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MELIH ERTAN CINAR, MURAT BİLECENOĞLU,
MEHMET BAKI YOKEŞ, HARUN GÜÇLÜSOY

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***Pinna nobilis* in the south Marmara Islands (Sea of Marmara); it still remains uninfected by the epidemic and acts as egg laying substratum for an alien invader**

Melih Ertan ÇINAR¹, Murat BİLECENOĞLU², Mehmet Baki YOKEŞ³ and Harun GÜÇLÜSOY⁴

¹Ege University, Faculty of Fisheries, Department of Hydrobiology, 35100, Bornova, İzmir, Turkey

²Aydın Adnan Menderes University, Science and Art Faculty, Department of Biology, Aydın, Turkey

³AMBRD Doğa Bilimleri, Hanımefendi Sokak 160/6 34384 Şişli, İstanbul, Turkey

⁴Dokuz Eylül University, Institute of Marine Sciences and Technology, 35330, İnciraltı, İzmir, Turkey

Corresponding author: melih.cinar@ege.edu.tr

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Abstract

A total of 12 *Pinna nobilis* beds were found and studied at depths varying between 2 and 6 m in the south Marmara Islands (Sea of Marmara). Fan mussel individuals in the beds were healthy with a few old dead specimens, indicating that the epidemic, which has devastated *P. nobilis* populations in the Mediterranean Sea, has not reached the Sea of Marmara, making the region a refuge area for the species. The average density of *P. nobilis* in the area varied between 0.6 ind.10 m⁻² and 24 ind.10 m⁻². The *P. nobilis* shells overall provided substrata or refuge for 14 species (10 sessile and 4 motile organisms), from macroalgae to fish. Shells of juvenile and adult specimens had different species assemblages. Four distinct assemblages were detected on shells, primarily formed by the red alga *Gracilaria bursa-postaris*, egg cocoons of the invasive alien *Rapana venosa*, the gastropod *Bittium reticulatum* and the serpulid polychaete *Spirobranchus polytrema*.

Keywords: Fan mussel; healthy individuals; critically endangered species; associated biota; Sea of Marmara; *Haplosporidium pinnae*.

Introduction

The fan mussel *Pinna nobilis* Linnaeus, 1758, endemic to the Mediterranean Sea, widely occurs in the shallow-water sandy environments (with or without seagrasses) of the Mediterranean Sea, but rarely in rhodolith and boulders beds, and lives as deep as 60 m (Butler *et al.*, 1993; Kersting & García-March, 2017). It is the largest bivalve species in the region, reaching a shell length of up to 120 cm and a life span that can exceed 45 years (Rouanet *et al.*, 2015). It plays important ecological functions in the benthic ecosystem, i.e. filtering seawater, removing suspended particulate matter and enhancing water transparency (Trigos *et al.*, 2014); providing a suitable substratum by its wide-surface shells for a large number of species belonging to different taxonomic groups (Addis *et al.*, 2009; Rabaoui *et al.*, 2009). This species has also a commensal relationship with the pea crabs *Nepinnotheres pinnotheres* and *Pinnotheres pisum*, and the shrimp *Pontonia pinnophylax* (Rabaoui *et al.*, 2008; Becker & Türkay, 2017).

As *Pinna nobilis* forms beds in the shallow-water benthic environments where several human activities (i.e.

fishing, recreation) take place, populations of the species have drastically declined over the last decades (Katsanevakis, 2006; Acarli *et al.*, 2011; Hendriks *et al.*, 2013). Consequently, it has been listed as an endangered species and is under protection according to the EU Habitats Directive 92/43/EEC (Annex IV), the Protocol for Specially Protected Areas and Biological Diversity in the Mediterranean (Barcelona Convention, Annex II) and national legislations of most Mediterranean countries, including Turkey.

