

The Effects of Mucilage Event on the Population of Critically Endangered *Pinna nobilis* (Linnaeus 1758) in Ocaklar Bay (Marmara Sea, Turkey)

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This paper aimed to understand the potential effects of the mucilage event on the critically endangered *Pinna nobilis* in Ocaklar Bay located at the southern Marmara Sea. Underwater surveys were carried out in October 2020 and July 2021. The study area covers 500 m² that was divided into 5 main zones having 100 m² areas (10×10 m). Then, each main zone was separated into sub areas covering 25 m² (5×5 m). The habitat structure, depth, and availability of the mucilage event were observed by SCUBA diving equipment in sub areas. During the underwater observations, the total number of dead and alive individuals was counted as 228 of which 130 individuals were alive and 98 were dead. The minimum and maximum population density (including both dead and alive individuals) of *P. nobilis* was found to be between 10 individuals per 100 m² and 112 individuals per 100 m² in the study area, respectively. The mortality rates were calculated as 35.96% and 16.12% for the years 2020 and 2021, respectively. This paper puts forward that the *P. nobilis* population could be resistant to extreme environmental stress and even juvenile individuals (smaller than 15 cm) were recruited in the study area during the mucilage event.

INTRODUCTION

The fan mussel *Pinna nobilis* is an indigenous species and the largest marine bivalve of the Mediterranean Sea. It can grow up to 1.2 m in length (Zavodnik et al., 1991) and survive up to 45 years (Rouanet et al., 2015). It lives with the tapered anterior buried in the soft bottom (seagrass meadows, mud, sandy mud, or gravel) (Tebble, 1966).

P. nobilis are filter-feeding organisms that can gather substances from their surroundings in order to feed. It is a good and sensitive bio-indicator for Mediterranean littoral

and water quality because of this property (Vicente et al., 2002; Natalotto et al., 2015). Many species, including annelids, ascidians, bivalves, bryozoans, cnidarians, crustaceans, echinoderms, macroalgae, gastropods, and sponges, use this species' hard surface as a viable habitat (Rabaoui et al., 2009; Acarli et al., 2010).

However, due to recreational and commercial fishing activities for food, the use of its shell for ornamental purposes, and inadvertent mortality by trawling and anchoring, the population of the fan mussel, which plays an ecological role, has been significantly diminished. The fan

mussel has been listed as an endangered and protected species Under Annex IV of the Habitats Directive (Council Directive 92/43/EEC). However, for the past five years, especially, *Haplosporidium pinnae*, a parasitic *Haplosporidium* species, has negatively affected the population of *P. nobilis* (Vázquez-Luis et al., 2017; Darriba, 2017; Carella et al., 2019; Katsanevakis et al., 2019). Thus, the species' status was upgraded from "vulnerable" to "critically endangered" at national level by the Spanish Sectoral Environmental Conference on July 17, 2017. Some biological aspects of *P. nobilis* such as reproductive cycle (Acarli et al., 2018), growth (Demirci & Acarli, 2019), culture (Acarli et al., 2011a), intraspecific relationships with other species (Acarli et al., 2019) have been studied by several scientists. Mass mortalities have been reported in the western Mediterranean, the Tyrrhenian Sea in Italy, and the Northern Aegean Sea (Catanesi et al., 2018; Carella et al., 2019; Katsanevakis et al., 2019; Nebot-Colomer et al., 2021). Meanwhile, numerous surviving individuals have been found in Spain Alfacos Bay (Ebro Delta) (Prado et al., 2020), the East of Corsica Diana Lagoon (Simide et al., 2019), Thau Lagoon (Foulquié et al., 2020), in Kalloni Gulf and Laganas Bay (Zotou et al., 2020) and in Schiaparelli Port-Cros Archipelago Marine Protected Area (Ruitton & Lefebvre, 2021). In Turkish exclusive economic zone, mass mortality has been reported for different locations in the Aegean Sea (Acarli et al., 2020; Öndes et al., 2020a), whereas the Çanakkale Strait, connecting the Aegean Sea and the Marmara, had 100% and 9.2% mortalities in the locations close by, respectively (Acarli et al., 2021).

