Mar. Sci. Tech. Bull. (2021) 10(4): 350-369
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.627562

Marine Science and Technology Bulletin

REVIEW ARTICLE

Population, aquaculture and transplantation applications of critically endangered species *Pinna nobilis* (Linnaeus 1758) in the Mediterranean Sea

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ARTICLE INFO

Article History:
Received: 01.10.2019
Received in revised form: 01.11.2021
Accepted: 01.11.2021
Available online: 23.11.2021

Keywords:
Critically endangered species
Pinna nobilis
Fan mussel
Aquaculture
Transplantation

ABSTRACT

The population of fan mussel, *Pinna nobilis* across the Mediterranean Sea has been affected by factors such as overfishing, fisheries processes, environmental pollution, destruction of habitat, tourism, etc. Therefore, the species *P. nobilis* was taken under protection by the Decisions of the Council of Europe and Barcelona Convention. However, its mortality rates of 100% have been reported to be due to *Haplosporidium pinnae*, a parasite in different Mediterranean regions. The status of *P. nobilis* has thus been revised to be reduced from "Vulnerable" to "Critically Endangered" and the importance of all the studies on the species further increased. The aim of the study is to present the current status of *P. nobilis*, the native to the Mediterranean, by combining the relevant studies on ecology, aquacultural process (larvae, spat settlement and rearing), culture methods and transplantation. The present study has provided comprehensive knowledge on the current status *P. nobilis* population, aquaculture and transplantation activities. Except for studies to determine stocks, in particular, those on collecting young individuals from nature and planting and growing them in predetermined sites as well as their production through various cultures from their larval phase onwards are of great importance in terms of rehabilitation and sustenance of the damaged *P. nobilis* population. Therefore, alternative and potential habitats should be created thanks to transplantation and aquaculture. Marine protected areas should be determined to enable a healthy *P. nobilis* population to be sustained.

Please cite this paper as follows:

Acarli, S. (2021). Population, aquaculture and transplantation applications of critically endangered species *Pinna nobilis* (Linnaeus 1758) in the Mediterranean Sea. *Marine Science and Technology Bulletin*, *10*(4), 350-369. https://doi.org/10.33714/masteb.627562

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Introduction

Fan mussels under the classis of Bivalvia are represented by the species of *Pinna nobilis* (Linnaeus 1758), *Pinna rudis* (Linnaeus 1758) and *Atrina fragilis* (Pennant 1777) in the Mediterranean basin. *A. fragilis* has distributed in southeast Africa, New Zealand, North Japan and East Coasts of Atlantic and *P. rudis* across East and West Atlantic, North American and South European shores while *P. nobilis* is native to the Mediterranean Sea. The species of the Pinnidae family are called fan mussel and noble pen shell. In recent years, *P. nobilis* population has significantly decreased along some Mediterranean seashores of France, Greece, Spain, Italy and Croatia (Vicente & Moreteau, 1991; Catanese et al., 2018). In other regions, its population density decreased from 17 individuals per 100m^2 (Richardson et al., 1999) to 1 individual per 100 m2 (Katsanevakis & Thessalou-Legaki, 2009). The future of the species has been established to be "Endangered" for the reasons below;

- various human activities have caused the destruction of coastal habitat,
- increased water pollution,
- damage due to fisheries activities,
- popular consumption of its delicious white muscular meat
- uses of pearly shells in weaving decorative and byssus threads into cloths,
- deep anchor dragging from boats to the destroy fan mussels to death,
- its usages as angling bait to lure and catch fish (Vincente & Moreteau, 1991; Hendriks et al., 2013; Deudero et al., 2015; Capó et al., 2015).

