	CS 8	Transects	-
Other Areas		Opportunistic; 45 min	Opportunistic

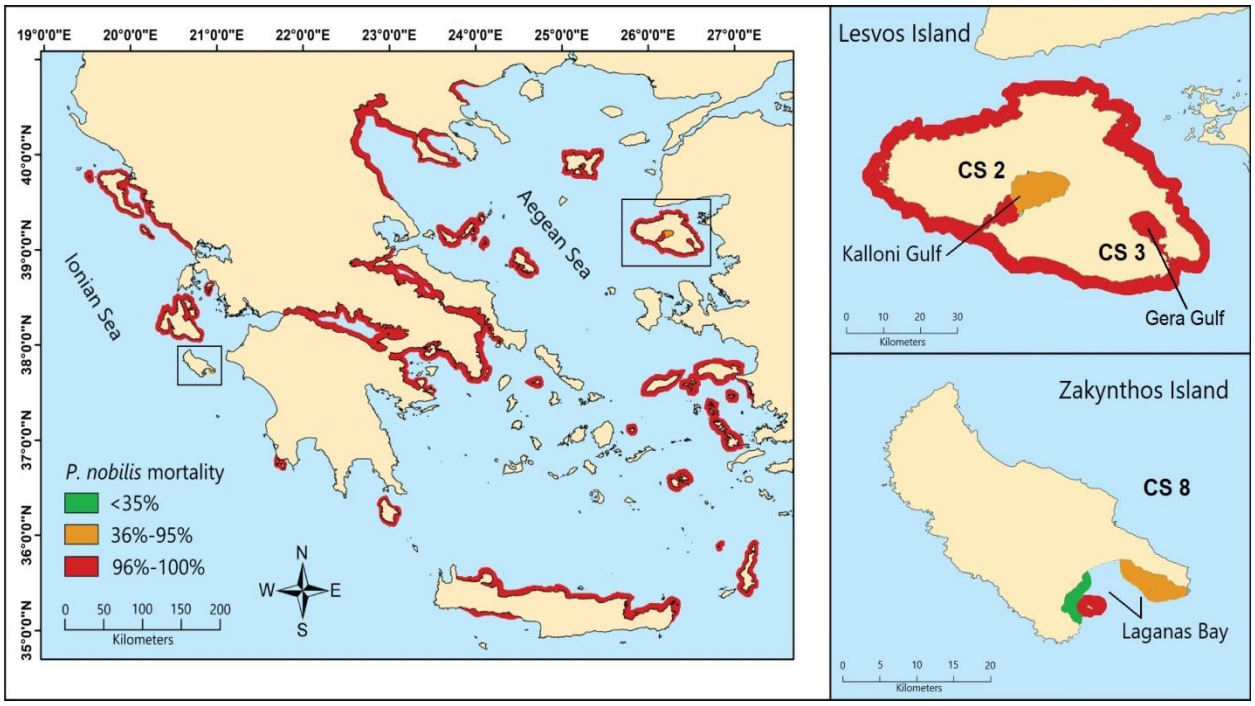

Figure 3. Integrated map of *P. nobilis* status in Greece by 2020 (Zotou *et al.,* 2020). Red coastlines represent the sites where fan mussel populations had collapsed. Inner Kalloni Gulf, and Laganas Bay (right panels) were the only known areas with surviving populations. Coastlines with no color represent fan mussel populations that had not been assessed.

In Gera Gulf (CS-3) which until 2016 held the greatest fan mussel population ever recorded in Greece (Tsatiris *et al*., 2018), in summer of 2019, only one single juvenile live individual was recorded (estimated

mortality rate 99.8%), while during a revisit in June 2020 no live individual was recorded. Hence, the average mortality rate estimation in Gera Gulf was 100%.

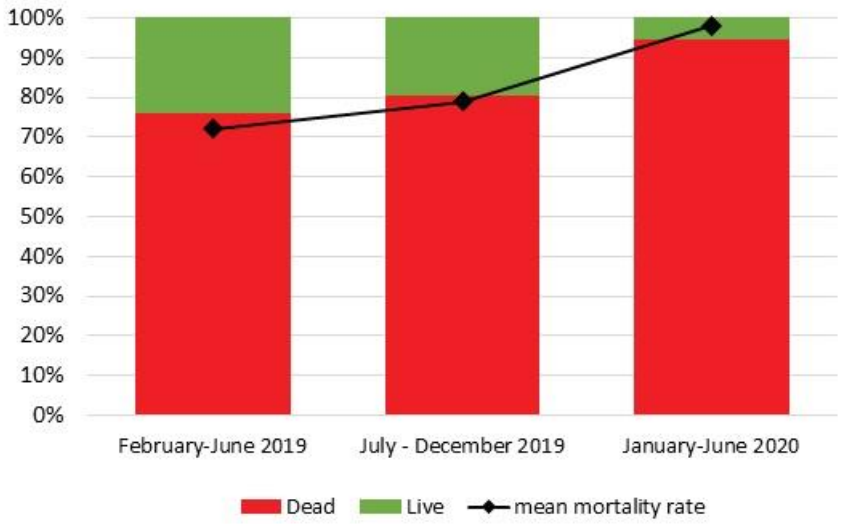

Figure 4. **Temporal progress of the MME in the Aegean Sea in 2019, compiling data obtained from all case studies and opportunistic records. The stacked bars were created by including all dead and live individuals recorded during each period, while the line refers to the average mortality rate among surveyed sites during each period (treating all sites equally in the estimation of the mean, ignoring differences in abundance) (Zotou *et al.,* 2020).**

