Tracking Population Status and Structure of Mediterranean Pen Shell Pinna Nobilis After a Mass Mortality Outbreak

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Abstract

The dramatic Mass Mortality Event, MME, of *Pinna nobilis* populations initially detected in 2016 in the western Mediterranean basin, has also spread rapidly to the central and eastern basin. Unfortunately, there is still a significant lack of information on the status and health of *P. nobilis*, since only a fragmentary picture of the mortality rate affecting these populations is available. Regarding the Italian coast, several surveys have given only localized or point-like views on the distribution of species and the effect of the MME. Therefore, for the first time, this study investigated *P. nobilis* densities, distribution and mortality in 164 surveys covering a total of 800 km along the southeast coast of Italy (Apulia region). The geographical scale of this investigation made it the largest ever conducted in Italy, and this was achieved through a rapid and standardized protocol. No live individuals were observed along the 92 km linear transects, allowing us to assess that the *P. nobilis* populations had totally collapsed, with a mortality rate of 100% in Apulia. The distributional pattern of the species showed a strong overlap with seagrass meadows on meso and macro geographical scale, however this was not the case on a micro scale. This result indicates that although there is a relationship between *P. nobilis* and seagrass meadows, it is not limited to the habitat patch but crosses the boundaries of seagrass. This observation led us to the conclusion that the distribution of *P. nobilis* shows a trophic link through the cross-boundary subsidy occurring from seagrass meadows to the nearby habitat, by means of the refractory detrital pathway.

Introduction

Pinna nobilis (Linneaus 1758) is one of the largest bivalve mollusks worldwide (Zavodnik et al., 1991) it is an endemic species of the Mediterranean were has been present since the Miocene Epoch (Molinari and Bernat, 2016). This species filter large quantities of seawater (Zavodnik et al., 1991; Schultz and Huber, 2013) retaining Phytoplankton, Zooplankton and indeterminate debris (Davenport et al., 2011) but its ecological role in the benthic food chain dynamics still remain poorly understood although the considerable biomass reached in some coastal habitat.

In the last five years, the pen shell has been disappearing from our seas due to an abnormal Mass Mortality Event (MME), the causes of which are still under investigation. In 2016, the MME was first detected across a wide geographical area of the Spanish coast (Vázquez-Luis et al., 2017), affecting specimens of all sizes, depth range, and habitat (Darriba, 2017; Vázquez-Luis et al., 2017). The disease spread rapidly from the west to east of the Mediterranean basin, reaching the coasts of France, Italy, Croatia, Greece, Turkey, and northern Africa (Cabanellas-Reboredo et al., 2019; Kersting et al., 2019), causing high mortality rates in infected populations. As a consequence, in December 2019, *P. nobilis* entered the IUCN Red List as a critically endangered species (Kersting et al., 2019).

In early 2017, the MME of *P. nobilis* was reported along the western coast of Italy (Campania and Sicily), affecting 85–100% of the population (Carella et al., 2019). In the summer of 2018, an infected population was recorded in the Mar Piccolo of Taranto (Panarese et al., 2019), and in 2019 the disease reached the coasts of the Northern Adriatic. Recently, abnormal mortalities have also been reported inside the Venice lagoon, which was thought to host one of the last healthy populations of the north Adriatic Sea (ISMAR, 2021). We currently have only fragmented knowledge of the mortality rate affecting populations, based on localized observations focused on specific areas. This is the case for the entire Mediterranean basin, except for a few studies covering large geographical areas such as Šarić et al. (2020) for the Croatian coast, Vázquez-Luis et al. (2017) for the Spanish coast and Zotou et al. (2020) for the Greek coasts.

Regarding the Italian coasts, in particular, there is a lack of surveys aiming to assess the species distribution and the effect of the MME on a large geographical scale. This constitutes the most significant information gap on the state of health of *P. nobilis* populations. It is essential to gather data from large-scale monitoring activities to understand the state of local populations, the pressures and the trends affecting them. For this reason, gaining an overview of the distribution of natural populations before the MME and on the incidence of mortality in local populations is the starting point for undertaking critical protection and management actions.

The objective of this study was to track, by means of comprehensive surveys, the progress of the MME and to provide updated information on the status of *P. nobilis* in southeastern Italy (Apulia region), in both the Adriatic and the Ionian Sea. Furthermore, a transversal investigation on species distribution patterns in relation to the substrate type was carried out among different habitat types and ecosystems to investigate the main drivers underlying its spatial distribution. For this purpose, a rapid and standardized protocol was developed through visual census surveys, which allowed us to assess the state of *P. nobilis* along 800 km of the coastline.

