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RESEARCH ARTICLE

Current status of critically endangered fan mussel *Pinna nobilis* (Linnaeus 1758) population in Çanakkale Strait, Turkey

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ABSTRACT

The population of *Pinna nobilis* (Linnaeus, 1758) has been severely damaged, especially by the effect of the disease, in addition to the causes such as fishing activities, overexploitation, environmental pollution, habitat degradation, tourism, and human impacts. Particularly, *Haplosporidium pinnae* caused gigantic destruction in the *P. nobilis* population. Accordingly, the present study aimed to determine the current status of the *P. nobilis* populations in the Çanakkale Strait. Samplings were carried out between July and August 2020 at 9 different stations in the Çanakkale Strait. The results revealed that mass mortality was observed at the two stations (Abide Beach and Kumkale Village Beach) located near the Aegean Sea. On the other hand, it has been determined that the lowest mortality rate (9.62%) has been found at Ilgardere station. Juvenile individuals have been observed in healthy and uninfected populations with high survival rates. This paper is the first document on the spatial distribution of *P. nobilis* in Çanakkale Strait. Therefore, the *P. nobilis* population should be continuously monitored to ensure the sustainability of the species.

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Introduction

The fan mussel *Pinna nobilis* is an endemic species and the largest marine bivalve in the Mediterranean Sea. It can reach to 1.2 m (Zavodnik et al., 1991) and live up to 45 years (Rouanet et al., 2015). It is distributed in the coasts covered by the seagrass meadows, mud, sandy mud, or gravel, and it is buried in the soft bottom (or holds on to soft bottoms) by its byssus (Tebble, 1966).

P. nobilis are filter-feeder organisms that feed suspended organic/inorganic matters. Due to this characteristic, it is a good and sensitive bio-indicator for Mediterranean littoral quality and water quality (Vicente et al., 2002; Natalotto et al., 2015). In addition to the hard surface of this species, it serves as potential living habitat for many species such as annelids, ascidians, bivalves, bryozoans, cnidarians, crustaceans, echinoderms, macroalgae, gastropods, sponges (Rabaoui et al., 2009, Acarli et al., 2010). On the other hand, the population of ecologically important fan mussel has been greatly reduced due to recreational and commercial fishing activities to supply food, shell usage for decorative purposes, and incidental killing by trawling and/or anchoring. The fan mussel has been announced as an endangered and protected species under Annex IV of the Habitats Directive (Council Directive 92/43/EEC). Particularly, species of Haplosporidium parasite, *Haplosporidium pinnae* caused great destruction to the *P. nobilis* population for the last four years (Vázquez-Luis et al., 2017; Dariba et al., 2017; Carella et al., 2019; Katsanevakis et al., 2019). Moreover, the status of the species has been updated from "Vulnerable" to "Critically Endangered" by the Spanish Sectoral Environmental Conference on 17 July 2017, at the national level.

In Turkey, some characteristics of the species have been investigated such as growth (Acarli et al., 2011a; Demirci and Acarli, 2019), spat settlement (Acarli et al., 2011b; Kurtay et al., 2018), and gonad development (Acarli et al., 2018). However, there is an exclusive study on the determination of *P. nobilis* population in the Aegean Sea and the Marmara Sea (Acarlı et al., 2020; Öndes et al., 2020a). However, there is no study on the *P. nobilis* population in the Çanakkale Strait. Therefore, the present study aimed to determine the *P. nobilis* population in the Çanakkale Strait and to provide recent knowledge to the scientific literature about the current status of the species.

Material and Methods

This study was carried out at 9 stations in the Çanakkale Strait (Figure 1). Çanakkale Strait is located in the north-western part of Turkey and is a part of the Turkish Straits System including Bosporus Strait, the Marmara Sea, and Çanakkale Strait (Kale, 2020). It is a transition zone between the Aegean Sea and the Black Sea. The region has a typical transition climate type characterized by rainy and cold winters and dry and hot summers (Kale, 2020). Cengiz and Akbulak (2009) reported that July is the warmest month and January is the coldest month. Kale (2017a) documented that air temperature had an increasing trend in the region similar to the report of Kale (2017b) which indicates that trends in the evaporation were increasing.