A strong volume of mucilage organic matter generated by planktonic algal bloom was first detected in mid-autumn

2007 in the Marmara Sea (Aktan et al., 2008). On the other hand, mucilage was observed in greater abundance in the Marmara Sea starting from January 2021 than in previous years (Balkis-Ozdelice et al., 2021). The rising temperature of seawater, agriculture activities, domestic and industrial waste discharges, and overfishing all contribute to planktonic algal blooms (Flander-Putrlé & Malej, 2008; Yentur et al., 2013; Savun-Hekimoğlu & Gazioğlu, 2021). Mucilage sinking or accumulating on the bottom has the greatest impact on benthic life (Schiaparelli et al., 2007). Currently, the mucilage event was observed during the study period in 2021 in Ocaklar Bay, Erdek on the southern coasts of the Marmara Sea. The aim of this study was to reveal the effect of mucilage on *P. nobilis* population observed in Ocaklar Bay in the southern Marmara Sea.

MATERIAL AND METHODS

This study was carried out in two different phases both in October 2020 and June 2021 in Ocaklar Bay, coasts in the southern Marmara Sea (Figure 1). The images before the mucilage event in October 2020 and during the



Figure 1. Study area

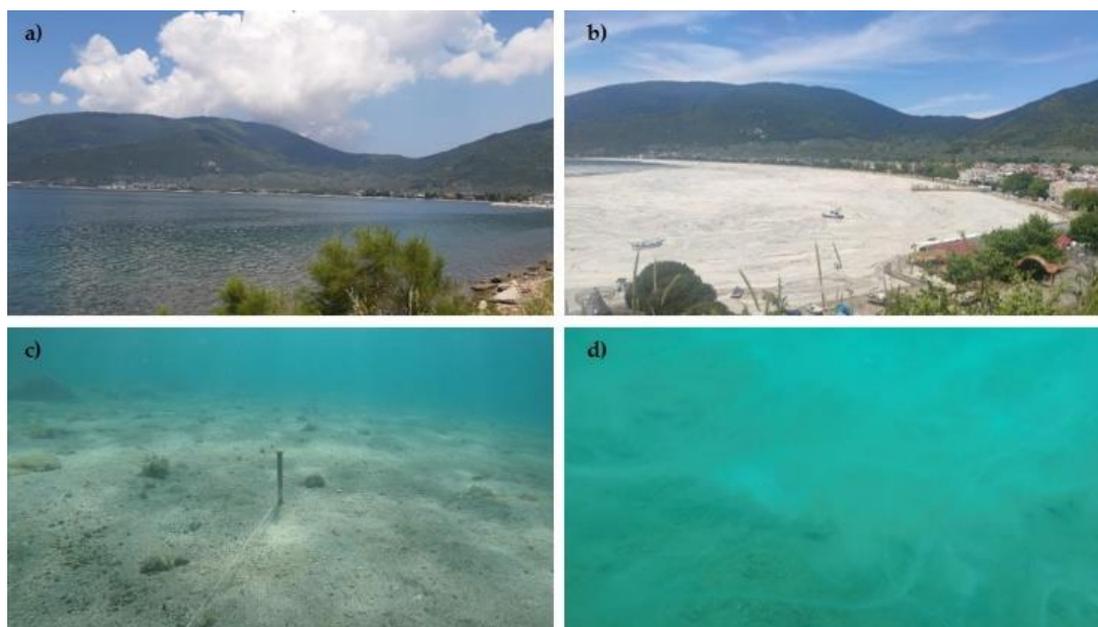


Figure 2. The surface images before (a) and during (b) the mucilage event, underwater images before (c) and during (d) the mucilage event in Ocaklar Bay