Material And Methods

To provide data on the extent and impact of the *P. nobilis* mass mortality event at a regional scale, field surveys were conducted by scuba divers who were purpose-trained. Thirty-one areas were investigated along the Apulia coast, 20 located in the Adriatic Sea and 11 in the Ionian Sea, covering a total coastline of approximately 800 km (Fig. 1). The extensive transects allowed us to cover all the habitat types through a cross-boundary approach. In each coastal area, three bathymetries were investigated (5m, 10m, and 15m), and the Navionics SonarChart was used to draw the transect, relying on the sonar cartographic bathymetry. The effective depth of the transects was checked through the vessel echo sounder to adjust the path, if necessary, with field data. For each bathymetry, one or more transects were defined by laying down a 600m-long rope stretched out parallel to the coastline. The rope was held down by anchors positioned every 100 meters, and the GPS tracking data was collected during the positioning. Two marker buoys were placed at the endpoints of each transect to signal their location above the surface. For each survey, the scuba divers covered a total linear transect covering from 400 to 600 m (depending on the diving time) by counting and recording specimens within a 1m span (50 cm from the line on each side), for a total survey area of 400–600 m^2 for each transect (Fig. 2). When the transect passed through seagrass meadows, the operator moved aside the seagrass leaves around the rope manually to detect all the *P. nobilis* specimens. From preliminary surveys, 50 cm to 1 m was found to be the optimal width for a scuba diver to investigate the microbenthic organisms thoroughly when spreading the seagrass meadows leaves. During the surveys, specimens that had clearly been dead for a long time (based on the shell state and fouling inside the shell) were not considered (Katsanevakis, 2019). After each survey, the rope, ballast, and buoys were winched on board. A total of 164 transects were completed, for an overall survey length of 92 km examined by the scuba diversthroughout the study.

Results

During our surveys, total mortality of *P. nobilis* was detected along the southeast coast of Italy, in the Apulia region. A total of 321 recently dead specimens were recorded in 164 transects along the 31 coastal areas investigated. No live individuals were observed along 92 km transects, allowing us to conclude that the *P. nobilis* populations had totally collapsed, with a mortality rate of 100% in the Apulia region. Dead specimens of all ages and sizes were found (both juveniles and adults) in all the depths and habitat types examined. Total mortality of *P. nobilis* was also recorded within the three marine protected areas of the region (Torre Guaceto, Porto Cesareo and the Tremiti archipelago), and no difference emerged between coastal and island ecosystems, with total mortality also observed in the Tremiti archipelago.

During the monitoring period, three lagoon systems were investigated: Varano, Alimini, and Acquatina. In the Alimini and Varano lagoons, no *P. nobilis* shells were observed. In the Acquatina lagoon, despite a previous report had testified to the presence of the *P. nobilis* (Pinna et al., 2018), total population mortality was detected.

The presence of *P. nobilis* shells was registered in all the 11 monitored sites of the Ionian Coast. In the Adriatic Coast, *P. nobilis* shells were found in the Tremiti Islands (A1) and among the southern sites from A7-A17. No traces of *P. nobilis* were found in the A2-A6 sites, which happen to be the northernmost sites of the Apulian coast (Fig. 2).

The density of the pen shells was recorded for each coastal area and was reported in terms of individuals/100m^2 (Table 1). Substantial differences emerged between the two basins in terms of the density of *P. nobilis*, with the Ionian Sea showing considerably higher densities than the Adriatic Sea, with 0.88 individuals/100m^2 and 0.09 individuals/100m^2 respectively. The values recorded in the Ionian Sea were higher than 0.1 individuals/100 m^2 in all the sites investigated. The highest mean value was recorded in the I10 site, located in the Gulf of Gallipoli, with a density of 3.94 individuals/100m^2, followed by I6, I7 and I8 sites, where density values of 0.50 individuals/100m^2, 0.80 individuals/100m^2 and 1.56 individuals/100m^2 were recorded, respectively. In the Adriatic Sea, instead, except for A16 and A17, the recorded values were lower than 0.1 individuals/100m^2 (Fig. 3) in every case.

Table 1
Exploited areas, Habitat type, densities, mortality rate of the area covered, transect length