The study of Zotou *et al*. (2020) documented 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 had affected populations in both protected and unprotected areas, in the Aegean and Ionian seas, at all longitudes and latitudes, while the only live remaining *P. nobilis* populations in Greek seas were recorded in Kalloni Gulf and Laganas Bay.

1.3 Zotou et al. (2021)

Zotou *et al.* (2021) presented the state of fan mussel populations as a compilation of independent targeted case studies and opportunistic records and observations, collected in the framework of other scientific projects, in various marine areas of Greece, by the end of 2021. The search for unaffected areas, any sign of recovery in impacted populations (such as recruitment or juvenile individuals), or the expansion of the disease in other locations are crucial information in order to estimate the extent of the MME. By the end of the study, the only known populations with surviving individuals were found in Kalloni Gulf (Aegean Sea) and Amvrakikos Gulf (Ionian Sea).

In the targeted case studies, a 45-min visual survey protocol was applied, according to which all *P. nobilis* individuals both alive and dead were counted in order to estimate the mortality rate in the area (Katsanevakis *et al*., 2019). Since the MME had affected Greek seas over the previous 3 years, all dead individuals were counted, without excluding specimens presumably dead for long periods. From the assessed locations of this study, some areas were visited for the first time after the MME, while some sites had been revisited. Thus, in Kalloni Gulf (Lesvos Island) 6 sites were surveyed between July and September of 2020 and 8 more sites were visited between May and July of 2021. Two sites were assessed in Gera Gulf (Lesvos Island) in the summer (June, July) of 2020 and another site was surveyed in June 2021. In June 2021, 21 sites in the National Marine Park of Alonnisos – Northern Sporades (N.M.P.A.N.S) were

visited in order to estimate the mortality rate of the fan mussel in the boundaries of the Marine Park. Around Peloponnese, 26 sites were assessed in August 2020 and another 10 locations were visited in July 2021. In Amvrakikos Gulf, 11 sites were visited in April 2021 and 9 more sites in July 2021. Through surveys for other research projects, opportunistic records for 19 sites were compiled between July and August 2020, and 24 more sites were assessed from April to July 2021 in various localities in Greece (Table 3). All data collected within the framework of this study were combined with those reported by Zotou *et al*., (2020) to depict the status of *P. nobilis* in the Greek seas by July 2021.

Table 3: Sampling methodology followed in each marine area for the years 2020-2021 and the estimated mortality rate. "45'protocol" refers to a 45-min standardized protocol of underwater visual observations, and "Opportunistic" refers to opportunistic records without applying a strict protocol (Zotou *et al*., 2021).

Marine Area	Year	# Sites	Mortality Rate	Sampling Method
Kalloni Gulf	2020	6	98.4%	45' Protocol
	2021	8	97.8%	45' Protocol
Gera Gulf	2020	2	100%	45' Protocol
	2021	1	100%	45' Protocol
N.M.P.A.N.S.	2021	21	100%	45' Protocol
Peloponnese	2020	26	100%	45' Protocol
	2021	10	100%	Opportunistic
Amvrakikos Gulf	2021	20	33%	45' Protocol
Other Areas	2020	19	100%	Opportunistic
	2021	24	99.9%	Opportunistic

In total 137 visual surveys were conducted between June 2020 - July 2021 from different research groups along the Greek coastline, at a depth range of 0.3 – 34 m. Overall 4971 *P. nobilis* individuals were recorded, among which 98.6% were dead and only 1.4% were alive, not including those found in Amvrakikos Gulf, where a large and healthy population (a few thousand individuals) with signs of successful recruitment of juveniles had been recently discovered by an ongoing research project that is focusing in the area (Y. Issaris, unpublished data). Preliminary data showed that the estimated mortality in Amvrakikos Gulf was low (33%) and the fan mussel population seemed to be largely unaffected by the MME. Of the 69 alive individuals found in the rest of Greece, 57.4% were juveniles and all of them were found in Kalloni Gulf (Figure 6), which indicated successful recruitment in specific locations, despite the ongoing MME at the time. Apart from Kalloni Gulf, only one additional live individual was counted in Thermaikos Gulf as an opportunistic record, among more than 100 dead individuals.