mucilage event in June 2021 were illustrated in Figure 2. A total of nine diving surveys were conducted by SCUBA diving equipment. The study area covered a total of 500 m² which was divided into 5 main zones (zones 1, 2, 3, 4, 5) that have 100 m² area (10×10 m). Then, each main zone was separated into sub areas covering 25 m² (5×5 m). The boundaries of the study area were determined with the help of a rope by driving the aluminium sticks to the seabed (see video from URL-1 at <https://youtu.be/OAeoPHISh-Y>). Each sub area has been coded (1A, 1B, 1C, 1D, 2A, 2B, ..., 5C, 5D). The numbers of alive and dead individuals in the sub areas were determined after the study area was prepared in October 2020. All dead and alive individuals were counted and measured in the sub areas in June 2021. The temperature and the depth of the sub areas were recorded by Oceanic GEO2. The habitat structure of the sub areas was noted to the underwater slate during the underwater surveys.

The maximum shell width (dorso-ventral) of all individuals (alive and dead) in sub areas was measured by in situ measurements without removing any dead/alive individuals. Measurements were performed using a scale with a precision of 0.1 mm and immediately recorded on the underwater writing slate (see video from URL-2 at <https://youtu.be/NMcGomt-7NY>). The data of the measured shell width of the individuals were used to calculate the total length of the individuals by adapting the equation which used raw data of Acarli et al. (2018).

$$TL = 2.7479W + 2.0128 \quad (r^2 = 0.7454) \quad (1)$$

In this formula, *TL* is the total length, *W* is the shell width of the individuals.

Table 1. Habitat structure, depth and temperature of the sub areas in the study area

Sub Areas	Habitat Structure	Min. Depth (m)	Max. Depth (m)	Temperature in June 2021 (°C)
1A	Boulder (20%), Sandy (80%)	1.8	2.1	27.8
1B	Boulder (25%), Sandy (75%)	1.5	2.1	26.1
1C	Boulder (10%), Sandy (90%)	2.1	2.4	25.6
1D	Sandy	2.1	2.4	25.6
2A	Sandy	2.4	2.7	25.6
2B	Sandy	2.4	3.0	25.6
2C	Covered by <i>Mytilus galloprovincialis</i>	2.7	3.7	25.6
2D	Covered by <i>Mytilus galloprovincialis</i>	3.0	3.7	25.6
3A	<i>Cymodocea nodosa</i> bed	3.7	4.6	25.6
3B	<i>Cymodocea nodosa</i> bed	4.0	4.9	25.6
3C	<i>Cymodocea nodosa</i> bed	4.6	5.5	25.0
3D	<i>Cymodocea nodosa</i> bed	4.9	5.5	25.0
4A	<i>Cymodocea nodosa</i> bed	5.5	6.1	24.4
4B	<i>Cymodocea nodosa</i> bed	5.5	6.1	24.4
4C	<i>Cymodocea nodosa</i> bed	6.1	7.0	24.4
4D	<i>Cymodocea nodosa</i> bed	6.1	7.0	24.4
5A	<i>Cymodocea nodosa</i> bed	7.9	8.8	23.9
5B	<i>Cymodocea nodosa</i> (40%), Sandy (60%)	7.9	8.8	23.9
5C	Sandy	8.8	9.4	22.2
5D	Sandy	8.8	9.4	22.2

RESULTS

The temperature ranged between 22.2°C and 27.8°C during the underwater surveys. The habitat structure, depth and temperature of the sub areas were presented in Table 1. The depth of the surveyed areas changed from 1.8 m to 9.4 m. The structure of the habitat consisted of boulders in the shallow waters while it was replaced with sandy and seagrass sediments in the deeper waters, and it was covered with sandy sediment in the deepest water of the study area.

During the underwater observations, the total number of dead and alive individuals was counted as 228 in October 2020. Among dead individuals, 73 were counted before the mucilage event while 25 dead individuals were counted after the mucilage. A total of 98 dead individuals was observed during the underwater observations. On the other hand, 130 alive individuals were observed in June 2021 in the study

area. It has been determined that 25.51% of the mortality rate occurred in 2021. In addition, it has been determined that two juvenile individuals (smaller than 15 cm) have recruited to the population during the mucilage event.