Since the late 2016, mass mortalities of *P. nobilis* have been described in the western Mediterranean and Aegean Seas, due to a suddenly developed disease, which was primarily caused by a sporozoan parasite, *Haplosporidium pinnae*, or by a set of pathogens including different bacteria and *H. pinnae* (Vázquez-Luis *et al.*, 2017; Catanese *et al.*, 2018; Cabanellas-Reboredo *et al.*, 2019; Katsanevakis *et al.*, 2019; Panarese *et al.*, 2019; Carella *et al.*, 2020; Scarpa *et al.*, 2020). Even in the Çanakkale Strait, which constitutes the southern gate of the Sea of Marmara, mass mortality of *P. nobilis* has been recently reported (Özalp & Kersting, 2020). This epidemic is seriously threatening the existence of *P. nobilis* in the region,

therefore, IUCN has recently categorized it as a critically endangered species (Kersting *et al.*, 2019).

The Sea of Marmara's two layered-water system provides a suitable environment for *P. nobilis*, due to its special characters such as high productivity and low salinity in surface waters (Özsoy & Altiok, 2016). The Black Sea-originated surface water layer (between 0-25 m depth) has lower salinity (around 18 psu near the İstanbul Strait and becoming more saline (22-24 psu) towards the Çanakkale Strait), but the Aegean Sea-originated deep water layer (>25 m depth) has high salinity (37-38 psu) across the Sea of Marmara (Özsoy & Altiok, 2016). *Pinna nobilis* has been sporadically recorded in the region since the first report by Forsskål (1775), but data on its population and health status have not been documented, except for a recent study by Öndes *et al.* (2020b), who mentioned a healthy, uninfected *P. nobilis* population around Erdek, which is close to the south Marmara Islands.

During an expedition with a completely different purpose (assessing alien species), we were able to dive and observe several sites in the south Marmara Islands. *Pinna nobilis* beds were frequently encountered in the shallow waters of certain areas and information about its population, health status, and shell-associated biota were gathered during the study.

Materials and Methods

Within the scope of the MARIAS project (Addressing Invasive Alien Species Threats at Key Marine Biodiversity Areas Project), a scientific cruise was organized between 05.09.2020-09.09.2020 to assess the composition and distribution of alien species (especially *Rapana venosa* and *Asterias rubens*) along the coasts of the south

Marmara Islands. For this purpose, 17 stations were selected from different parts of the region (Fig. 1), and the depths 0-25 m (if possible) were swept by SCUBA divers. The selection of stations was random, but attention was paid to put at least one station in all large islands and to put stations in different geographic directions of islands.

At each station where a continuous (covering large area, i.e. at least 50 m on a horizontal line) *P. nobilis* bed existed (at around 3 m depth), five replicate plots, each with an area of 10 m² (5 m x 2 m), were considered to count living and dead *P. nobilis* individuals. The replicates were randomly deployed and set at least 20 m apart from each other. The adult and juvenile specimens, which differ from each other in having different shell length (juvenile <20 cm) and morphology (thick raised scales on shell in juveniles), were counted separately. The species settled on shells of *P. nobilis* were identified by under-water observations and examinations of photographs taken at 7 stations (1, 3, 5, 9, 11, 15 and 17). The coverage of species (both sessile and motile) on shells was estimated using the photoQuad software (Trygonis & Sini, 2012; freely available at: <http://www.mar.aegean.gr/sonarlab/photoquad/index.php>). For the assessment of the species' percentage coverage, 100 points were uniformly applied over each shell.

In order to assess the species assemblages on *P. nobilis* shell surfaces, coverage values of species were considered, and the average coverage values derived from replicates were square-root transformed prior to the analysis. The Bray-Curtis index (Bray & Curtis, 1957) was applied to construct the triangular resemblance matrices. The assemblage multivariate pattern was explored and visualized using the Principal Coordinate Analysis (PCoA) (Torgerson, 1958) applied on the Bray-Curtis resemblance matrices at the species level.