In Kalloni Gulf the estimated mortality rate during the 2020 survey was 98.4%, while during 2021 the mortality rate was 97.8%. The fan mussel population in Kalloni Gulf had been severely affected, however the few surviving individuals and the juveniles among them indicated some resistance against the pathogen compared to the rest of the Greek coastline (Figure 5). Surviving populations were found near the salt pans in the inner part of the gulf, indicating that increased salinity halted the disease. In Gera Gulf, N.M.P.A.N.S., and Peloponnese and all other surveyed areas there were no signs of recovery, since no live individual was found and the mortality rate was 100%. From the opportunistic records dataset, only 1 individual was found alive, among more than 766 dead fan mussels, which led to a total mortality rate estimation of 99.9%.

Additional anecdotal information supported that scattered individuals were surviving in various locations and depths in the Greek seas. One specimen was caught on a fisher's net in Thrakiko Pelagos in February 2021 (A. Christidis, pers. comm.) and another fan mussel was caught in Samos Island (A. Miliou,

pers. comm.). A citizen also reported a small surviving population in the NW coasts of Skyros Island (A. Christidis, pers. comm.), which however could not be confirmed and was thus not included in the study of Zotou *et al*. (2021).

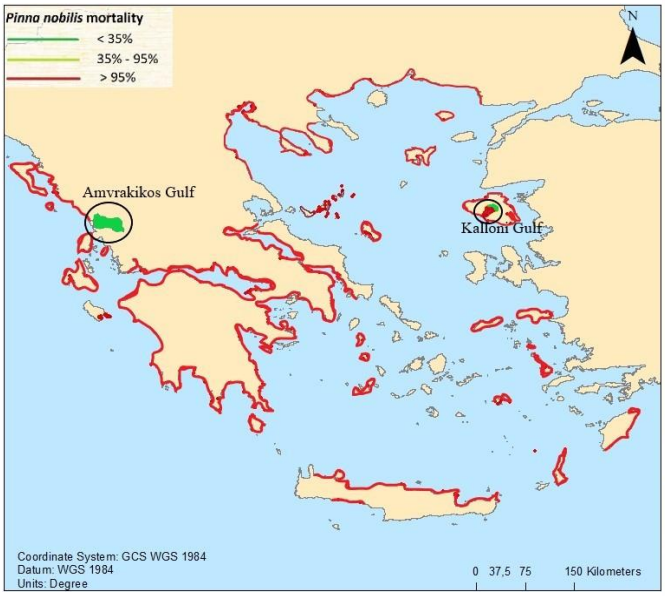

Figure 5: Integrated map of the current status (July 2021) of *P. nobilis* in the Greek seas (Zotou *et al*., 2021)

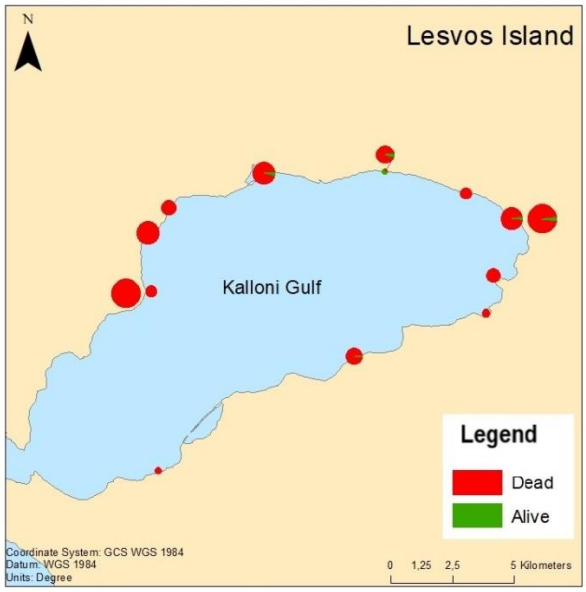

Figure 6: Mortality assessment of *Pinna nobilis* populations in Kalloni Gulf. The size of the pie charts represents the number of recorded individuals. The red and green color in the pie charts show the proportion of dead and live individuals, respectively (Zotou *et al*., 2021)

The study of Zotou *et al*. (2021) documented the large-scale progression of the *P. nobilis* MME throughout the Greek seas during 2020 and 2021 and the collapse of fan mussel populations in most of the marine areas investigated. In Greece, there are still areas that are unassessed (Figure 5), whereas even in assessed localities surveys were mostly restricted in shallow waters. Intensification of efforts to identify the remaining populations is needed to investigate anecdotal information about surviving individuals in various locations and depths across the Greek seas, to better examine possible methods of *P. nobilis* populations' restoration. Characteristic example is the case of Amvrakikos Gulf, where a healthy important population was discovered in 2021, being previously unknown.

Proposals for conservation

2 IDENTIFICATION OF PRESSURES AT NATIONAL LEVEL

In addition to the *MME* which caused the collapse of *P. nobilis* population in the Mediterranean Sea, habitat degradation (especially of *P. oceanica* meadows due to trawling and uncontrolled anchoring), marine pollution, fishing activities, invasive species and climate change have been listed as major threats for fan mussel survival (Basso *et al.,* 2015). Due to these anthropogenic threats, the species has been protected under the European Union 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.