The number of individuals observed in each sub area, and the minimum and maximum length of alive and dead individuals are given in Table 2. No (dead or alive) *P. nobilis* individuals were encountered in sub areas 1D and 2B. However, no viable individuals were seen in sub areas 5C and 5D. On the other hand, *P. nobilis* individuals, which were observed to be dead during the mucilage event in the Marmara Sea, were found in sub areas 2D, 3A, 3B, 3C, 3D, 4A, 4B, 4C, and 4D. The total length of the dead individuals that died during the mucilage event were ranged between 32.24 cm and 52.85 cm. On the other hand, the minimum and maximum lengths of alive individuals were measured as 14.65 cm and 58.62 cm, respectively (Table 2).

Table 2. Total numbers and total length measurements of *P. nobilis* in the sub areas observed in October 2020 and June 2021

Sub Areas	N	Alive in June 2021		Dead in October 2020			Dead in June 2021		
		TL _{min} (cm)	TL _{max} (cm)	N	TL _{min} (cm)	TL _{max} (cm)	N	TL _{min} (cm)	TL _{max} (cm)
1A	2	37.19	39.93	-	-	-	-	-	-
1B	6	14.93	39.11	-	-	-	-	-	-
1C	2	35.26	35.81	-	-	-	-	-	-
1D	-	-	-	-	-	-	-	-	-
2A	2	14.65	19.05	-	-	-	-	-	-
2B	-	-	-	-	-	-	-	-	-
2C	3	39.38	54.77	-	-	-	-	-	-
2D	4	39.66	49.83	1	44.61	44.61	1	44.88	44.88
3A	25	33.06	55.32	13	39.11	54.22	4	40.76	49.83
3B	21	38.29	58.62	7	40.76	45.43	2	49.28	50.38
3C	11	38.29	51.75	11	40.76	51.75	2	41.58	49.00
3D	10	23.45	49.28	5	39.11	54.77	6	40.76	51.20
4A	7	42.96	49.83	11	39.11	57.80	2	45.98	52.85
4B	7	32.79	49.00	5	35.54	52.02	5	41.86	49.00
4C	9	38.01	48.73	8	34.99	51.48	1	32.24	32.24
4D	14	29.77	54.22	6	32.24	50.10	2	43.23	51.48
5A	5	41.86	47.90	2	47.35	48.73	-	-	-
5B	2	47.63	48.73	1	51.48	51.48	-	-	-
5C	-	-	-	1	56.97	56.97	-	-	-
5D	-	-	-	2	38.83	45.98	-	-	-
Total		130			73			25	

Note: N: sample size; TL_{min}: minimum total length; TL_{max}: maximum total length