Considering the above all, *P. nobilis* has been listed as the species endangered in the Mediterranean ecosystem and thus taken under strict protection according to Annex II of the Barcelona Convention (SPA/BD Protocol 1995) and the European Council Directive 92/43/EEC (Annex IV). In addition, the central marine zone and southernmost coasts of the Iberian Peninsula (Spanish Western Mediterranean) showed a 100% mortality in all length groups of the population (Vázquez-Luis et al., 2017). The status of the species was therefore reconsidered to be increased from "Vulnerable" to "Critically Endangered" at national level by the Spanish Sectorial Environmental Conference on July 17/2017. Darriba (2017) was the first to report the presence of haplosporidan parasite as the earliest histopathology. *Haplosporidium pinnae*, a species of *Haplosporidium* parasite in particular has significantly damaged *P. nobilis* population over the last five years. Occasional western Mediterranean and the Tyrrhenian Sea in Italy have recently exhibited 100% mortalities (Catanese et al., 2018; Carella et al., 2019). Moreover, Katsanevakis et al. (2019) performed a study on mass mortalities of *P. nobilis* in and around the island of Lesvos in the Northern Aegean Sea However, many surviving individuals have been documented in France Port-Cros Archipelago Marine Protected Area (Ruitton & Lefebvre, 2021), Thau Lagoon (Foulquié et al., 2020), and the East of Corsica Diana Lagoon (Simide et al., 2019), in Spain Alfacts Bay (Ebro Delta) (Prado et al., 2020) and in Greek Kalloni Gulf and Laganas Bay (Zotou et al., 2020). Different stations in the Aegean Sea coasts of Turkey were recorded to exhibit 100 % mortalities (Acarli et al., 2020; Öndes et al., 2020) whereas the Aegean and Marmara mouths of Çanakkale Strait showed mortalities of 100% and 9.2% in 2020, respectively (Acarli et al., 2021a). Künili et al. (2021) mention that *H. pinnae* infected *P nobils* individuals and caused death in Çanakkale Strait. In addition, Cinar et al. (2021) indicated that at seven locations, a total of 191 *P. nobilis* individuals were discovered, with 88% of them being dead in the Marmara Sea. The authors claimed that the cause of mortality could be linked to an epidemic sickness or a catastrophic mucilage occurrence after November 2020. Recently, Acarli et al. (2021b) investigated the effects of the mucilage event on the population of critically endangered *Pinna nobilis* in Ocaklar Bay (Marmara Sea, Turkey) and the authors documented that the mortality rate was found 35.96% before the mucilage event while it was calculated as 16.12% after the mucilage event in the study area. Acarli et al. (2021b) asserted 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.

The intense amount of mucilage organic matter due to planktonic algal bloom was first reported in mid-autumn 2007 for the Marmara Sea (Aktan et al., 2008). However, mucilage was observed to be more intense in the Marmara Sea this year than in the previous years (Balkıs-Ozdelice et al., 2021). The planktonic algal bloom occurs as the result of higher seawater temperatures, agricultural activities, domestic and industrial waste discharges, and overfishing (Flander-Putrle & Malej, 2008; Savun-Hekimoğlu & Gazioğlu, 2021). Benthic organisms are the most affected by the mucilage sunken or accumulated on the bottom (Schiaparelli et al., 2007). It has been observed that *P. nobilis* covered of mucilage, one of the benthic organisms in the coast of Erdek-Ocaklar Bay in the south of