Long before the expansion of the disease which caused the MME of *P. nobilis* in Greek seas in 2018 (Katsanevakis *et al*., 2019), its populations had been substantially decreasing in Greece in the past decades (Katsanevakis *et al.,* 2008). Despite the existing legal framework for its protection, poaching of fan mussels has been widely spread in Greece and posed a significant risk for many local populations, as the species is considered a delicacy in many coastal communities and there was low awareness about its protection status. A marked size segregation with depth was observed in Lake Vouliagmeni (Greece) and was attributed exclusively to fishing mortality (Katsanevakis 2007). The survival of large individuals in shallow waters was strikingly low due to fishing mortality (by free divers), whilst at a depth of 12 m, large individuals did not suffer from poaching, protected by the turbid waters of the marine lake. A temporal pattern of fishing mortality was found by the latter study. Fishing mortality was greater during summer and early autumn, especially during August, as at that time the lake is crowded by summer visitors who spend their holidays by the lake and many of them collect fan mussels for food or for their shells, targeting mostly large individuals. On the contrary, during winter and spring, the lake is depopulated and the water is too cold for free-diving, which explains the temporal pattern of fishing mortality.

Additionally, incidental killing by trawlers, bottom nets, or anchoring consist common cause of fan mussel mortality in Greece (Katsanevakis, 2007; Katsanevakis & Thessalou-Legaki, 2009; Katsanevakis *et al*., 2011). *Posidonia oceanica* and *Cymodocea nodosa* meadows degradation, as a result of coastal development, trawling and anchoring, has also affected fan mussels populations in Greece (Katsanevakis *et al.,* 2008).

3 RECOMMENDATIONS ABOUT SPECIFIC ACTIONS THAT CAN IMPROVE THE CONSERVATION OF INDIVIDUALS

Proper management aimed at **minimizing accidental mortality** due to human interactions (e.g. anchoring and fishing activities) or poaching should be pursued in the remaining fan mussel populations in Greece, 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 neighboring Gera Gulf where the population was entirely lost in 2019 (Zotou *et al.,*

2020; Zotou *et al.,* 2021). 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 (Zotou *et al.,* 2021).

Continuous monitoring of fan mussel populations and especially the search for juveniles and recruits is needed in order to observe a possible "natural recovery" of the species. **Larvae collectors** are a useful tool for early detection of new recruits (Kersting *et al*., 2020) and **collected juveniles could be cultivated ex situ or be transplanted in parasite-free locations** (Katsanevakis, 2016; Zotou *et al*., 2020). A large healthy *P. nobilis* population in the Sea of Marmara (Turkey) is protected from infection by the low salinities of the region, and can potentially export larvae to various locations in the northern Aegean Sea. By applying **ocean circulation models**, the export and fate of larvae from the Sea of Marmara to the northern Aegean through the Dardanelles strait will be predicted and collectors will be deployed in sites with high predicted recruitment to (1) **investigate how the Sea of Marmara population contributes to the restocking of the northern Aegean populations**, and (2) **collect *P. nobilis* juveniles for transplantation and restocking actions**. Furthermore, the **population dynamics** and recovery potential of *P. nobilis* populations in two gulfs in Lesvos Island (Greece) Gera Gulf & Kalloni Gulf will be investigated. In Gera 100% mortality was observed in a previous thriving population (abundance: ~215,000 individuals), while in Kalloni, the same was observed except in the northern (inner) part of the Gulf, where there were still (by the end of 2021) live individuals likely protected from infection by the extreme conditions of temperature and salinity. Through **a network of larvae collectors** (Figure 7) (1) we will investigate if the surviving population acts as a source for **recolonization** of other sites, and (2) to optimize survival chances, all collected *P. nobilis* will be **relocated** to the northern Kalloni Gulf. In this way we will try to enhance this surviving population. In total **40 larvae collectors** will be installed around Lesvos Island (focused mainly in Kalloni and Gera gulfs), at Lemnos Island, Samothraki Island and at Thracian Sea (Figure 8). In order to finalize the specific locations at Lemnos Island, Samothraki Island, and Thracian Sea, we are currently developing an **ocean circulation model**, which will reveal the optimal locations that potentially receive larvae by sea currents from the Sea of Marmara.

In sites where *P. nobilis* juveniles are detected to grow under unfavorable conditions (e.g. at depths shallower than 1 m with high chances of stranding by wave action; in areas with high boating or touristic activities), **juveniles will be transplanted** across deeper parts of the respective *P. nobilis* population's extent or at sites with such environmental conditions that the chances of parasitic infection are substantially reduced. The transplanted individuals will be tagged and monitored throughout the duration of the Project. Depending on the recruitment success and if possible, at least 25 individuals will be translocated between areas or introduced in the new sanctuaries found by monitoring actions which characterize new environmental candidate areas to host fan mussels.