Site	Locality	Basin	Type of environment	Substrate type	Exploited area (m^2)	N° P.nobilis	Population density ($/100m^2$)	Mortality incidence
A1	Tremiti Arcipelago (MPA)	Adriatic Basin	Island	*P.oceanica*, sandy, rocky	1900 m^2	6	0.31	100%
A2	Lesina		Coastal	sandy	1800m^2	0	0	n.a.
A3	Capoiale		Coastal	sandy	1800 m^2	0	0	n.a.
A4	Vieste		Coastal	sandy, rocky	1800 m^2	0	0	n.a.
A5	Manfredonia		Coastal	*Cymodocea nodosa*, sandy	1800 m^2	0	0	n.a.
A6	Margherita di Savoia		Coastal	sandy	1800 m^2	0	0	n.a.
A7	Bisceglie		Coastal	*P.oceanica*, sandy, rocky	3600 m^2	2	0.06	100%
A8	Mola di Bari		Coastal	*P.oceanica*, sandy, rocky	3600 m^2	2	0.06	100%
A9	Monopoli		Coastal	*P. oceanica*, sandy, rocky	3000 m^2	2	0.07	100%
A10	Torre Canne		Coastal	*P. oceanica*, sandy, rocky	3300 m^2	1	0.03	100%
A11	Torre Guaceto (MPA)		Coastal	*P. oceanica*, sandy, rocky	3100 m^2	4	0.13	100%
A12	Brindisi		Coastal	*P. oceanica*, sandy, rocky	5300 m^2	2	0.04	100%
A13	Lido Cerano		Coastal	*P. oceanica*, sandy, rocky	5400 m^2	2	0.04	100%
A14	Frigole		Coastal	*P. oceanica*, sandy, rocky	3600 m^2	1	0.03	100%
A15	Torre Specchia		Coastal	*P. oceanica*, sandy, rocky	3600 m^2	2	0.06	100%
A16	Alimini		Coastal	*P. oceanica*, sandy, rocky	3400 m^2	14	0.41	100%
A17	Porto Miggiano		Coastal	*P. oceanica*, sandy, rocky	3600 m^2	8	0.22	100%
L1	Varano Lagoon		Lagoon	*C. nodosa*, *Zoostera sp.*, sandy, muddy	6000 m^2	0	0	n.a.

Site	Locality	Basin	Type of environment	Substrate type	Exploited area (m^2)	N° P.nobilis	Population density ($/100m^2$)	Mortality incidence
L2	Acquatina Lagoon		Lagoon	*C. nodosa*, *Zoostera sp.*, sandy, muddy	1800 m^2	6	0.33	100%
L3	Alimini Lagoon		Lagoon	*C. nodosa*, Zostera sp., sandy, mudy	1800 m^2	0	0	n.a.
I1	Lido Azzurro	Ionian Basin	Coastal	*P. oceanica, C. nodosa*, sandy	1800 m^2	2	0.11	100%
I2	San Pietro Island		Island	*P. oceanica, C. nodosa*, sandy/rocky	1800 m^2	7	0.39	100%
I3	San Vito		Coastal	*P. oceanica*, *C. nodosa*, sandy, rocky	1800 m^2	3	0.17	100%
I4	Torre Ovo		Coastal	*P. oceanica*, sandy, rocky	1800 m^2	3	0.17	100%
I5	Torre Colimena		Coastal	*P. oceanica*, sandy, rocky	3600 m^2	10	0.28	100%
I6	Torre Lapillo (MPA)		Coastal	*P. oceanica*, sandy, rocky	3600 m^2	18	0.50	100%
I7	Porto Cesareo (MPA)		Coastal	*P. oceanica*, sandy, rocky	4100 m^2	33	0.80	100%
I8	Sant'Isidoro (MPA)		Coastal	*P. oceanica*, sandy, rocky	1800 m^2	28	1.56	100%
I9	Santa Caterina		Coastal	*P. oceanica*, sandy, rocky	3400 m^2	16	0.47	100%
I10	Baia verde		Coastal	*P. oceanica*, sandy, rocky	3600 m^2	142	3.94	100%
I11	Torre San Giovanni		Coastal	*P. oceanica*, sandy, rocky	3000 m^2	7	0.23	100%
ADRIATIC SEA			Coastal & Island		52500 m^2	46	0.09	100%
IONIAN SEA			Coastal & Island		30300 m^2	269	0.88	100%
LAGOON SYSTEMS			Lagoon		9600 m^2	6	0.06	100%

Discussion

The target of the present study was to assess the mortality incidence on *P. nobilis* in local populations along the southeast coast of Italy (the Apulia peninsula) following the mass mortality event. At the same time, species distribution and densities were investigated in the Adriatic and the Ionian Sea, allowing us to build a picture of species populations before the MEE.

Spatial distribution

Concerning the data collected on *P. nobilis* distribution in the Apulia region before the MME, unfortunately, there is a lack of information at the regional scale and the reports found in the scientific literature are limited only to semi-enclosed systems such as the Taranto basins (Centoducati et al., 2007; Tiscar et al., 2019; Tursi et al., 2018) and the Aquatina lagoon (Marrocco et al., 2018; Pinna et al., 2018). No large-scale monitoring program on *P. nobilis* has been carried out previously along the Apulian coast, although these types of surveys are indispensable for the management of a protected species and must become mandatory for a critically endangered species such as the *P. nobilis*. To address this information gap, a data-gathering survey was undertaken to assess the current status of the *P. nobilis* population. In this study, thirty-one areas distributed along the entire Apulian region coast were explored with standardized visual-census surveys. Recently dead specimens with still intact shells allowed us to map *P. nobilis* distribution and densities in each area before the Mass Mortality Event.