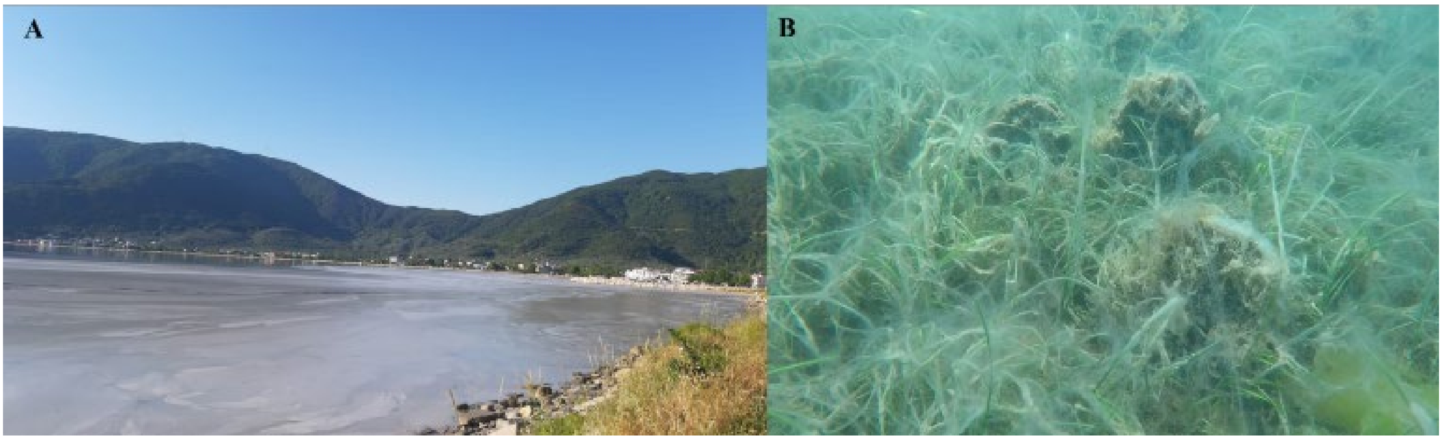

Figure 1. Mucilage on the sea surface of the Erdek-Ocaklar Bay in May, 2021 (A) and underwater view of mucilage on *P. nobilis* and *Posidonia oceonica* in the Erdek-Ocaklar Bay in June 2021 (B)

Marmara Sea in March 2021 (Figure 1), effects of which this event will later be understood more clearly.

P. nobilis hosts various species of microorganisms thanks to the width of its surface area and structure of calcium carbonate as well as its individual contributions (Acarli et al., 2010). The associated studies found that species of Bivalvia, Gastropoda, Ascidiacea, Polyplacophora, Echinodermata, Demospongiae, Gymnolaemata, Maxillopoda, Polychaeta, Cyanobacteria, Anthozoa and macroalgae were covered on the shells of the *P. nobilis* (Giacobbe, 2002; Addis et al., 2009; Acarli et al., 2010; Rabaoui, et al., 2015). The present study exhibited that any damages to *P. nobilis* population expose to macrobenthic species on its shell to danger as well.

The purpose of the present paper is (i) to summarize the basic information to recognize the species, (ii) to define the culture methods applied considering other pinna species and to reveal the importance of its aquaculture in conservation programs, and (iii) to define the role and importance of transplantation studies as part of rehabilitation programs.

General Characteristics of Fan Mussels

Morphology

Pinnids are of a triangular shape due to their adaptation to tying to the substrate via byssal threads (Figure 2). Generally, the shell has many functions, including serving as a skeleton for attaching muscles, protecting against predators and in burrowing species, and helping keep mud and sand of the mantle cavity (Gosling, 2003). It is composed of three layers: an outer horny periostracum and two calcified layers beneath it. The inner layer is consisting of aragonitic nacre, which is only found in the anterior part of the shell (García-March & Vicente, 2006). The ligament is located in the dorsal region with the mere function to hold the valves together. The ventral part of the shell has a small opening for the byssus to exit. Byssus consists of many filamentous fibrils that could grow up to 16 cm. Byssal extensions have sticky discs on each (de Gaulejac, 1993).

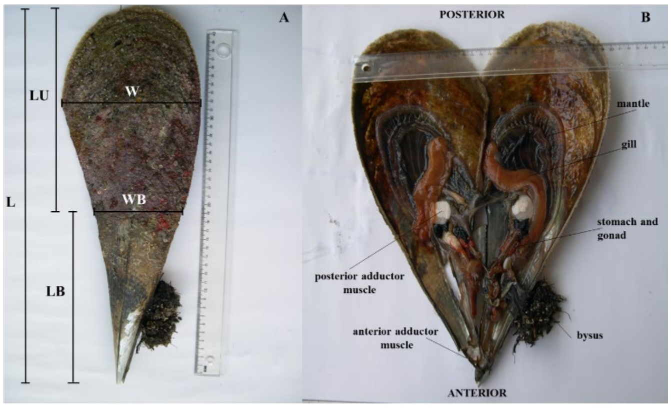

Figure 2. Morphometric parameters (A) and internal organs (B) of *P. nobilis* (*L*: total length; *LB*: bury in shell length; *LU*: unbury in shell length; W: width; *WB*: bury in shell width)

Members of the Pinnidae have two adductor muscles: the large posterior adductor is powerful and centrally located in the shell and the small anterior one is located at the umbonal vertex (Grave 1911; Coronel, 1981 in Escamilla-Montes et al., 2017). The posterior adductor and posterior foot retractor muscles are so close to each other that they appear to be one muscle (Czihak & Dierl, 1961 as cited in Basso et al., 2015). The mantle is not attached to the shell as occurs in other species (e.g., some Mytilidae) and therefore it is very retractable and without a pallial line (García-March, 2005).