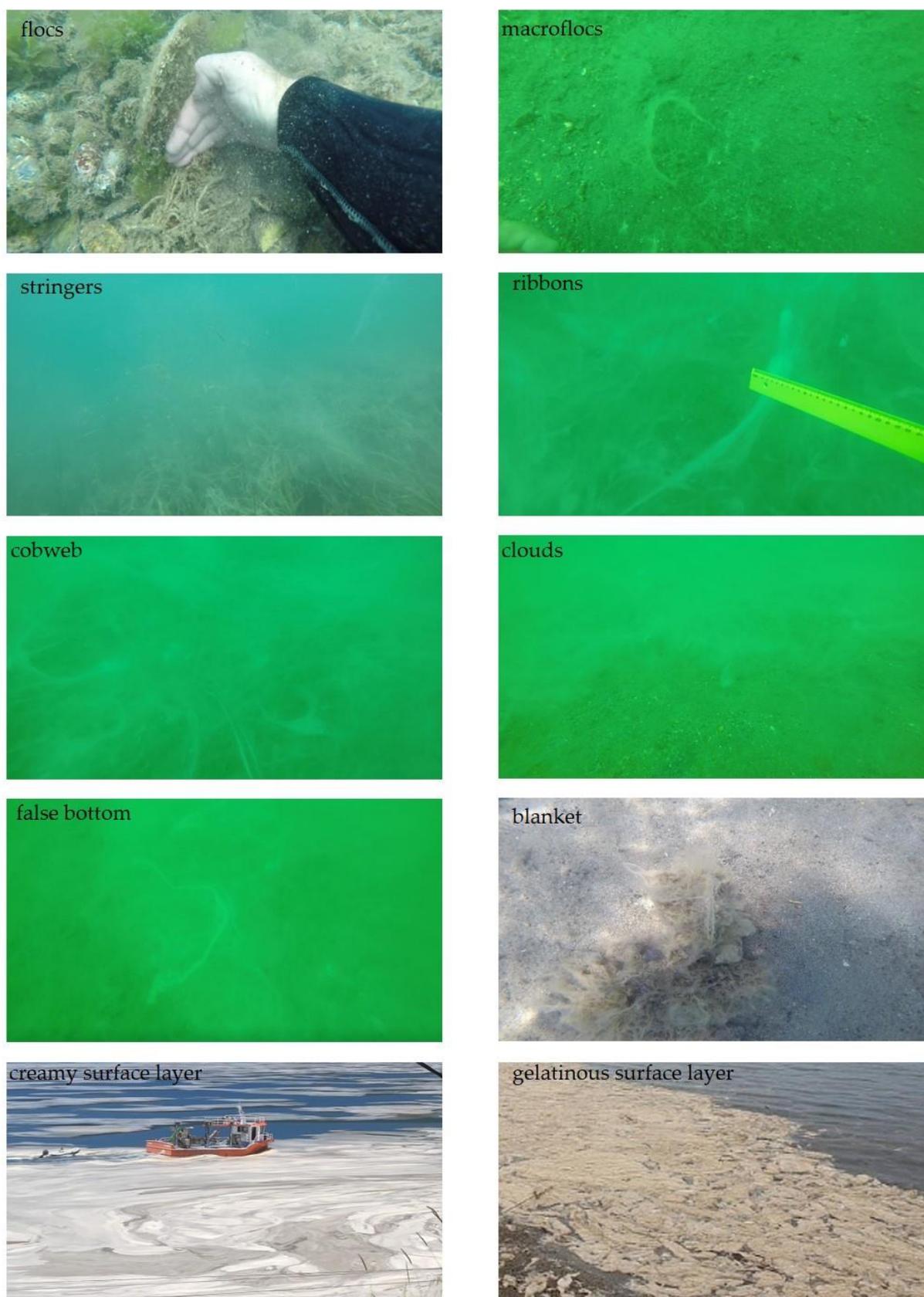


Figure 3. Types of mucilage observed in Ocaklar Bay during study period

The mucilage types were classified during the underwater observation as blanket type, cobweb, ribbons, stringers, flocs, macroflocs, cloud, and false bottom in June 2021. In addition, the mucilage types covering the sea surface were also observed as creamy surface layer and gelatinous

surface layer (Figure 3). During the underwater observations, it was determined that the mucilage event had a lively filamentous appearance at depths of 8 m and above with dense sandy ground and that they moved towards the shore with the current. It was observed that the dead form of the

mucilage settled on the *Cymodocea nodosa* meadows. The divers could easily clear the dead forms on *C. nodosa* with simple hand movements (see video from URL-3 at <https://youtu.be/Oij- -Uurlc>). It was generally observed that the mucilage could not reach shallow waters by accumulating on the *C. nodosa* meadows lying parallel to the coast in the study area.

It is promising that the survival rate was found to be 100% in 5 sub areas (1A, 1B, 1C, 2A, 2C) in the study area. Unfortunately, no live individuals were found in the sub areas 5C and 5D. However, all deaths in the sub areas 5C and 5D were determined to occur during the mucilage event. It was determined that dead individuals detected during the underwater observations carried out in October 2020 before the mucilage event were observed intensively between zones 3 and 4 (Figure 4). The population density (dead and alive individuals) of *P. nobilis* was found to be between 10 individuals per 100 m² and 112 individuals per 100 m² in the study area (Figure 5).