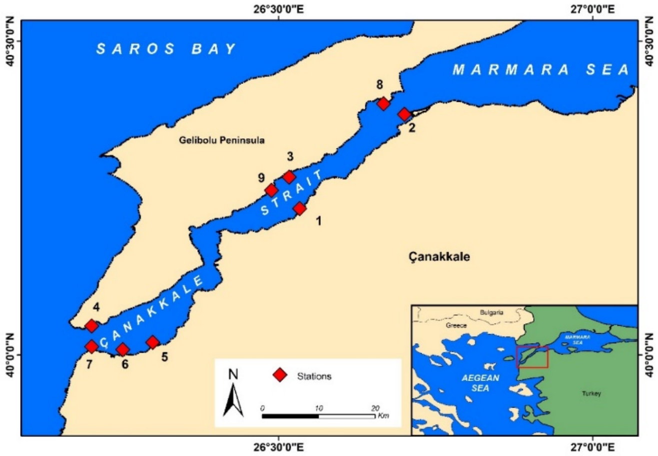

Figure 1. Study area (Station Names; 1: Yapıldak, 2: Çardak Kum Adası, 3: Cennet Bay, 4: Abide Beach, 5: İntepe, 6: Kumkale Harbour, 7: Kumkale Village Beach, 8: Hamza Bay, 9: Ilgardere)

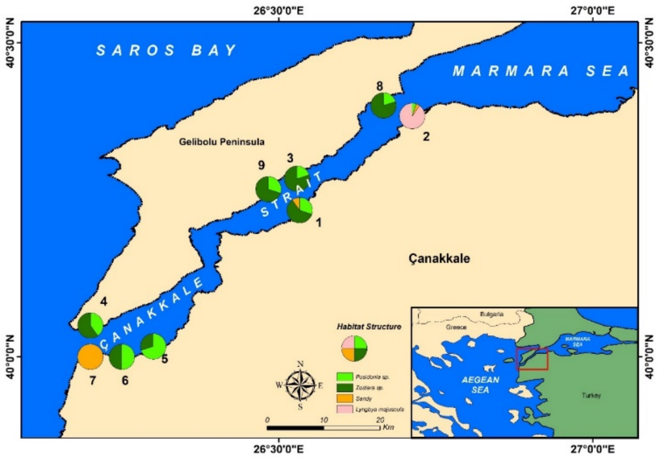

Figure 2. Habitat structure of the sampling stations in the Çanakkale Strait (Station Names; 1: Yapıldak, 2: Çardak Kum Adası, 3: Cennet Bay, 4: Abide Beach, 5: İntepe, 6: Kumkale Harbour, 7: Kumkale Village Beach, 8: Hamza Bay, 9: Ilgardere)

Monitoring fish and underwater habitats require non-destructive investigation techniques and this is usually succeeded by divers carrying out underwater visual census (UVC) (Pelletier et al., 2011). UVC is the most common approach to estimate the abundance, diversity, and size of fishes

Table 1. Descriptive information of the sampling locations

Station	Coordinates	Surveyed Area (m²)	Depth Range(m)	Habitat Structure
Yapıldak	40°14'017"N 26°32'460"E	1500	2-6	*Posidonia* sp. (30%) *Zostera* sp. (60%) Sandy (10%)
Çardak Kum Adası	40°23'003"N 26°42'542"E	3500	5	*Lyngbya majuscula* (90%) Dead *Posidonia* sp. + Sandy (10%)
Cennet Bay	40°17'544"N 26°31'128"E	2000	2-4	*Posidonia* sp. (20%) *Zostera* sp. (80%), (from shore to 2.5 m depth)
Abide Beach	40°03'148"N 26°12'497"E	2500	3-5	*Posidonia* sp. (40%) *Zostera* sp. (60%)
İntepe	40°01'025"N 26°18'861"E	2500	0-7*	*Posidonia* sp. (70%) *Zostera* sp. (30%)
Kumkale Harbour	40°00'053"N 26°15'624"E	3500	0-5*	*Posidonia* sp. (50%) *Zostera* sp. (50%)
Kumkale Village Beach	40°00'446"N 26°12'505"E	3000	4-6	Sandy (100%)
Hamza Bay	40°24'649"N 26°40'833"E	3000	4-5	*Posidonia* sp. (20%) *Zostera* sp. (80%)
Ilgardere	40°16'683"N 26°29'631"E	1500	3-4	*Posidonia* sp. (30%) *Zostera* sp. (70%)