The Pinnidae are characterized by waste channels, the ducts through the mantle in an antero-posterior direction from the palps to the of the mouth to the contact with the posterior-dorsal end of the gills, in which they combine with the inner lobe of the mantle edge to finally complete the division between the inhalant and exhaling chambers. These channels allow the removal of pseudo feces and other debris from the inhalant chamber and can be used to clean the cavity of sand and debris introduced by the waves during storms before closing the leaflets (García-March, 2005).

P. nobilis' functional morphology, in particular the unique pallial organ, the equally unparalleled buccal (previously pallial) gland, stomach and its contents are described (Morton & Pulijas, 2019). The function of the pallial organ has been controversial over time. It was previously believed to be a cleaning organ responsible for extracting the broken pieces of the shell in the pallial cavity (Yonge, 1953). However, later, it was discovered in *Atrina pectinata* that the organ proves thigmotropic and highly secretory, with a pH in its head of 2 to 4, and it is therefore supposed to have a defensive function, to protect the animal from the entry of epibionts and predator attacks (Liang & Morton, 1988). In addition, Morton & Pulijas (2019) found that the pallial gland produces sulphuric acid that engaged in prey capture and function as a shell cleansing pad.

Habitat

Fan mussels are generally brown, transparently beige to white and yellow in color and fanlike in shape. They are known to be able to live in the depth of 60 m regardless of variations in species and age. Umbonally attached to and partly embedded in the ground by their byssal threads (Figure 1), they inhabit areas of sandy, sandy pebbled and muddy seabed through seaweeds, *Posidonia oceanica* (Figure 3) (Tebble, 1966; García-March & Vicente, 2006).

Though variable in size, their lengths range from 20 to 40 cm (Fischer, 1987). *P. nobilis* is known to live until 45 years with a maximum (Rouanet et al., 2015) length of 120 cm (Zavodnik et al., 1991). It is necessary to count adductor muscle scar rings inside the shell to determine the age of an individual fan mussel. Because the first year-scar ring is invisible or too vague to notice, the age of the fan mussel is determined by adding 1 age to already counted scar rings (Richardson et al., 1999).

Nepinnotheres pinnotheres (Crustacea: Decapoda) (Linnaeus 1758) is known as pinna pea crab brown in color (Hayward & Ryland, 1995) (Figure 3), which is a parasitic species found in the mantle cavity of *P. nobilis* (Becker & Turkay, 2017). The adult female is significantly bigger than the mature male (Becker & Türkay, 2017). *N. pinnotheres* is believed to live within *P. nobilis* as a temporary refuge. Therefore, more than one male pinna pea crab has been encountered within the same *P. nobilis* individual (Rabaoui et al., 2008). Acarlı et al. (2019) reported that *N. pinnotheres* did not directly influence the intact *P. nobilis* since damages to the tissue of the infected fan mussels were in the process of the study. On the other hand, the same researchers noted that the physical condition of fan mussels might be threatened by adverse environmental conditions or invasion of infectious diseases such as mycobacterium and haplosporidian parasites.

Feeding

P. nobilis is a suspension feeder or filter feeder organism that feeds on suspended organic and inorganic matters, zooplankton, phytoplankton, bacteria, and viruses in the water column (Gosling, 2003; Davenport et al., 2011; Najdek et al., 2013; Trigos et al., 2014). Thus, it contributes to the improvement of water quality, playing a vital role in the ecology of the Mediterranean Sea (Natalotto et al., 2015). Gills are equipped with functions of feeding and respiration. Food particles captured by the mantle and gills and covered with

Figure 3. *Pinna nobilis* on the seagrass meadows (*Posidonia oceanica*) in the Karantina Island (İzmir Bay, Aegean Sea) in 2008