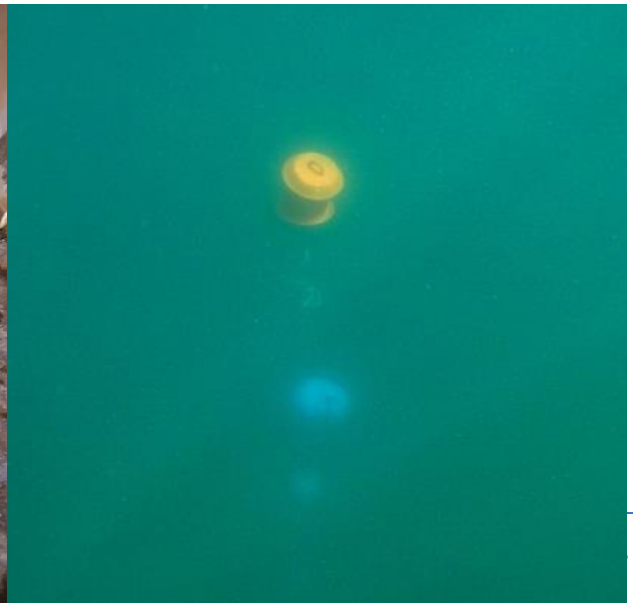

Figure 7. The larvae collectors which consist of a weight which stabilizes them on the sea bottom, a rope where the larvae collector bags are attached and a buoy which holds the structure upright in the water column (Photos: O. Papadakis)

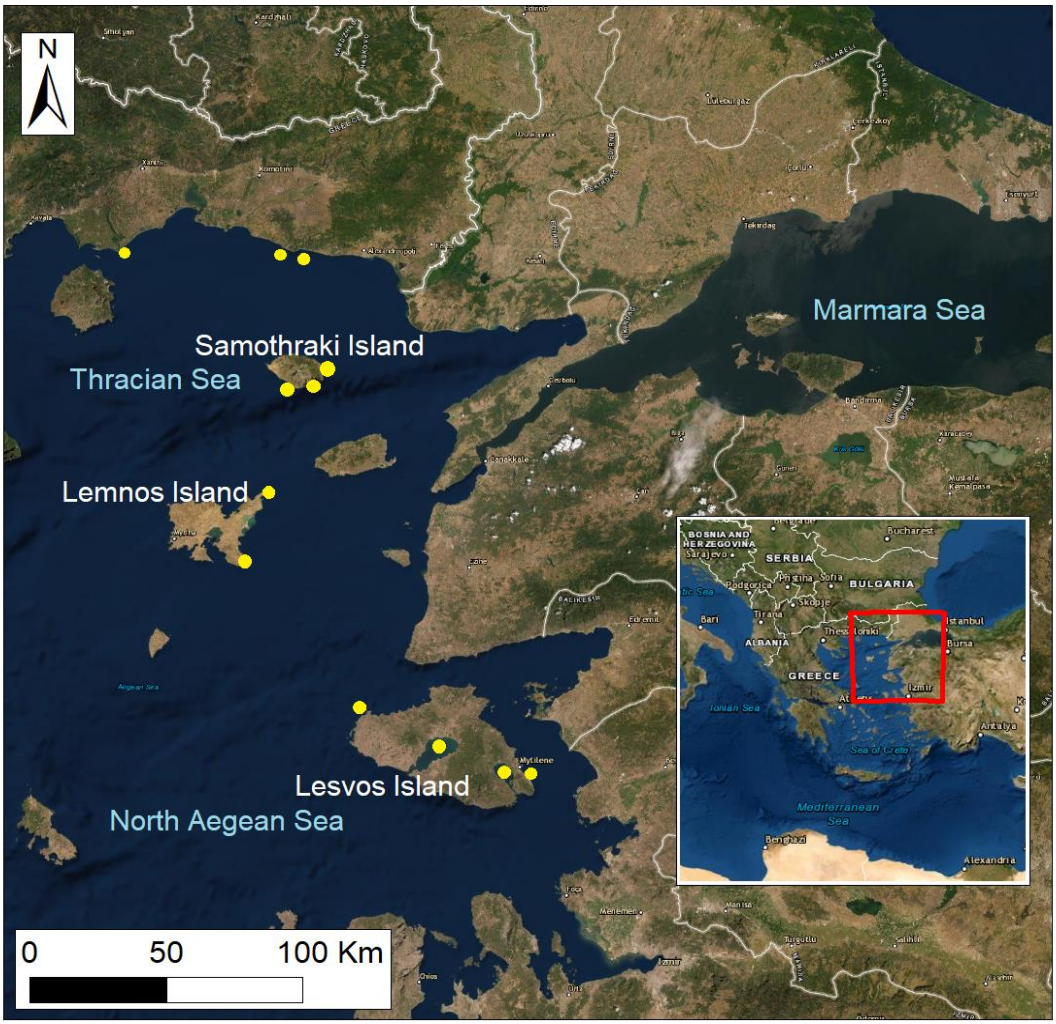

Figure 8. Map of the North Aegean; Thracian and Marmara Seas depicting the selected sites where the larvae collectors will be installed (yellow).