Along the Ionian coast, *P. nobilis* was detected in all the areas studied, highlighting a continuous distribution of the species. This result indicated that the Ionian coastal region presents environmental conditions compatible with the species requirements throughout the entire coast extension. However, the distribution along the Adriatic coast was not continuous. *Pinna nobilis* occurrence was recorded in the areas surveyed in the south, from A7 to A17, but no traces were found along the northernmost areas except the Tremiti archipelago. These results indicate that the northernmost Adriatic coast of the region probably does not meet the environmental conditions suitable for hosting this species. The Gulf of Manfredonia may have represented the sole exception in the past. In this area, we collected multiple reports from fishermen indicating the presence of the species in a local *Cymodocea nodosa* meadow before the 1980s. Still, no shell traces were observed during the surveys. Therefore, we can assume that excessive fishing and anthropogenic activities in this area are likely to have caused the species to disappear many decades ago.

Mortality incidence

Data regarding the effects of the MME in Apulian populations is scarce. Panarese et al., in 2019 reported the advent of the disease in Mar Piccolo di Taranto but without describing the disease incidence. In this study, we recorded a mortality incidence of 100% in all habitat types, bathymetries and basins, demonstrating the seriousness of the situation along the entire Apulian coast. To achieve an exhaustive evaluation of the state of *P. nobilis* populations, different environments such as, inshore, offshore, and lagoon and marine protected areas were investigated.

While the availability of nutrients and the trophic conditions are assumed to be very different between offshore and inshore systems, the archipelago of Tremiti islands, located 13 miles from the coast, showed no differences in mortality incidence from sites along the coast, evidencing the same critical conditions in both inshore and offshore environments.

Many Mediterranean lagoon systems, including the Ebro Delta, Mar Menor Lagoon in Spain (Prado et al., 2021), the Rhone delta, Leucate and Thau in France (Foulquie et al., 2020; García-March et al., 2020; Katsanevakis et al., 2021), Venice, Grado-Marano and Faro in Italy (Curiel et al., 2021; Donato et al., 2021; Russo, 2017), Bizerte in Tunisia (Katsanevakis et al., 2021) are considered the last healthy shelters for *P. nobilis* populations in the central and eastern Mediterranean basin (Foulquie et al., 2020). These areas seem to offer a degree of resistance against the disease and are all characterized by high seasonal fluctuations of environmental parameters, such as temperature and salinity. It has been supposed that the effect of these fluctuations could make these environments less suitable for the spread of the disease and reduce the rate of transmission (Foulquie et al., 2020; Prado et al., 2021). For this purpose, three lagoon systems were investigated in the present study: Aquatina di Frigole, Varano, and Alimini. Aquatina di Frigole is the sole lagoon in the region where previous studies have reported the presence of *P. nobilis* (Marrocco et al., 2018). The results of the surveys highlighted a critical condition, showing that also the population of *P. nobilis* in the Aquatina di Frigole lagoon have totally collapsed. Considering that the lagoon refuges currently represent the main source of larval production for *P. nobilis* recruitment (Foulquie et al., 2020) the collapse of these populations confirms the severity of the situation for species conservation along the southeast coast of Italy.

Regarding the timeframe of the spread of the MME along the Apulian cost, the first report of the infection dates back to 2018 (Tiscar et al., 2019), in the Mar Piccolo di Taranto. Compared to the first MME event observed in the Spanish coast in 2016 (Darriba, 2017; Vázquez-Luis et al., 2017), over a period of two years, the disease has spread from the western to the eastern basin of the Mediterranean Sea. Our surveys, carried out in 2020, showed that 91% of the shells were still undamaged and with joined valves. Based on the state of conservation of the shells (Scarpa et al., 2021) it is possible to hypothesize that the death of the specimens was a recent phenomenon that had occurred in Apulia in the two years preceding our surveys, and most probably shud should be dated back to 2019.