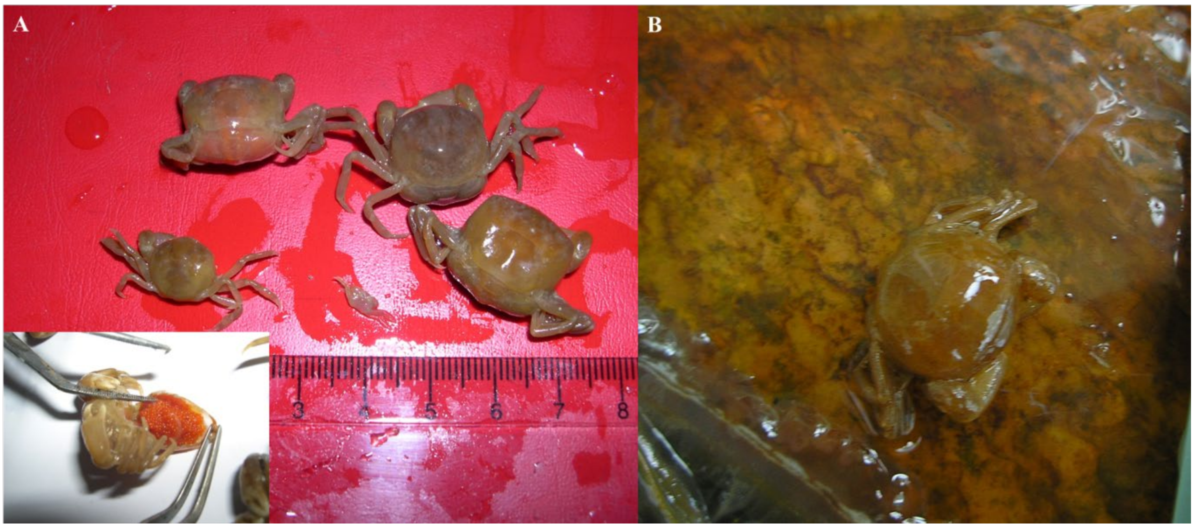

Figure 4. Photographs of *N. pinnotheres* (A) and *N. pinnotheres* on the *P. nobilis* inner shell (B)

mucus are transported by actions of cilia in the gills to labial palps through which they would be selected and processed. While discarded particles are moved to the sides of the palps, those suitable to be ingested are carried to the mouth, from which they pass through the stomach where they are digested by digestive enzymes. Indigestible part is passed through intestines and discharged out of the anus (de Gaulejac, 1993) (Figure 4).

Species of fan mussels do not have a siphon mechanism but a pallial cavity instead, in which inhalant and exhalant chambers are completely separated by lamellibranch gills. In the absence of siphons, the mantle cavity and large posterior extension of the shell serve the function of siphonage (Butler et al., 1993). The species of *P. nobilis* keep its valves open as a gaping process in order to filter the water around it, which shows that an individual fan mussel has been in the process of feeding and respiration thanks to its wide-open valves. However, insufficiently gaped or completely closed valves imply that filtering has decelerated or ceased. The rate of filtration and valve gaping is determined by the existence of factors such as particle concentration, temperature, salinity, and stress (Gosling, 2003). Butler et al. (1993) reported that the species of *P. nobilis* can inhabit oligotypic environment, clean water and little resuspension of sediment. Sediment loading which harms gill cilia can affect activities of feeding and respiration (Katsanevakis, 2005; García-March et al., 2008; Coppa et al., 2010). Garcia-March et al. (2008) found that the gaping activity of *P. nobilis* is directly influenced by current intensity and direction rather than factors such as temperature, dissolved oxygen, turbidity, and chlorophyll-*a*. The gaping activity of the population is usually synchronous and a periodic cycle from 21.9 to 24h of gaping activity occurs in all *P. nobilis* individuals during the year.

Aquaculture

Because species of fan mussels such as *Pinna bicolor*, *Pinna rugosa*, *Atrina pectinata* and *Atrina maura* are popularly consumed and marketed for high prices in Pacific and Asian countries (Cendejas et al., 1985), they are of commercially great potential. As a result, studies have been made on the aquaculture production of the species in order that its sustainable output should be obtained and natural stocks are protected.

As the species of *P. nobilis* has currently been under serious danger of extinction, in particular, the presence of aquaculture output, as well as control measures, is required in terms of protection of the species and regeneration of the stocks in the habitat. As a consequence of the diseases, stocks of *P. nobilis* have been on the brink of disappearance in most locations of the Mediterranean Sea. The fact that disease-free locations are determined where production is to be restarted requires the collection of spat from the environment and rearing larvae and thus juveniles.