The healthy populations of fan mussels in Kalloni and Amvrakikos Gulfs (and possibly other healthy populations that will be found during the extensive shallow and deep surveys) will be **monitored** regularly for the duration of the project. A sufficient number of individuals will be **tagged** to estimate growth and mortality rates. Environmental conditions will be monitored continuously with **temperature loggers** and regularly for **pH and oxygen**. The University of the Aegean also has a permanent buoy in Kalloni Gulf fully equipped with a variety of sensors (CTD, pH, chlorophyll, nitrates, gamma-radiation etc), which will

provide a time-series of the average conditions in the Gulf. **Tissue samples** will be collected in a non-lethal way to determine the possible presence of the haplosporidian parasite (and other pathogens such as *Mycobacteria* sp. and *Vibrio mediterranei*) in the area.

We have already made preliminary discussions with local authorities in Lesvos and they are positive in the idea of establishing a **protected zone in Kalloni Gulf**, where all boat activities will be restricted, recreational activities will be strictly regulated, and any kind of commercial or recreational fishing will be prohibited, so that the surviving fan mussel population is adequately protected. Most of this population thrives in shallow waters (0.5-3.0 m) and thus many human activities (even walking in shallow waters) threaten the live individuals. In the framework of this project we will **map the surviving population** and make all the needed actions in collaboration with local authorities to establish such a protected area. If similar sites of high value for the protection of *P. nobilis* are found during the planned surveys, we will expand the efforts of such protection efforts also in other prefectures in Greece.

4 LIST OF AREAS POTENTIALLY CAPABLE OF HOSTING POPULATION TO CREATE NEW RESERVOIRS, FOR THEIR FURTHER EVALUATION DURING THE PROYECT

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 (Zotou *et al.,* 2020).

Although mass mortality of fan mussels has been observed in the entire marine territory of Greece, there are still a few unaffected or less severely affected populations in Kalloni and Amvrakikos Gulfs (Zotou *et al.,* 2021). In this action, all the available information about the survival rates of fan mussels in Greece, in relation to environmental conditions, will be integrated and analyzed to identify the optimal conditions for the survival of the species and thus optimal sites. A Generalized Additive Modelling approach will be followed to model the survival rates in relation to environmental covariates and map sites of optimal conditions.

Until now the only known areas which are capable of hosting healthy populations are in Kalloni gulf in Lesvos Island and Amvrakikos gulf in the Ionian Sea (Figure 5). The **shallow and deep underwater visual census** which will be conducted until autumn of 2022 will probably reveal more areas potentially capable of hosting populations.

5 PROPOSAL OF SITES TO CONDUCT FURTHER SHALLOW AND DEEP CENSUS

Mass mortality of fan mussel populations in Greek waters is still in progress. By the end of 2018, there were many unaffected populations both in the Aegean (mostly in northern sites) and the Ionian Sea. However, in 2019, 2020 and 2021 many of these populations have been reported to be affected. Intensification of efforts to identify the remaining populations will be conducted, in order to investigate anecdotal information about surviving individuals in various locations and depths across the Greek seas, as well as in sites which have not been visited during the previous studies and could potentially hold surviving populations.

Scientific divers will conduct shallow underwater visual surveys (<20 m depth) in all Greek sites (both in the Aegean and Ionian Seas) where *P. nobilis* had been reported to be abundant, in marine MPAs, as well as in a large number of other sites in which the presence/abundance of *P. nobilis* had never been investigated in the past. In addition to exhaustive shallow water census which will be conducted in Kalloni and Amvrakikos Gulfs, where the only remaining surviving populations had been found by 2021 (Zotou *et al.,* 2021), in total, at least 80 more sites will be surveyed in both the coastline of the Greek mainland and in Greek islands (Figure 9). At sites where healthy populations remain, distance sampling surveys will be conducted for estimating the abundance of the species by properly accounting for detectability issues. The ratio of alive and dead individuals will be estimated.

Deep underwater visual surveys (at depths between 20 and 40 m) will be conducted at 20 sites (both in the Aegean and Ionian Seas). Sites with known previous presence of abundant *P. nobilis* populations will be selected (Figure 9). The same field protocols as in shallow surveys will be applied. Special focus will