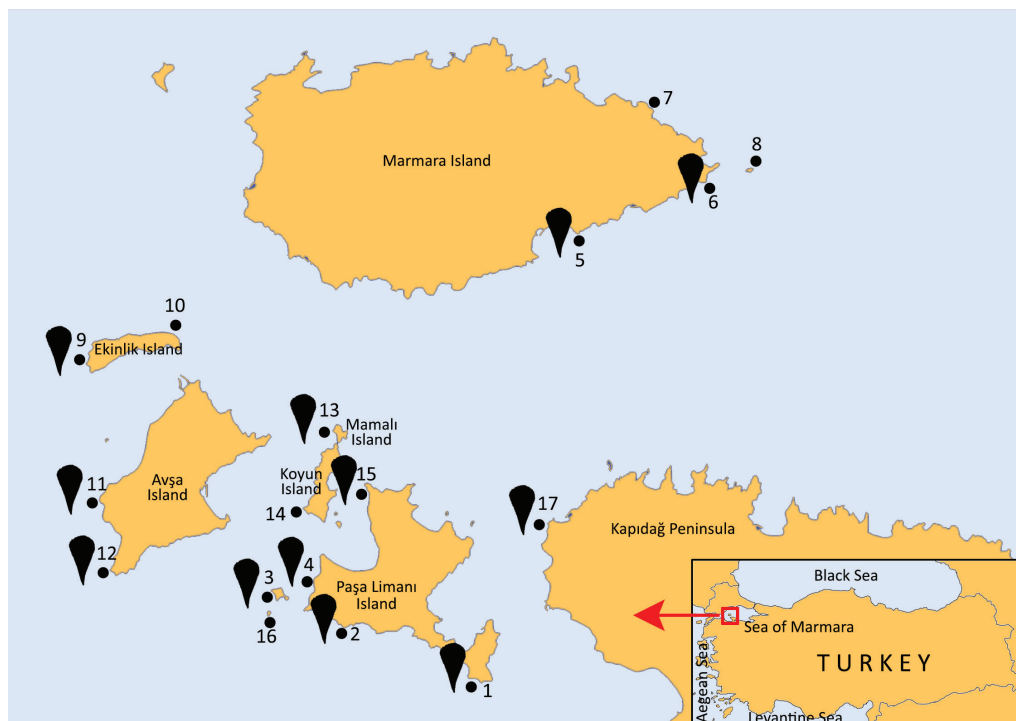


Fig. 1: Map of the investigated area (south Marmara Islands) with the locations of sampling sites. The black triangle shapes indicate the *Pinna nobilis* beds in the region.

Permutational Analysis of Variance (PERMANOVA) was applied to check 1) if the density of *P. nobilis* differed among stations (Euclidian distance matrix) and 2) if shells of adult and juvenile individuals of *P. nobilis* possessed different epibiotic communities (Bray-Curtis distance matrix). In the first PERMANOVA design, stations (12 levels) where *P. nobilis* was found, were selected as a fixed factor. In the second design, only one factor (the shell size) was considered (two levels, fixed).

Results

Density of *Pinna nobilis*

Continuous *Pinna nobilis* beds were found at 12 out of 17 stations in the study area. The depth range distribution of the bed was between 2-6 m depths, but some solitary individuals were observed at 12 m (max depth range of the species in the area). It largely inhabited sandy substrata with shell fragments, but specimens were also observed in *Cymodocea nodosa* beds (Fig. 2). The *P. nobilis* populations were mainly composed of adult specimens, but juvenile individuals (almost 5-10% of the populations) were also encountered. The density of the species differed among the sampled stations (PERMANOVA, $P < 0.01$) (Table 1). The highest density of the species (40 ind.10 m⁻²) was detected at station 3. The average density of the species ranged from 0.6 ind.10 m⁻² (± 0.54 SD, station 11) to 24 ind.10 m⁻² (± 11 SD, station 2) (Fig. 3). At four stations (1-3 and 9), the average density of the species was higher than 10 ind.10 m⁻².