Reproduction and Maturation

No studies have been encountered on determining the reproduction period of *P. rudis* and *A. fragilis* like *P. nobilis*. *P. bicolor* is gonochoristic (Roberts, 1984) whereas *P. nobilis* is of successively (alternately) hermaphrodite characteristic with its gonads developing through digestive organs. The color of gonads varies from light orange to dark brick hue, which is however not indicative of sexuality or any sexual phases (Figure 5) (Acarlı et al., 2018). Having matured, reproductive cells are

Figure 5. Gonadal appearance of *Pinna nobilis* (original)

water columns where the process of larval development is later completed (de Gaulejac, 1993). The reproductive cycle in fan mussels is influenced by endogenous factors such as energy reserves and genetic elements (Lee et al., 2015; Wang et al., 2017) and exogenous factors including variations in temperature, photoperiod and food quality and quantity (Baik, et al., 2001; Angel-Dapa et al., 2015; Qui et al., 2014; Gongora-Gomez et al., 2016a). Reproductive periods of *P. nobilis* in different regions are presented in Table 1. Histological studies show that the reproductive period of *P. nobilis* occurs when the temperature has been high. *P. nobilis* can be cited to be a summer breeder species according to the classification by Boolotian et al. (1962) considering spawning in terms of comparison of spawning periods (Table 1). These differences may occur due to depth, thermocline position, degrees of latitudes and other regionals (Hernandis et al., 2018).

Table 1. Spawning periods of *Pinna nobilis* in different regions

Locality	Spawning period	References
France	June-August (26°C)	de Gaulejac (1995)
Spain	May-August (20-26°C)	Deudero et al. (2017)
Turkey	May-September (24-26°C)	Acarli et al. (2018)

As for the collection of sexually mature individuals, it is necessary to choose periods when condition index is high and muscle index low (Acarli et al., 2015; Angel-Dapa et al., 2015; Acarli et al., 2018). Maeno et al. (2009) and Chung et al. (2006) reported that the maturation progress of fan mussel broodstocks in suspension culture resembled that of those in natural beds. Angel-Dapa et al. (2015) informed that gamete quality, in other words, broodstock condition and origin (depth, phases of tidal cycle), as well as environmental conditions, could have effects on larval survival and growth rates in the studies of larval production. Species of microalgae such as *Pavlova* sp. and *Chroococcus* sp. have been found to be effective nutrient sources on the maturation of adult individuals. 18: 1n-9 content is related to condition index and gonad index and can be used as the degree of maturation for gonads in them (Qiu et al., 2014). To enable the species under protection to sustain, rearing processes should be carried out. The first stage is therefore to determine the period of reproduction.

Production of Larva

When spawning has occurred, external fertilization of sperm and egg takes place and larval development from trochophore to pediveliger phase is planktonic with eventual settlement and metamorphosis (de Gaulejac & Vicente, 1990). Mature fan mussel individuals collected from nature are exposed to temperature shock (Angel-Dapa et al., 2015; Trigos et al., 2018), hydrogen peroxidation (McCoy & Chongpeepien, 1988) or UV (Yang et al., 2006) to obtain their gonadal cells.

The eggs having been incubated, larvae are fed on phytoplankton species of *Isochrysis galbana*, *Tetraselmis* sp., *Chaetoceros calcitrans*, *Pavlova lutheri* in such a way to be 5000cells/ml for about 5-6 weeks to continue until the juvenile phases (Kawahara et al., 2004). However, the length of the larval period could vary according to the species, larva and mature quality and food (Gosling, 2003). Ohashi et al. (2008) were the first to successfully study the process of growing larvae and juveniles of *A. pectinata* in indoor tanks, though the study showed that the rate of survival was not desirable upon fertilization, hatching and attachment. During the latter, the rate of survival and length of spats were 0.004 % and 514.1±25.9 µm, respectively.