DISCUSSION

This is the first study reporting the impacts of the mucilage events on *P. nobilis* populations in a previously unaffected marine area along the coast of Ocaklar Bay in the southern Marmara Sea. The presence of temperature anomalies with hydrodynamic regime (i.e., current speed and water mass turnover) may trigger the increasing frequency of mucilage outbreaks (Danovaro et al., 2009). Savun-Hekimoğlu & Gazioğlu (2021) indicated that the

temperature in the Marmara Sea had risen by 2 to 2.5°C over the last 20 years, well above the global norm. Likewise, Acarli & Ayaz (2015) reported that sea surface temperature (SST) was recorded as 22°C and 21°C in July 2011 and July 2012 in Ocaklar Bay, respectively. In the present study, the temperature of seawater ranged between 22.0°C and 27.8°C in accordance with the depth. The variation in the temperature of seawater on the bottom was greater than Acarli & Ayaz (2015) and supported the findings of Savun-Hekimoğlu & Gazioğlu (2021) which reported an increase in the temperature of seawater in the Marmara Sea. Balkis-Ozdelice et al. (2021) noted that the mucilage event was found to be more extreme in 2021 than in previous years. Therefore, it can be concluded that the rise in the temperature of seawater has triggered the mucilage event.

Stachowitsch et al. (1990) defined mucilage types that are macroflocs, stringers, clouds, creamy surface layers, and gelatinous surface layers. This classification was taken into account not only on the size and shape, but also takes relative position in the water, stability, behaviour, and effect on benthos in consideration. On the other hand, Precali et al. (2005) described the mucilage types as blanket type, cobweb, ribbons, stringers, flocs, macroflocs, cloud, and false bottom, creamy surface layers and gelatinous surface layers in the Adriatic Sea. Mr. Engin Algan, the head of Marmara Island Fisheries Cooperative in Turkey, informed that the mucilage event was appeared firstly in October 2020 and clings to purse-seine nets in the fishing gears used by fishermen in Marmara Island (Personal communication).