Dead individuals of *Pinna nobilis*

Individuals in the *P. nobilis* beds in the region showed no signs of the disease. Dead individuals were very rare, observed only at stations 3 (five individuals) and 5 (one individual). However, dense epibiotic settlements on shells indicated that they were old dead individuals. No apparent human impact (e.g. anchorage) was detected in the *P. nobilis* beds, except for station 17 where a ghost net completely covered 5-6 *P. nobilis* individuals (Fig. 2B).



Fig. 2: General view of a *Pinna nobilis* bed at station 9 (Ekinlik Island) (upper picture) and fishing net-covered *P. nobilis* individuals in a *Cymodocea nodosa* bed at station 17 (Kapıdağ Peninsula) (lower picture).

Epibiotic communities of *Pinna nobilis*

The large surface area of *P. nobilis* shells attracted many species, from algae to fish. A total of 14 species [10 sessile (see Fig. 4) and 4 motile (*Bittium reticulatum*, *Gibbula adansoni*, *Paracentrotus lividus* and *Tripterygion tripterenotus*)] were observed on shells. The species occupying large areas on shells differed among stations

Table 1. Overall PERMANOVA tests applied on the density of *Pinna nobilis* at locations (based on Euclidian distance) and the epibiotic assemblages (based on Bray-Curtis similarity) on shells of different growth stages of *P. nobilis* (juvenile and adult).

	Degree of Freedom	Sum of Squares	Mean Squares	Pseudo-F	P(perm)
Station	11	2848.8	258.9	17.8	0.001
Residual	46	669.07	14.5		
Total	57	3517.9			
Growth stage	1	3078.4	3078.4	2.68	0.04
Residual	5	5737.1	1147.4		
Total	6	8815.5			

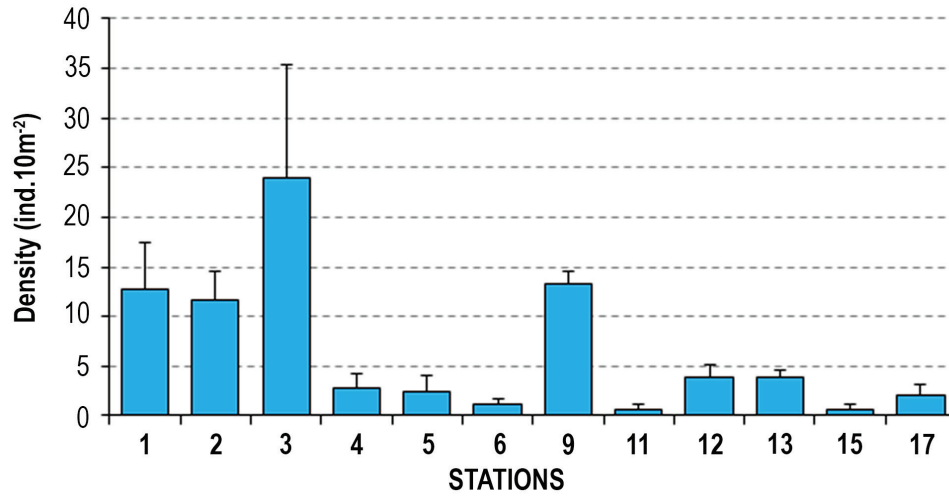


Fig. 3: Mean population densities of *Pinna nobilis* at stations (Bar indicates + standart deviation).