Most of the studies on *P. nobilis* include those on population. Although a limited number of studies have been made involving juvenile production and growth, the first larval output process was performed by Trigos et al (2018), which involve the process from egg fertilization to pediveliger stage and tried different rearing tanks, larvae density, light conditions and food of amounts to determine the optimal condition for larval development. The results showed that 16 L tanks with a concentration of 2 larvae ml, 21°C constant temperature, 12/12 h photoperiod and feeding on an optimal mixture of 25 cells per µL of *Chaetoceros calcitrans* +33.3 cells per µL of *Pavlova lutheri* + 100 cells per µL of *Isochrysis galbana* seem to be the best conditions to rear *P. nobilis* larvae. The larva achieved the stage of pediveliger in 7 days with a length of 110 µm. However, it failed to metamorphose by the end of 22 days. The problem seems to be that Vibrio is suspected to have caused mortalities over 80 % during the first 2-9 days based on light conditions and food doses. The process of larval culture exhibited low survival rates and it was thus interpreted that hydrophobicity of pediveliger larvae resulted in their buoyancy on the water with eventual dehydration and/or starvation (Robles-Mungaray, 2004 as cited in Gómez Hernández, 2011) (Maeda-Martínez, 2008; Angel Dapa, 2015). Pediveliger larva has a thin and fragile shell and gradually stores lipids with the result that it has a positive buoyancy prior to metamorphosis (Maeda-Martínez, 2008). Successful completion of the larval stage with the metamorphosis of *P. nobilis* can only come true due to the formation and development of the culture hatchery protocol (temperature, salinity, density, feeding strategies, tank cleaning, water change, etc.).

Spat Collection

First young individuals obtained after the larval period are called spats with transparent shells and interior organelles visible to the naked eye (Figure 6). Yielding of spat collection is affected by environmental factors such as temperature, currents, water depth and type of collector material in particular (Narváez et al., 2000; Saucedo et al., 2005; Yıldız et al., 2005; Yildiz et al., 2013; Halla et al., 2018). A variety of natural and artificial collectors are used to obtain bivalve spats, including monofilament gillnets, PVC glass, onion bag, netlon, bivalve cultch (oyster, mussel, cardium, etc.), wood, fiberglass, car tires and tiles (Lök & Yolkolu, 1999; Helm & Bourne, 2004; Buitrago & Avarado, 2005; Velasco & Barros, 2010; Soria et al., 2015; Gregori et al., 2019). Although numerous collector designs and materials have been thus far tested, the productivity of a collector generally vary based on the species, the region and the duration of soaking the collector. Of such collectors, the most efficient are nylon filaments or onion bags made of polyethylene mesh for collecting fan mussels (Cendejas et al., 1985; Beer & Southgate, 2006: Kersting & Hendriks, 2019). The time of planting collector is generally important. Once it has been planted before reproduction, unwanted or untargeted species attached to the surface of the collector and the area to which species could attach would thus decrease. However, when it has been planted upon spawning period, its efficiency lowers then the rate of collecting target species drops as well (Lök & Acarli, 2006; Yıldız et al., 2010; Yiğitkurt et al., 2017). The most available period for *P. nobilis* to attach is the summer months when temperature and presence of nutrients are high (Acarli et al., 2011a; Theodorou et al., 2015). On the other hand, the rate of attachment close to the water surface was found to be much better than in the depth of 8 m (Kurtay et al., 2018). Therefore, pelagic bivalve larvae tend to swim up to surface water where food concentration is high (Bayne 1976). Bivalve larvae exhibit a negative phototropic behavior to attachment when they avoid light and prefer sheltered substrates in shadow areas (Baker, 1997; Saucedo et al., 2005; Lök & Acarli, 2006). In this context, Acarli et al. (2011a) observed that the spat settlement of *P. nobilis* was significantly higher within mesh collectors (92%) than their exterior.