Note: * indicates that no specimen was observed.

in clear waters. On the other hand, the estimation of the abundance by UVC approach is exclusively problematic in species that highly aggregated and/or observing at low densities due to their high mobility at both temporal and spatial scales (Irigoyen et al., 2018). The statistical strength of investigations including UVC approaches may be improved by increasing the number of replicates or the area explored. Alternatively, video-based techniques have become commonly used tools to observe underwater macrofauna and habitats (Pelletier et al., 2011). Therefore, SCUBA diving equipment was used to determine the current status of the *P. nobilis* population. Transect and visual census methods were used to estimate the abundance of the populations of *P. nobilis*. Depending on underwater visibility (2.5-10 m), divers detected the number of alive and dead *P. nobilis* individuals in a certain area underwater. At the same time, the UVC observation methodology was also supported with video-based tools (GoPro Silver model, and Nikon Coolpix 5600 model). The depths and temperature were measured with the Oceanic Geo 2 dive computer. The divers used an underwater tablet as an approximate estimator to measure the shell lengths of *P. nobilis* (dead or alive) underwater. Then, all measurements were photographed by the divers and all taken photos were analysed to determine unburied shell lengths by using an image processing toolbox in Matlab. The actual lengths of *P. nobilis* were calculated by the formula between the unburied shell length and total shell length given in Equation 1. Raw data provided by Acarli et al. (2018) were used in order to calculate this relationship.

$$a = 0.8061b + 28.61 \ (r^2 = 0.717) \qquad (1)$$

In this equation, a is calculated total length, b is unburied shell length.

Descriptive information about the sampling locations such as habitat characteristics, depth, and surveyed area are given in Table 1. Since the underwater habitat differs in each region, the divers first determined the transect distance, and then the surveyed area was calculated separately for the number of dead, alive and total (dead + alive shell) individuals per 1000 m². Shell lengths were measured by UVC techniques.

Results

During the underwater observations, the temperature was recorded as 27°C for Abide beach and Kumkale Harbour, 26.8°C for Kumkale Village Beach, 26.5°C for İntepe, 26.3°C for

Figure 3. Illustrations of alive *P. nobilis* observed during underwater observations in different habitats in the Çanakkale Strait

Ilgardere, 26°C for Cennet Bay Yapıldakaltı, Hamza Bay, and Çardak Kum Adası. It has been observed that *P. nobilis* individuals in small size groups were between 108 mm and 200 mm in length and they distributed throughout 1.5-2 m water depth. It has been determined that the majority of the individuals in the large size groups (>30 cm) are distributed between 4 and 6 m water depth. In addition, 90% of the individuals were observed in seagrass meadows of *Posidonia* sp. and *Zostera* sp. while the rest were completely found on the sandy habitat (Table 1). Habitat structure of the sampling stations in the Çanakkale Strait is presented in Figure 2.

Minimum and maximum shell length values were given in Table 2. A total of 494 fan mussel individuals were observed during underwater observations. Some illustrations taken during the underwater observations were shown in Figure 3. Total numbers of individuals observed at all stations in the Çanakkale Strait are demonstrated in Figure 4. It has been determined that 71.25% of *P. nobilis* individuals were dead. The spatial distribution of dead and alive individuals in the Çanakkale Strait was given in Figure 5. No individuals were observed at İntepe and Kumkale Harbour stations located near the Aegean Sea. Mass mortality was observed in Abide Beach and Kumkale Village Beach nearly located to the Aegean Sea whereas the least mortality was found in Ilgardere station (9.62%) located in the central part of the strait.