Total Number of Dead and Alive Individuals

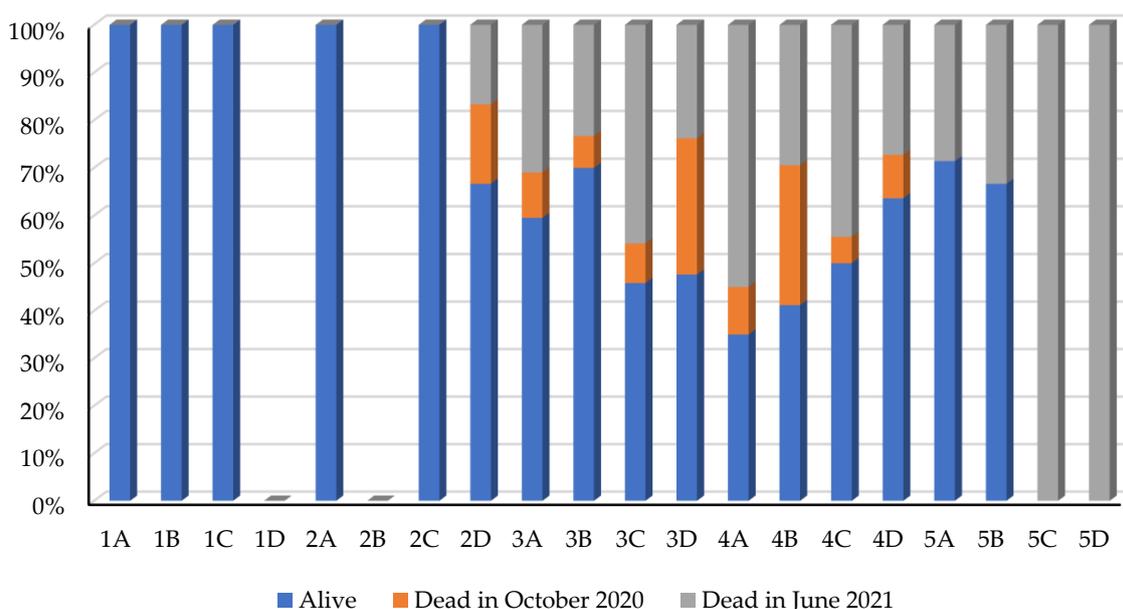


Figure 4. Total number of dead and alive individuals in sub areas

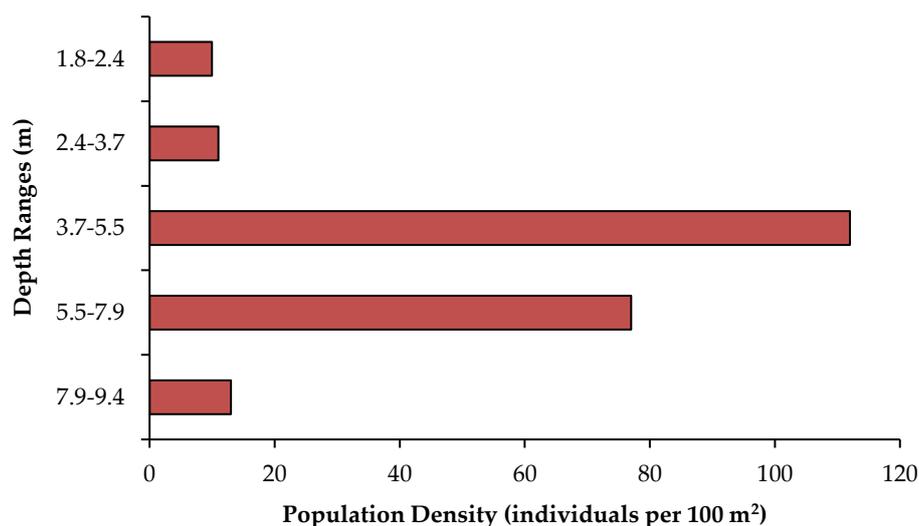


Figure 5. The population density (dead and alive individuals) of *P. nobilis* in Ocaklar Bay

When the first mucilage event occurred, it was described as the creamy surface layer type in the sea surface of Ocaklar Bay in March 2021. Subsequently, the mucilage types were also classified during the underwater observation as blanket type, cobweb, ribbons, stringers, flocs, macroflocs, cloud, and false bottom in June 2021 as defined by Precali et al. (2005). Altın et al. (2015) reported that by covering the benthic habitat and generating anoxic conditions, blanket type mucilage could induce a decrease in the amount of food in the bottom or prohibit food intake in the benthic welling. In addition, the benthic organisms include the communities of bivalve, echinoids, crustacean, polychaetas holothurians and sipunculids, sponges, sea squirts, sea stars and barnacles could be affected due to anoxic conditions caused by mucilage (Stachowitsch, 1980; Rinaldi et al., 1995; Pellegrini et al. 2003; Schiaparelli et al., 2007). The hypoxia-sensitive groups are fish, crustaceans, echinoderms, molluscs (most to least) (Gray et al., 2002). Ascidians, anthozoans and molluscs are distinctly more tolerant than polychaetas, decapods, and echinoderms to hypoxia (Riedel et al., 2014). In this study, the mortality rate was found to be 16.12% in the *P. nobilis* population in July 2021 during the mucilage event. On the other hand, Crustacean species such as *Eriphia verrucosa* and *Carcinus aestuarii* were not seen during underwater surveys carried out according to the data obtained through citizen science. Higher resistance of mollusc species than other species to the environmental stress could cause the occurrence or absence in the study area.

Katsanevakis (2007) documented that the natural mortality rate is lower in *P. nobilis* population. However, some authors noted that death was encountered in *P. nobilis* populations due to human activities, including tourism and fishing activities (Deudero et al., 2015; Öndes et al., 2020b). It has been observed that there is no tourism-related damage