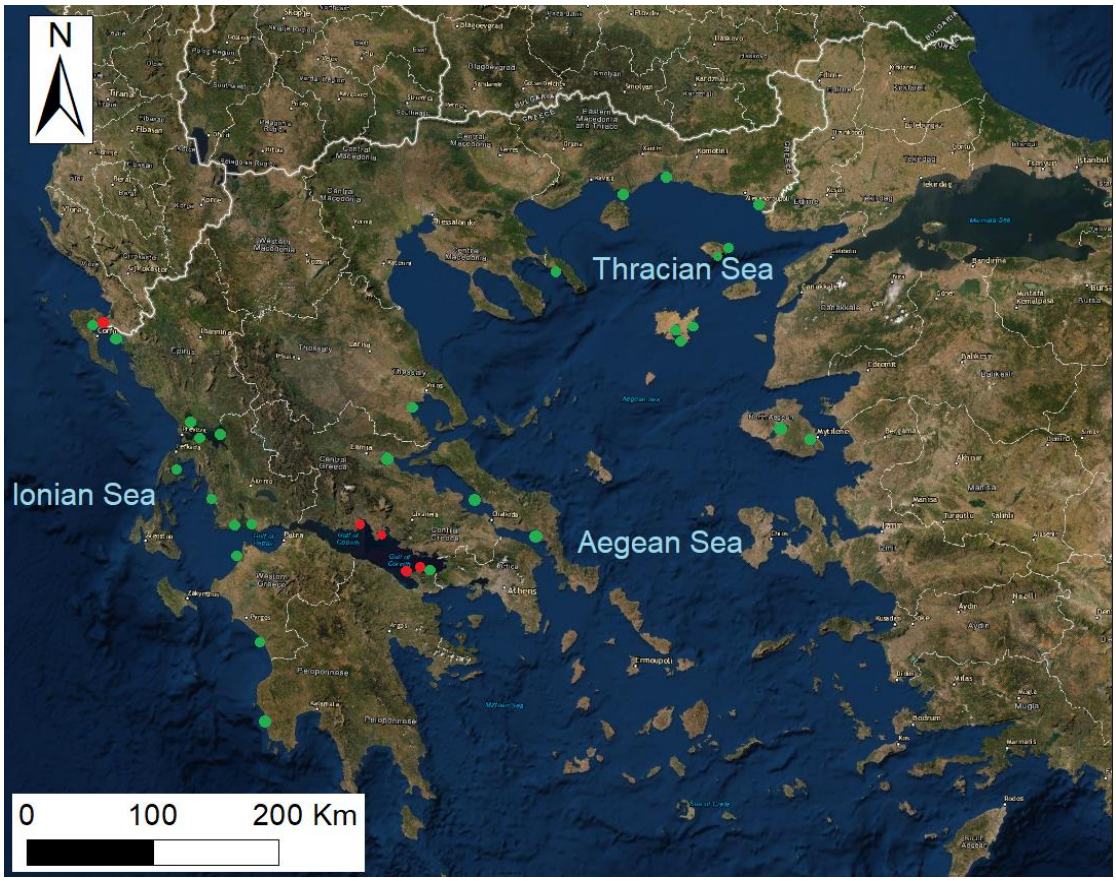

Figure 9. Map of Greece depicting the sampling locations selected for the shallow (green) and deep (red) underwater visual surveys. More than one sites will be surveyed at each location. The locations can be updated based on new information received in the following period about surviving populations.

be given in the deep areas of the marine Lake Vouliagmeni (Korinthiakos Gulf), where even in the summer there is a very intense thermocline with temperatures between 10-14°C all year long at depths >20 m, while in the shallow waters temperature varies from 14°C in winter to 30°C in summer. As the low temperatures in the deeper parts of the Lake are assumed to protect the species from infection, the survival of fan mussels in the deep areas of the Lake will be thoroughly investigated. High (100%) mortality has been recently (August 2020) reported in the shallow parts of the lake, in which previous investigations estimated the total abundance of the species at >6,000 individuals.

Networking

6 SPECIFIC SYNERGIES TO BE STARTED WITH OTHER PROJECTS AND OTHER COUNTRIES

We will aim for synergies with the following teams (beyond Pinnarca), working on Pinna nobilis:

- HCMR team (Greece). The Hellenic Centre for Marine Research (HCMR) has been active in surveying numerous sites across Greece for surviving populations. This team has received funding from the Green Fund of Greece for the project PINNA STATUS "Study of the Mass Mortality of *Pinna nobilis* and evaluation of the species in Greece". The University of the Aegean team has been collaborating with the HCMR team in assessing the status of the fan mussel in Greece and have produced common publications (Zotou et al. 2020, 2021).
- AUTH team (Greece). The Laboratory of Animal Physiology, Department of Zoology, School of Biology, Faculty of Science, Aristotle University of Thessaloniki (AUTH) has been active in monitoring, through molecular analyses, the presence of fan mussel pathogens in Greece. The University of the Aegean team has been collaborating with the AUTH team and have produced common publications (Zotou et al. 2020).

7 LIST OF STAKEHOLDERS OF INTEREST FOR THE CONSERVATION OF THE SPECIES, PUBLIC AND PRIVATE AT NATIONAL LEVEL (INSTITUTIONS)

Below is a list of national stakeholders of interest for the conservation of the fan mussel in Greece.