Habitat association and trophic ecology

In our surveys, *P. nobilis* showed transverse distribution among habitat types occurring both in marine and lagoon systems, inside and outside seagrass meadows. Nevertheless, when the pen shell distribution was analysed on a spatial macro and mesoscale, the results showed an overlap with the distributional range of seagrass meadows. In fact, all the coastal areas where the *P. nobilis* was found were also characterized by the presence of seagrass meadows, either directly in the area under examination or in proximity. In fact, in the northern Adriatic coast of the region, where extended seagrass meadows are absent, no trace of *P. nobilis* was encountered, except in the Tremiti archipelago where both *P.oceanica* meadows and pen shells were found to be present. Focusing specifically on the occurrence data, it emerged that *P. nobilis* is associated with various seagrass species such as *P. oceanica*, *C. nodosa* and *Zostera sp.*, which in turn are distributed in heterogeneous habitat including those of marines and lagoon-estuarines. From these results it can therefore be concluded that there is a macro and mesoscale association between *P. nobilis* and seagrass meadows *sensu lato*. This hypothesis is consistent with much of the data in literature reporting ubiquitous distribution of the species both in lagoon-estuarine ecosystems (Curiel et al., 2021; Donato et al., 2021; Foulquie et al., 2020; Katsanevakis et al., 2021; Prado et al., 2021; Tsatiris et al., 2018) and in marine ecosystems (Basso et al., 2015; Katsanevakis et al., 2021; Kersting et al., 2019; Šarić et al., 2020; Vázquez-Luis et al., 2017; Zotou et al., 2020).

However, taking a closer look at their micro-scale distribution, the pen shells in our surveys were commonly recorded also outside the seagrass meadows boundaries, at times up to one kilometer away. Hence, seagrass sheltering can potentially be ruled out as the sole explanatory factor for the distribution pattern of the species. The pattern emerging from our study led us to hypothesise that a trophic link with the seagrass detritus food-chain may explain both the macro-mesoscale association with seagrass species and the microscale cross-boundary distribution. In fact, seagrass detritus is highly refractory, since it is largely exported to the nearby areas where it can represent the major food source for other invertebrates (Boncagni et al., 2019a; Danovaro, 1996). This hypothesis is consistent with the stomach contents observations reported by Davenport and co-authors (2011) indicating detritus as the bulk component, accounting for 95% of the total ingested material.

One of the main factors underlying the distribution pattern in benthic invertebrates is indeed food availability (Palmer et al., 1996; Tregenza, 1995). According to the Ideal Free Distribution theory (IFD), in ecology the individuals in a population disperse to different resource patches within their environment, minimizing competition and maximizing fitness (Whitham, 1980). When the IFD assumptions are met, the number of individuals who aggregate in patches is proportional to the amount of food resource available in each one. Accordingly, the distribution of large, long life and sessile organisms such as *P. nobilis* would be expected to depict the species trophic supply, by analyzing the resources available in those patches.

Studies on the seagrass system energy flow have shown that seagrass debris must be fractionated before entering the food chain (Danovaro, 1996). In this way, plant material becomes fine particulates moving in the boundary layer over the sediment-water interface (Danovaro et al., 1998; Danovaro and Fabiano, 1997). These processes take time, and while the matter is transported, heterotrophic bacteria grow exponentially, turning it into a high quality and protein enriched food for consumers. Hence, bacteria adhering to seagrass detritus may play a key role in this benthic food chain and sediment-water interface consumers may incorporate more energy from associated microbes than from the detritus itself (Boncagni et al., 2019b; Danovaro et al., 1998; Rakaj et al., 2019, 2018). On the basis of these considerations, it is reasonable to conclude that the quantity, composition and origin of the suspended particles are regulated by a drift mechanism and that this mechanism may explain local densities of *P nobilis* as a response to sinking rates and resuspension effects. The assumption of the species' ability to feed on seagrass detritus, together with the high biomasses reached (large size specimens and high density), lead us to suppose that *P. nobilis* played a key role in the processing of matter and in the energy pathway deriving from seagrass detritus in Mediterranean coastal areas. This makes the repercussions of the MME not only a problem of conservation, but also and above all, an ecological-functional issue.

We can, therefore, conclude that Mediterranean seagrass meadows not only constitute a habitat for *P. nobilis*, but most of all, a food source through refractory detritus generation which is transferred and transformed outside the meadows. Unfortunately, literature is lacking on this topic and further investigations are needed to define the trophic role and function of these filter feeders in the different seagrass meadows.

Local densities

The density values that emerged were significantly different among basins. In the Adriatic Sea, where all the coastal values were recorded, the densities were consistently lower than those reported in the Ionian Sea, except for the two southernmost areas. In the Adriatic basin, it was also possible to recognize a north-south trend when considering the densities of pen shells in the coastal areas. In the central area of the region, the values recorded were fewer than 0.07 individuals/100 m^2(A7-A15). Instead, in the southernmost areas, density values of between 0.22 and 0.41 individuals/100 m^2 were recorded (A16; A17). Although the values recorded along the southern coast of the region were much greater than those recorded in the central coast, they were far lower than those reported by Čižmek et al. (2020) in the Croatian coast (North Adriatic Sea). Similar values to ours within the same basin were reported by Celebicic et al. (2018) in Bosnian waters (0.12 individuals/100m^2).