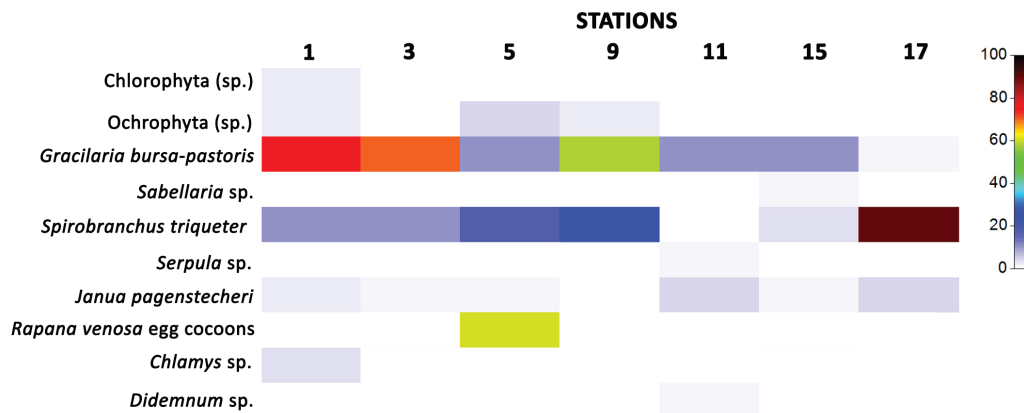


Fig. 4: The mean percent coverages of the species associated with *Pinna nobilis* shells.

(Fig. 4). The red alga *Gracilaria bursa-pastoris* had the highest percent coverage on shells (coverage: 58-75%) at three stations (1, 3 and 9), whereas the serpulid *Spirobranchus triqueter* covered the majority of shell surface (90%) at station 17. At only station 5, the alien invader gastropod *R. venosa* densely laid eggs on shells of *P. nobilis*, occupying almost 60% of the total shell surface.

In this small spatial scale, four different epibiotic communities were assessed, based on the Bray-Curtis similarity value higher than 50% (Fig. 5). The first two axes of the PCoA graph explained 77% of the total variance. Shells of adult and juvenile (at stations 11 and 15) individuals of *P. nobilis* possessed significantly different epibiotic communities (Table 1, Permanova, $P < 0.05$). Shell surfaces of adult *P. nobilis* were densely occupied by alga, *R. venosa* egg cocoons or serpulid polychaetes (Figs 5, 6), with almost no empty surfaces available, whereas the majority of shell surfaces of juvenile *P. nobilis* were empty, with only 16-18% of surface area being covered, mainly by filamentous algae (10%), *Bittium reticulatum* and serpulid polychaetes (4-5%).

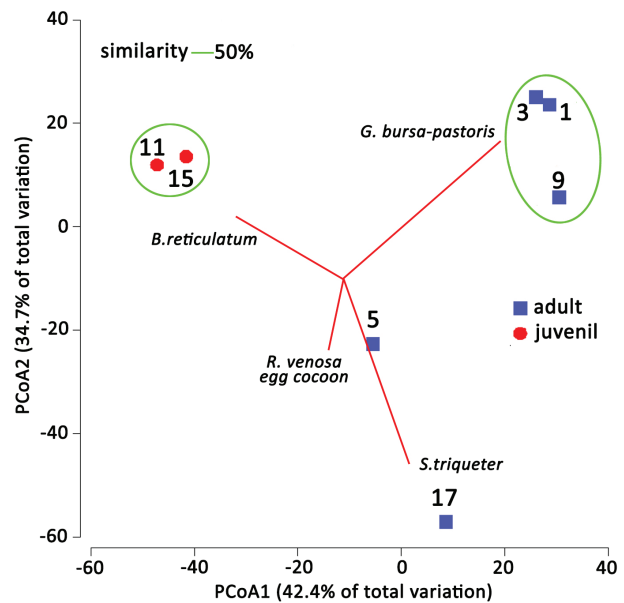


Fig. 5: Principal Coordinate Analysis (PCoA) showing the similarity among stations based on coverages of species associated with *Pinna nobilis* shells. Vectors represent the species that are responsible for dissimilarity among different assemblages. Stations 11 and 15 had juvenile *Pinna nobilis*.