Name	**Role**	**e-mail**
Dr Yiannis Issaris	Researcher – Hellenic Centre for Marine Research	issaris@hcmr.gr
Dr Christina Pavloudi	Researcher – Hellenic Centre for Marine Research	cpavloud@hcmr.gr
Athanasios Lattos	PhD student - Aristotle University of Thessaloniki	aqualattos@gmail.com

Ioannis Mitsopoulos	Managing Director - Natural Environment and Climate Change Agency	i.mitsopoulos@prv.ypeka.gr
Anastasia Miliou	Scientific Director – NGO Archipelagos	a.miliou@archipelago.gr
Spyros Iosifidis	Deputy Head – Management Unit of National Park of Alonissos Northern Sporades & Protected Areas of Eastern Thessaly	s.iosifidis@necca.gov.gr
Dr Charalambos Dimitriadis	Scientific Officer – National Park of Zakynthos	xdimitriadis@marine.aegean.gr
George Tentes	Sustainability Consultant – Green2Sustain	george@green2sustain.gr

8 REFERENCES

Basso, L., Vázquez-Luis, M., García-March, J., Deudero, S., Alvarez, E. *et al.*, 2015. The pen shell, *Pinna nobilis*: A review of population status and recommended research priorities in the Mediterranean Sea. *Advances in Marine Biology*, 71, 109-160.

Cabanellas-Reboredo, M., Vázquez -Luis, M., Mourre, B., Alvarez, E., Deudero, S. *et al.*, 2019. Tracking a mass mortality outbreak of pen shell *Pinna nobilis* populations: A collaborative effort of scientists and citizens. *Nature Scientific Reports*, 9, 13355.

Catanese, G., Grau, A., Valencia, J.M., García-March, J.M., Álvarez, E. *et al.*, 2018. *Haplosporidium pinnae* sp. nov., a haplosporidan parasite associated with massive mortalities of the fan mussel, *Pinna* nobilis, in the Western Mediterranean Sea. *Journal of Invertebrate Pathology*, 157, 9-24.

García-March, J.R., Tena-Medialdea, J., Henandis, S., Vázquez-Luis, M., López, D. *et al.*, 2020. Can we save a marine species affected by a highly infective, highly lethal, waterborne disease from extinction? *Biological Conservation*, 243, 108498.

Katsanevakis, S., 2007. Growth and mortality rates of the fan mussel *Pinna nobilis* in Lake Vouliagmeni (Korinthiakos Gulf, Greece): a generalized additive modelling approach. *Marine Biology*, 152 (6), 1319-1331.

Katsanevakis, S., Leftaditou, E., Galinou-Mitsoudi, S., Koutsoubas, D., Zenetos, A., 2008. Molluscan species of minor commercial interest in Hellenic seas: Distribution, exploitation and conservation status. *Mediterranean Marine Science*, 9 (1), 77-118.

Katsanevakis, S., Poursanidis, D., Issaris, Y., *et al*., 2011. "Protected" marine shelled molluscs: Thriving in Greek seafood restaurants. *Mediterranean Marine Science*, 11, 429–438.

Katsanevakis, S., Thessalou-Legaki, M., 2009. Spatial distribution, abundance and habitat use of the protected fan mussel *Pinna nobilis* in Souda Bay, Crete. *Aquatic Biology*, 8, 45-54.

Katsanevakis, S., Tsirintanis, K., Tsaparis, D., *et al*., 2019. The cryptogenic parasite *Haplosporidium pinnae* invades the Aegean Sea and causes the collapse of *Pinna nobilis* populations. *Aquatic Invasions,* 14(2), 150-164.

Kersting D.K., Vázquez-Luis M., Mourre B., et al. (2020) Recruitment disruption and the role of unaffected populations for potential recovery after the *Pinna nobilis* mass mortality event. *Frontiers in Marine Science*, 7, 594378.

Tsatiris, A., Papadopoulos, V., Makri, D., Topouzelis, K., Manoutsoglou, E. *et al.*, 2018. Spatial distribution, abundance and habitat use of the endemic Mediterranean fan mussel *Pinna nobilis* in Gera Gulf, Lesvos (Greece): comparison of design-based and model-based approaches. *Mediterranean Marine Science*, 19 (3), 642-655.

Vázquez-Luis, M., Alvarez, E., Barrajon, A., García-March, J.R., Grau, A. *et al.*, 2017. S.O.S. *Pinna nobilis*: a mass mortality event in western Mediterranean Sea. *Frontiers in Marine Science*, 4, 109.

Zotou, M., Gkrantounis, P., Karadimou, E., *et al*., 2020. *Pinna nobilis* in the Greek seas (NE Mediterranean): On the brink of extinction? *Mediterranean Marine Science*, 21, 575–591.

Zotou, M., Papadakis, O., Ragkousis, M., Issaris, Y., Gerakaris, V., Çinar, M.E., Katsanevakis, S., 2021. *Pinna nobilis*, in the brink of extinction in Greek seas. *Hydromedit: 4th International Congress on Applied Ichthyology, Oceanography and Aquatic Environment*, 4-6 November 2021, Mytilene, Greece.