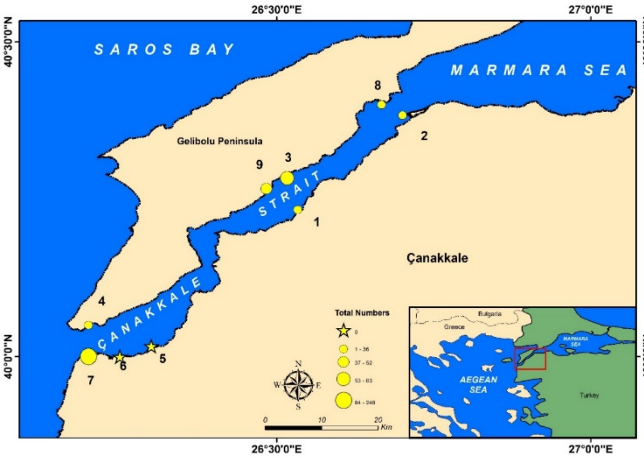

Figure 4. Total numbers of individuals observed in the Çanakkale Strait (Station Names; 1: Yapıldak, 2: Çardak Kum Adası, 3: Cennet Bay, 4: Abide Beach, 5: İntepe, 6: Kumkale Harbour, 7: Kumkale Village Beach, 8: Hamza Bay, 9: Ilgardere)

Table 2. Minimum and maximum shell length values of *Pinna nobilis* individuals measured by the underwater visual census in the Çanakkale Strait

Station	Alive		Dead		Total	
	N	Min-Max (cm)	N	Min-Max (cm)	N	Min-Max (cm)
Yapıldak	9	12.5-22.5	27	22.6-36.0	36	12.5-36.0
Çardak Kum Adası	7	22.4-28.2	17	28.3-35.3	24	22.4-35.3
Cennet Bay	65	11.3-30.0	18	30.1-37.4	83	11.3-37.4
Abide Beach	0	-	27	28.8-38.2	27	28.8-38.2
İntepe	0	*	0	*	0	*
Kumkale Harbour	0	*	0	*	0	*
Kumkale Village Beach	0	-	248	24.0-42.7	248	24.0-42.7
Hamza Bay	14	13.6-15.8	10	15.9-24.1	24	13.6-24.1
Ilgardere	47	10.8-29.1	5	29.2-34.7	52	10.8-34.7

Note: No *P. nobilis* individual was observed in this station.

On the other hand, the mortality rate was lower in Hamza Bay station, and Çardak Kum Adası stations located near the Marmara Sea. However, in the central part of the strait, one station (Yapıldak station) has a very high mortality, whereas the other two stations (Cennet Bay station, Ilgardere station) that very close to this station (Yapıldak station) have a very low mortality rate. Table 3 provides further information about the findings on the mortality at all stations.

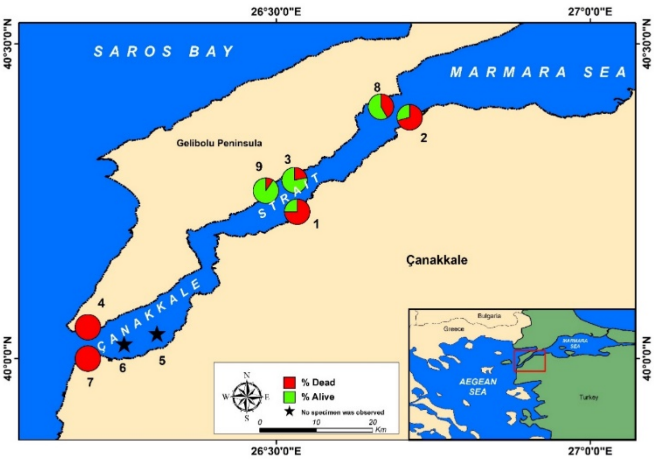

Figure 5. Spatial distribution of dead/alive individuals in the Çanakkale Strait (Station Names; 1: Yapıldak, 2: Çardak Kum Adası, 3: Cennet Bay, 4: Abide Beach, 5: İntepe, 6: Kumkale Harbour, 7: Kumkale Village Beach, 8: Hamza Bay, 9: Ilgardere)