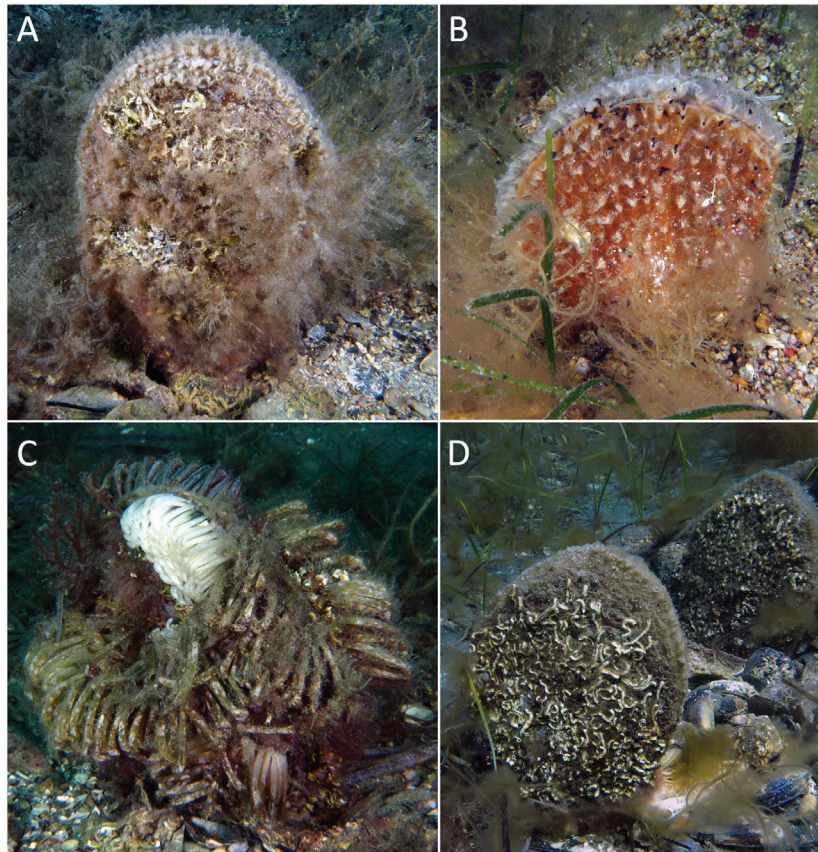


Fig. 6: Different species assemblages on shells of *Pinna nobilis* in the south Marmara Islands. A. *Gracilaria bursa-pastoris* dominated assemblage, B. *Bittium reticulatum* dominated assemblage, C. *Rapana venosa* egg cocoon dominated assemblage, D. *Spirobranchus triqueter* dominated assemblage.

Discussion

Pinna nobilis occurs widely in the shallow-water bare sand or sea-grass occupied sandy habitats along the coasts of Turkey with the exception of the Black Sea. Its first record in the region was given by Forsskål (1775) from the Sea of Marmara and the Aegean Sea. The northern distribution boundary of the species in the region was determined in the Istanbul Strait; between Kızkulesi and Tophane (cited as *Pinna pectinata* var. *fragilis* by Marion, 1898). In the following years, mainly in the Aegean Sea, data on its distribution (Geldiay & Kocataş, 1972; Pergent & Pergent, 1985; Okuş *et al.*, 2007; Gönülal & Güreşen, 2014), growth (Demirci & Acarli, 2019), commensal relationship with other species (Acarli *et al.*, 2019), and culture and growth (Acarli *et al.*, 2011; 2018) have been acquired.