On the other hand, in the Ionian areas, the values recorded were consistently higher than 0.1 individuals/100m^2. The highest density was recorded in the Gulf of Gallipoli (with a mean value of 3.94 individuals/100m^2), whilst the mean density value for the entire basin was 0.88 individuals/100m^2. The values recorded in the Mar Grande di Taranto, were higher than those reported by Centoducati et al. (2007) (0.1–0.7 ind/ha^2). From interviews with fishermen it emerged that illegal trawling in this area has strongly impacted the natural populations of the Mar Grande di Taranto, and a partial reduction in recent years could explain the slight increase in density compared to the 2004 survey data (Centoducati et al. 2007).

Analyzing the data collected for Aquatina di Frigole - the only lagoon system in which the presence of *P. nobilis* specimens was detected- the density value recorded of 0.33 individuals/ 100m^2 is far lower when compared with that reported in the Thau lagoon, Grado-Marano lagoon and Mljet lagoon (Curiel et al., 2021; Foulquie et al., 2020; Mihaljević et al., 2021), but is higher than the density values recorded in the Lake Faro (Sicily) in 2010 when density values of 0.18 individuals /100m^2 were recorded. In this area, the density values decreased dramatically after the MME, dropping to 0.07 individuals /100m^2 in 2020, although some live specimens are still present (Donato et al., 2021).

In interpreting our data, it should be considered that the surveys were carried out employing an extensive sampling protocol conceived to assess wide surface densities on coastal areas investigating across several habitat types. Therefore, literature density values focused only on local areas or habitat patchiness that were not randomly selected must be contextualized when compared with these data. In addition, given the scale of the prsented surveys, emphasis must be given to *P. nobilis* absence data of which the literature appears poor. Indeed, contrary to presence data, reliable absence data are difficult to obtain requiring much greater effort to rule out a rare occurrence (Gu & Swihart, 2004). The absence data obtained in this study derive from the merger of two different data types. The first one come from the local ecological knowledge arisng from the local fishermen intervew, which allowed us to confirm our data exluding pointlike occurrence in the same area. The other one dervies from a comprehensive view during the field surveys. Indeed the scuba diver overview was at least 10 times wider than 50 cm for side around the rope hence, the absence perception can be extended over a much larger investigated surface. Considering these two types of information together, we can assume the absence data exhaustive and full of information.

Conclusion

This study investigated different basins, habitat types, and bathymetries along the Apulian coast. The shells spatial distribution that arise from this study allowed us to obtain important information on the species throfic ecology. Indeed, the species distributional pattern showed a strong overlap with seagrass meadows on meso and macro geographical scale, however this was not the case on a micro scale. This result indicates that although there is a strong relationship between *P. nobilis* and seagrass meadows, it is not limited to the habitat patch but crosses the boundaries of seagrass. This result led us to the conclusion that the distribution of *P. nobilis* display a trophic link through the cross-boundary subsidy occurring from seagrass meadows to the nearby habitat, by means of the refractory detrital pathway.

No live specimens of *P. nobilis* were found in more than 800 km of coastal line, leading us to the conclusion that the coastal and lagoon population had totally collapsed in the region after the MME. The seriousness of the situation on the Apulian coasts, just as in the other Mediterranean ecoregions, indicates that the MME that began in 2016 is still in progress, and no local population in the Mediterranean can be considered safe. Given the gravity of the current situation, it is vital for species preservation to extend the survey across the entire Italian coast to gain a overall picture of the status of the *P. nobilis* population on a national scale. Indeed, other regions may reveal the existence of natural shelters, where live populations of *P. nobilis* may still persist. If this is the case, it is essential to identify and protect them in time. This initiative should be conducted in parallel by all the nations of the Mediterranean basin to implement standard guidelines for the monitoring, protection and recovery of this critically endangered species.