Discussion

This study was carried out in 9 stations in order to determine the fan mussel population and to provide recent knowledge on the current status of the species in the Çanakkale Strait. The current status of the population should be monitored since the *P. nobilis* species could not preserve its existence in the Mediterranean and had high mortality rates. Cabanellas-Reboredo et al. (2019) indicated that the main reasons are higher salinity and water temperature (>13.5°C) for the increase of the parasite infections in *P. nobilis*. The authors also documented that mortality was found at the temperature of 26.5°C. Šarićet al. (2020) reported mass mortality between 13°C and 25°C temperature in the Adriatic Sea. However, non-infected areas where less exposed to major sea currents have also been detected by the authors. Trigos (2015) indicated that the high temperatures of the seawater during the summer months could have decreased the physiological capacity of *P. nobilis* to resist parasite infections since respiratory requirement will be increased at high temperatures. In the present study, the temperature was recorded between 26°C and 27°C during the underwater observations in July and August 2020. The temperature (when combined with other stressors) may have a fatal impact on the population of *P. nobilis* particularly at stations that mass mortality (100%) has occurred. However, the mortality rate was found in other stations. Šarić et al. (2020) reported that areas less exposed to sea current were at lower risk of pathogen transmission. Although the transmission of the disease is commonly through the currents, the present study reports a different case for this common phenomenon. The spread of the disease occurs in the opposite direction to the currents in the strait. The current in the Çanakkale Strait has a two layer structure. The upper layer flows from the Marmara Sea to the Aegean Sea while the lower layer flows from the Aegean Sea to the Marmara Sea. The upper layer of the current sometimes reverses because of the meteorological conditions and the shape of the coasts (Başar, 2010). Similarly, Jarosz et al. (2012) estimated that large amounts of lower part of the current (approximately 43%-45% of the flow) reversed back to the Aegean Sea. Therefore, this inverse relationship between the spread and expected route of the disease could be related to the currents directions. The decrease of the mass mortality and lower spread of the disease could be associated with the reverse current.

Mass mortality (100% death) was observed in Kumkale Village Beach station and Abide Beach station located near the Aegean Sea similar to Öndes et al. (2020a). On the other hand, the mortality rate was found lower in the stations located near the coasts of the Gelibolu Peninsula (Cennet Bay, Hamza Bay, and Ilgardere stations). It is thought that the stations on these coasts are less affected by human activities since the Gelibolu Peninsula is under protection by legislative regulations. Spatial distribution, abundance and mortality of *P. nobilis* are affected by several factors such as environmental stress including variations of temperature, salinity, depth, wave height (García-March et al., 2007; Kurihara et al., 2018; Maoxiao et al., 2019) and human impacts (Deudero et al., 2015; Öndes et al., 2020b). Moreover, Deudero et al. (2015) claimed that anthropogenic impacts much more affected the coastal benthic communities than global environmental changes.