on the stocks. The damage caused by the mooring anchors was not observed in the study area. The mortality rate of old dead was calculated as 35.96% in the present study. When the inner shell surface of the dead individuals was examined, it was determined that many macrobenthic organisms settled on these dead individuals. Considering the density and size of these macrobenthic organisms (Bivalve, Gastropod, tragona [described by Acarli & Ayaz, 2015]), the death of *P. nobilis* was predicted to be long time ago. Deaths that occurred before the year 2020 are not thought to be due to the disease. Because it has been determined that healthy individuals perform the closure of the shells quickly during underwater surveys. However, Acarli et al. (2021) highlighted that the closure of the shells of infected individuals was quite slow in *P. nobilis* stocks in the Çanakkale Strait where high mortality occurred. It can be concluded that anthropogenic activities could cause higher mortality than natural mortality although the mucilage event has already influenced the population in the study area. It was mentioned that individuals up to 15 cm in length are less than 1-year-old in some studies (Kožul et al., 2011; Acarli et al., 2011b; Demirci & Acarli, 2019). Katsanevakis (2006) pointed out that individuals in the group of the youngest age had the highest densities in the bathymetric zone between 1 and 3 m depth, and the density of the youngest age group was higher in poorly sorted sediments (the quartile deviation of grain size distribution is larger than 1). The author has also noted that no *P. nobilis* individual has been reported deeper than 22 m depth. It was promising that recruited individuals were also observed in the study area. Healthy juvenile individuals were found between 1.8 m and 2.4 m in June 2021 although they were not observed during the underwater surveys in 2020 (see video from URL-4 at <https://youtu.be/F1NBga3mFgM>). The total lengths of healthy juvenile individuals were measured as 14.65 cm and

14.93 cm. It can be concluded that juvenile individuals attached to the ground and grow up during the mucilage event. Moreover, no dead *P. nobilis* was observed until 3.0 m water depth. There was only one dead individual in sub area 2D (depth ranged between 3.0 m and 3.7 m) before the mucilage event. Then, one more *P. nobilis* individual was found to be dead in the sub area 2D during the mucilage event. Adult *P. nobilis* individuals (larger than 15 cm) were observed to become denser after the depth of approximately 3 m. It suggests that adverse environmental effects such as mucilage event in the coastal waters up to 3 meters may not have a negative effect on the survival rate of the fan mussel alone. It's possible that at low depths, simple wave movement and tidal currents were enough to disperse the mucilage (Devescovi & Iveša, 2007). The fact that the effect in this bathymetric zone is lower than at other depths suggests that the effect of mucilage may have been further reduced due to wave activity at these depths.

Although there are several documents reporting mass mortality in *P. nobilis* population in the Marmara Sea (Çınar et al., 2021), the results of the present paper are promising for ensuring the sustainability of *P. nobilis* stocks in the Marmara Sea. Transplantation applications of the *P. nobilis* as recommend by Acarli (2021) must be acted to ensure the sustainability of healthy stocks. Therefore, further researches must remain to carry out to monitor the establishment and maintenance of healthy *P. nobilis* populations although several impacts of natural, environmental, and/or anthropogenic factors on the population have occurred.

CONCLUSION

Healthy *P. nobilis* populations were found at different locations in Ocaklar Bay, along the coasts of Kapıdağ Peninsula. In order to revitalize the damaged *P. nobilis* populations, it is very important to identify healthy populations or populations that are resistant to disease and mucilage and to protect these areas. Spats should be collected via collectors from healthy and/or resistant populations and they should be reared under controlled environment. Then, transplanting the juveniles collected from healthy and/or resistant populations to a new location and/or regions with collapsed populations would be effective in revitalizing the population. The survival of the juvenile individuals (smaller than 15 cm) during the mucilage event is promising in terms of ensuring the sustainability of the stocks. Herewith, a very important finding was obtained that the *P. nobilis* stock in the study area could be maintained despite the mucilage event.

Although the present study is a micro-study investigating the effects of mucilage event on *P. nobilis* population, it puts forward that *P. nobilis* population could be resistant to the

extreme environmental stress although it could not survive the disease caused by *Haplosporidium pinnae* parasite. Thus, studies should be continued for the monitoring of *P. nobilis* population in nearby areas (i.e., Kapıdağ Peninsula) affected by mucilage event.

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Compliance With Ethical Standards

Authors' Contributions

DA and SA designed the study and carried out underwater observations. SK managed the draft of the manuscript, statistical analyses and data management. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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