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Figures

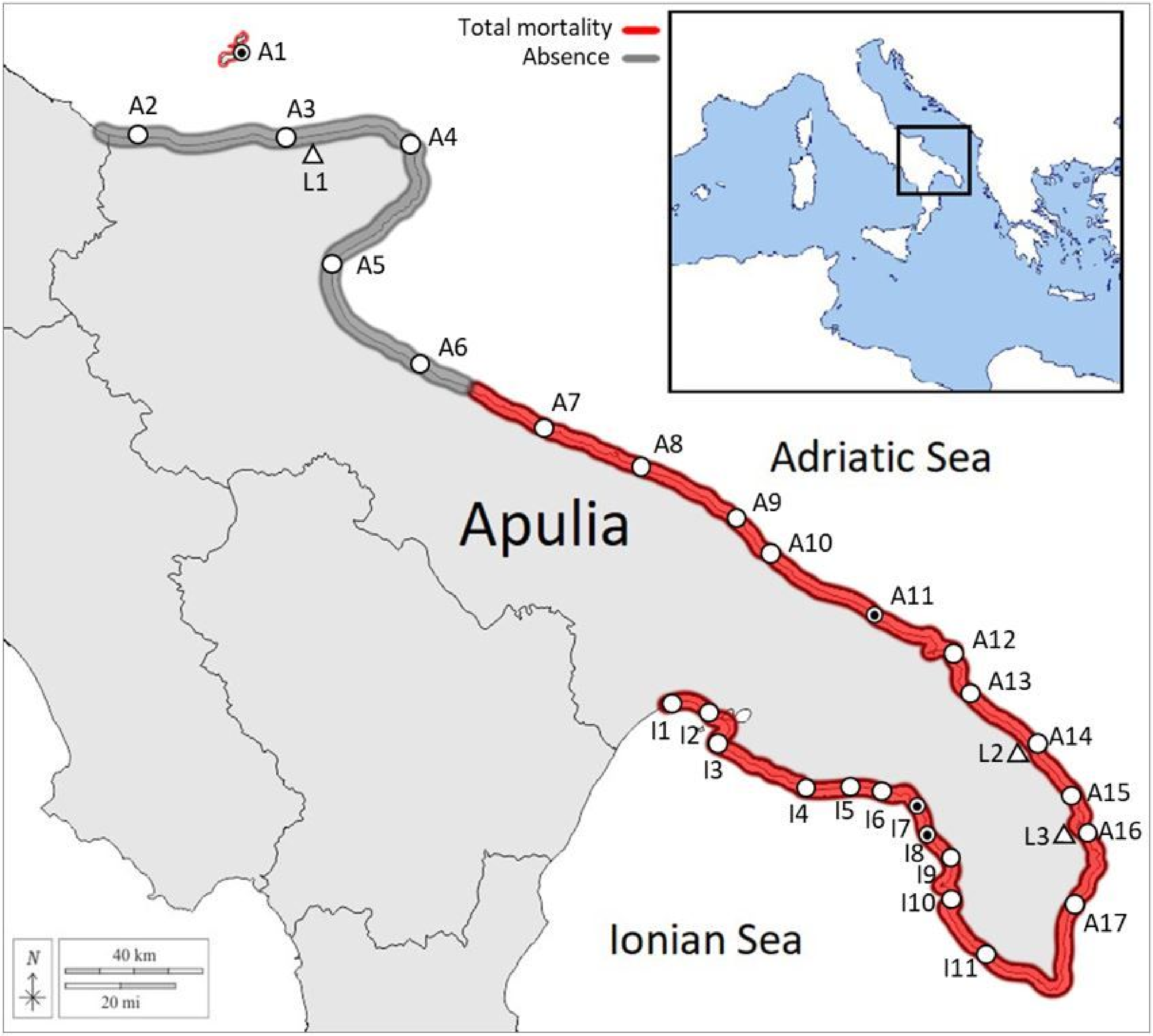

Figure 1

Survey areas and P.nobilis distribution; Grey line absence; Red line dead populations;