Acarli et al. (2018) indicated that the spawning period of *P. nobilis* was ranged from May to September (primarily in July) in İzmir Bay, Turkey. The settlement of *P. nobilis* was determined in August in the same region (Acarli et al., 2011b). Some authors reported that *P. nobilis* has reached 150 mm in length after one-year growth experiments (Kožul et al., 2011; Acarli et al., 2011a; Demirci and Acarli, 2019). In this study, individuals have generally been assumed to be over 1 year old in lights of the previously conducted researches and investigations on the reproduction and growth of the species, length measurements, and the prominence of the serrated protrusions on it. . During the underwater observations, healthy and uninfected juvenile individuals were determined. Newly attached individuals of the *P nobilis* species are resistant to manipulation and accordingly their survival rate is quite high (Acarli et al 2011b). Šarić et al. (2020) indicated that mortality in juveniles was lower, better reaction to *H. pinnae* in Sakarun (Dugi Otok) station. *Haplosporidans parasitize* infect marine and freshwater invertebrates such as mollusc and crustacean (Stentiford et al., 2013; Arzul and Carnegie, 2015). *Bonamia ostreae* and *H. pinnae* belong to the Haplosporiidae family (Catanese et al., 2018). *B. ostreae* has distributed in many countries where oyster stocks are depleted (Culloty and Mulcahy, 2007). It was first detected in 1979 in Brittany-France and later distributed in Spain, Denmark and England. Deaths (up to 90%) have been detected in uninfected populations. Factors such as high stocking density, handling, lack of nutrients, high temperature, and salinity have caused the spread of bonamiosis (Crawford, 2016). Mortality increases particularly at high water temperature (Engelsma et al., 2010). Arzul et al. (2009) declared that the mortality is more effective at higher temperature (25°C) compared to the lower temperature values (4°C and 15°C). Engelsma et al. (2010) indicated that a positive relationship was found between prevalence of *B. ostreae* and salinity. The authors indicated that higher salinity is more suitable for *B. ostreae.* Cáceres-Martínez et al. (1995) pointed out that a significant positive correlation between the mean total length and the presence of *B. ostreae* could be detected in an oyster at all sizes but the mortality rate is lower in individuals smaller than 2 cm shell length. Thus, infection increased as the size increased. Disease susceptibility seems to be age-related. Robert et al. (1991) and Culloty and Mulcahy (1996) noted that the age of 2 years comes out to be the critical age for the development of the disease. It can be concluded that the anamnesis of *B.* ostreae and *H.* pinnae shows similarities. Since the survival rate is high in juvenile individuals and the mortality rate is higher in older individuals, it is thought that it may be in the same situation in *P. nobilis* as in *O. edulis*. Therefore, continuously monitoring of surviving juvenile and adult individuals is essential to ensure the sustainability of the critically endangered *P. nobilis* stocks in order to make more accurate assessments regarding the distribution and degree of impact of the parasite, and to rehabilitate other populations which the disease has observed.

It is very important to identify uninfected populations in coastal waters of Turkey to continuously monitor and to protect these regions in terms of ensuring the continuity of stocks. In previous studies, healthy *P. nobilis* populations were observed in the Erdek-Ocaklar coasts and Marmara Island (in the Marmara Sea), which are relatively close to the stations located near the Marmara Sea in the Çanakkale Strait (Pers. obser. in the Marmara Sea by SA and DA). Similarly, Öndes et al. (2020a) reported the existence of healthy individuals on the Erdek coasts. Juveniles can be collected with the help of collectors from uninfected areas and transported to new areas. These areas are important for rehabilitating and revitalizing damaged fan mussel populations. Again, it can be aimed to create restocking programs from resistant individuals (resilient juveniles and adults) by transplantation and monitoring. The information provided on artificial recruitment, experimental juvenile growth, and re-implantation in nature, may serve in the future to help reinforce or even re-establish populations in locations devastated by the recent mass mortality event.

Conclusion

In conclusion, mortality has been observed in *P. nobilis* population in the Çanakkale Strait despite the fact that the mortality decreased gradually from the Aegean Sea to the Marmara Sea. In this context, it is known that the Marmara Sea has healthy populations, considering our personal observations. The Çanakkale Strait currently acts as a barrier. The monitoring of the populations distributed in this area is very important in terms of the course of the disease. Particularly, it is of great importance to transplantation of resilient individuals in the Çanakkale Strait in new areas (the natural population of fan mussel has occurred or not) that are infected and/or not infected for the rehabilitation of these areas. Alternatively, new healthy populations might be established by transplanting juvenile individuals from non-infected populations from the Marmara Sea to suitable areas in the Çanakkale Strait. Marine protected areas should be established for protecting and monitoring the healthy populations and/or infected (but has lower mortality) populations. Thus, the sustainability of the population could be ensured. In addition, the natural populations might be improved by aquaculture approaches (breeding program, larval culture, spat collecting, rearing, etc.) starting from the larval stage.

Compliance with Ethical Standards

Authors' Contributions

Author SA designed the study, DA carried out underwater surveys. SK performed GIS-related analyses. All authors wrote the first draft of the manuscript together and equally contributed for preparing the manuscript. 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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