Little has been published about the density of *P. nobilis* in the region. In the cartography study carried out by Pergent & Pergent (1985) near Urla Harbour (Aegean Sea), it was indicated on the map that the *P. nobilis* density among *Posidonia oceanica* meadows reached up to 4 ind.10m⁻². The mean density of *P. nobilis* was estimated as 1.1 individuals.10m⁻² (highest density as 10 individuals.10 m⁻²) in the middle Aegean Sea (from Şakran to Özbek) (Öndes *et al.*, 2020a), and as 2.4 ind.10m⁻² in the Sea of Marmara (Erdek) (Öndes *et al.*, 2020b). The present study indicated that this species formed beds at different parts of the south Marmara Islands and built up

a mean density from 0.6 to 24 ind.10m⁻², with a maximum of 40 ind.10m⁻². Such a high density (90 ind.10 m⁻²) was also reported from the Çanakkale Strait (Özalp & Kersting, 2020). In the Greek coasts of the Aegean Sea, the density of the species locally exceeded 2 ind.10m⁻² in Souda Gulf (Crete) (Katsanavakis & Thessalou-Legaki, 2009), 1 ind.10m⁻² in Gera Gulf (Lesvos) (Tsatiris *et al.*, 2018), and 0.7 ind.10m⁻² (with a mean estimate of 0.3 ind.10m⁻²) in the southern Aegean Islands (Vafidis *et al.*, 2014). In the other sub-regions of the Mediterranean Sea, the average population density of this species was estimated as 2 ind.10m⁻² in the Adriatic Sea (Šiletić & Peharda, 2003), and 0.1 ind.10m⁻² in the western Mediterranean Sea (Coppa *et al.*, 2010).

Across the Mediterranean Sea, the *P. nobilis* populations have been subjected to a mass mortality event first appearing on the Spanish coast in Autumn 2016 (Vázquez-Luis *et al.*, 2017), possibly due to the sporozoan parasite *Haplosporidium pinnae* (Catanese *et al.*, 2018) or a mix of pathogens including *Vibrio* spp., *H. pinnae*, *Mycobacterium* sp. and *Perkinsus* sp. (Lattos *et al.*, 2020; Carella *et al.*, 2020). The pathogens seem to be host-specific as they do not infect the congeneric species *P. rudis*. The death rate is very high, reaching up to 90%-100% in the western Mediterranean (Vázquez-Luis *et al.*, 2017), 36%-100% in the Adriatic Sea (Çizmek *et al.*, 2020), 75%-100% in the Aegean Sea (Katsanavakis *et al.*, 2019; Öndes *et al.*, 2020b), 99% in the Çanakkale Strait (Özalp & Kersting, 2020). It seems that only two

years later, this epidemic reached from the Spanish coast to the north Aegean Sea (Katsenavakis *et al.*, 2019). The summer marine current system in the Mediterranean (Catanese *et al.*, 2018), an intermediate host (planktonic species) (Cabanellas-Reboredo *et al.*, 2019) or the movement of vessels (through ballast water) (Šarić *et al.*, 2020) might help these pathogens to spread across the Mediterranean. There has been evidence that the disease is closely related to the environmental variables, being active in temperatures above 13.5°C and in a salinity range between 36.5–39.7 PSU (Cabanellas-Reboredo *et al.*, 2019). It seems that the mortality is now at the gate of the Sea of Marmara (Özalp & Kersting, 2020). Nevertheless, the low mortality rate found in Erdek (10%, Öndes *et al.*, 2020b) and the southern Marmara Islands (<1%, old dead, present study) indicated that this severe epidemic has not reached to the region. The low salinity (around 22 PSU in the southern Marmara Islands) (Çinar *et al.*, 2020) in the Sea of Marmara due to influx by the Black Sea water seems to act as a barrier for these pathogens. However, to reach a reliable conclusion, the *P. nobilis* populations in the region should be monitored. Kersting *et al.* (2020) indicated the importance of unaffected population of *P. nobilis* on its survival in the Mediterranean Sea, which would act as a source population through which larvae are dispersed to affected areas via sea currents, a golden chance for its recovery.