Marine Protected Areas ● Coastal ecosystems △ Lagoon ecosystems

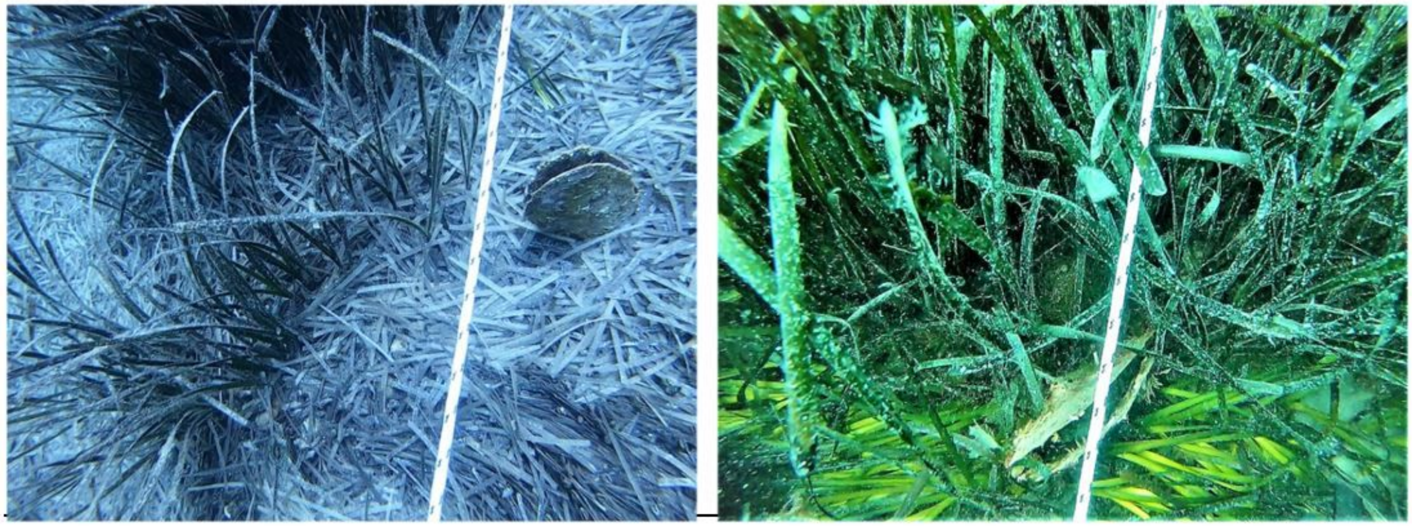

Figure 2

Underwater images showing field surveys with Pinna nobilis shells beside the rope; a: 15m; b: 5m

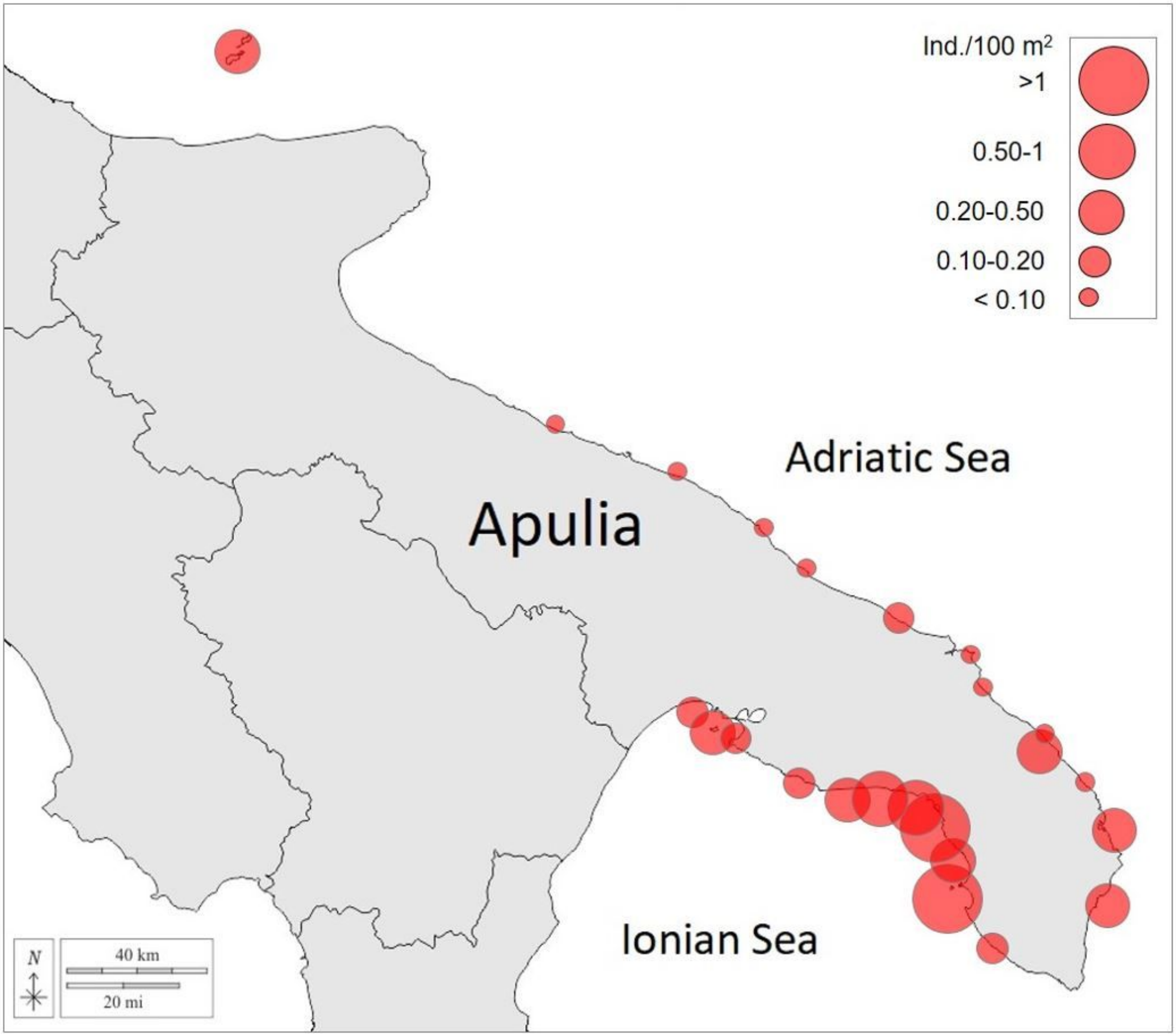

Figure 3

Local densities of P. nobilis in terms of dead shells/100m^2