The first data presented here regarding the biota associated with *P. nobilis* shells from the Sea of Marmara exerted very interesting results. The juvenile and adult *P. nobilis* shells included different epibiotic compositions. Unlikely, surfaces of juvenile shells were largely bare and occupied (especially on posterior margin) by the herbivorous *Bittium reticulatum*, which was assumed to feed on filamentous algae newly growing on shells. Contrary to other studies (Cosentino & Giacobbe, 2007; Rabaoui *et al.*, 2009), this study clearly indicated that the size of *P. nobilis* shells played an important role in the structure of the associated biota. In accordance with the present study, Banach-Esteve *et al.* (2015) postulated that juvenile and adults presented different species composition aggregated to shells. The shells in the present study had different species assemblages mainly characterized by abundant occurrence of; 1) *Gracilaria bursa-pastoris*, 2) *Bittium reticulatum*, 3) *Rapana venosa* egg cocoon, 4) *Spirobranchus triqueter*. These assemblages seem to be developed in specific biotic and abiotic conditions. The availability of reproducing individuals of *R. venosa* (present at station 5), the development stage of the species (juvenile or adult) and different environmental conditions (especially at station 17, high turbidity) at stations might have contributed in shaping the epibiotic communities.

It is important to note that shells of *P. nobilis* acted as egg laying substrata of the invasive gastropod *R. venosa*. Although the gastropod attaches its egg cocoons to every available hard substrata (including the shells of other *R. venosa* individuals, pers. obser.), it seems that its preferential substratum at station 5 was shells of *P. nobilis*. The availability of adult gastropod species seemed to be responsible for this dense settlement, but if any envi-

ronmental variable played a role in this phenomenon is unknown at this stage and it needs further investigation. On these shells, other sessile species such as *S. triqueter* were represented by low percent coverages. Egg cocoons formed a layer of 5-6 cm thickness on shell surfaces. The effect of dense egg cocoon aggregations on the feeding activity of *P. nobilis* and the epibiota is unknown at this stage. Cabanellas-Reboredo *et al.* (2010) reported the invasive alga species *Lophocladia lallemandii* on shells of *P. nobilis* and proved that the alga slightly decreased the trophic level of *P. nobilis*. Kersting & García-March (2017) found that the reduction in growth of juveniles of *P. nobilis* in the Columbretes Islands over the years co-occurred with the spread of *L. lallemandii*.

Contrary to the south Marmara Islands, *Pinna nobilis* shells were almost completely covered by *S. triqueter* in the Kapıdağ Peninsula (station 17), where dense human settlements occurred. Though not measured, relatively dense suspended material that diminished underwater visibility were observed in this area and it might have drastically affected the species composition associated with *P. nobilis*. *Spirobranchus triqueter*, a serpulid polychaete species, is known to densely settle on bottoms with shell fragments in the Sea of Marmara (Çinar *et al.*, 2015). It is considered as an opportunistic species, making use of available space quickly (Riley & Ballerstedt, 2005), occurring predominantly in stressed environments such as harbors (Çinar, 2006). It was also reported on *P. nobilis* shells, but with a low cover percentage (<4%) (Šiletic & Peharda, 2003; Plečaš, 2017).

The epibiotic communities of *P. nobilis* were rarely a subject of study in Turkey. Çinar *et al.* (2001) and Çinar (2003) reported relatively dense populations of *Platynereis dumerilii* (88% of total number of nereidid individuals), and *Salvatoria clavata*, *Haplosyllis spongicola* and *Syllis prolifera* (36% of total syllid individuals) on shells collected from the east coast of the Aegean Sea.

Conclusions

The present study indicated that the *Pinna nobilis* individuals in the south Marmara Islands were healthy and have not been affected by the rapidly spreading disease occurring in the Mediterranean and Çanakkale populations. It is very crucial to protect the populations from any anthropogenic impacts, as they would possibly act as a source population for the recovery of this species in the Mediterranean Sea. Therefore, the south Marmara Islands, which represent an important reservoir of *P. nobilis*, should be declared as a marine protected area and a monitoring program should be implemented. The reasons why *P. nobilis* populations in the Sea of Marmara (at least in the south Marmara Islands) have not been affected by the disease would be an interesting subject of study. Is it a matter of time or a matter of environmental conditions of the Sea of Marmara?

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