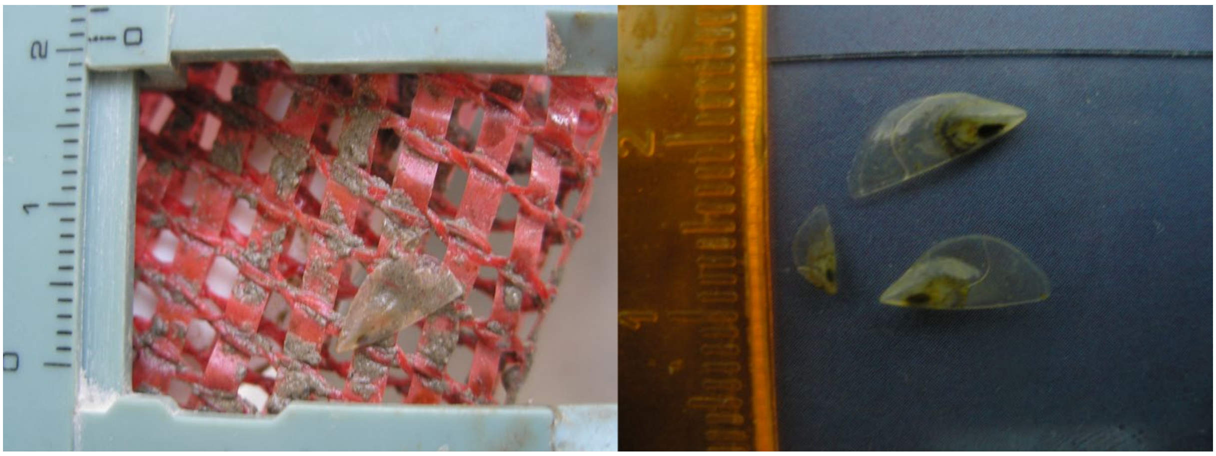

Figure 6. *P. nobilis* spat collection trials (Acarli et al., 2011a)

Culture Study

All other species of the Pinnidae family including *P. nobilis* and *P. bicolor* are those whose shells grow the fastest in all bivalve species (Richardson et al., 2004). The upper section of the shell in water grows faster than the lower part on the bottom. The ones to inhabit deep water show a slower growth but achieve greater length than those in shallow water (García-March et al., 2007). The growth of the Pinnidae family is influenced by food quality and quantity (Safi et al., 2007; Acarli et al., 2011b), temperature (Leyva-Valencia et al., 2001), salinity, reproduction (Narváez et al., 2000), pollutants (Góngora-Gómez et al., 2015), age and shells size (Demirci & Acarli, 2019). Of those, food supply and optimum temperature are considered to be the most important for bivalves without which sustainable growth is difficult or almost impossible. The relationship between shell length and the total weight *of P. nobilis* was described with the equation $W=0.003L^{3.6451}$ (r^2= 0.9625), the result of which revealed that there was a positive allometric growth between shell length and total weight ($P<0.05$) (Acarlı et al., 2011b).

The juveniles are observed to grow faster as in other bivalve species but growth shows a deceleration later on both in culture and in situ growth studies (Katsanavakis, 2007; Aucoin & Himmelman, 2011; Demirci & Acarli, 2019). The rearing studies are generally based on the principle that the cultivation of juvenile individuals obtained from natural environments should be performed on suitable grounds. Culture trials and choice of system and field involve important criteria such as growth, mortality, meat yield, condition factor and biochemical composition of fan mussels (Arizpe, 1995). The process involves that the techniques of suspended culture and bottom culture are employed considering the area where individuals would be placed in the water column.

Suspension Culture

The purpose of the floating (long line or float raft) is to increase the amount of yield by means of excessive water volume and get rid of the pressure of predation present on the bottom as well, for which juveniles are placed in the materials such as boxes, tray pearl baskets, lantern nets, etc. hung down from the system above. On their bottom is placed sufficient amounts of sand into which fan mussels could be buried and planted (Wu & Shin, 1998) or instead rearing process can be performed without placing sand in the same materials cited above (Acarlı et al., 2011b) (Figure 7). Values of shell width, dry tissue weight and condition index for *P. bicolor* juveniles (30-40 mm shell width) in raft culture were found to be higher than those on the sea bottom (Wu & Shin, 1998). Koźul et al. (2011) reported that *P. nobilis* grew very rapidly and reached from 29 mm to 157 mm over a year. Kozul et al. (2013) also placed the cages with juveniles at three depths: 1 m, 3 m and 5 m to finally see that following the two-year growth period the average length was 244±22.9 mm at 1m, the specimens averaged 244±25.3 mm at 3 m and the average length was 231.1±22.5 mm at 5 m. The rearing processes within cages managed to solve some problems primarily caused by human activities for purpose of improving natural fan mussel populations. Beer & Sauthagate (2006) placed *P. bicolor* species in pocket panel nets to find that they grew from 75.5±1.19 mm to 175.5 ± 3.9 mm in hinge length by the end of an 80-week period when the deceleration of the growth was associated with the reproduction activity. There was a positive effect of the suspended culture system and pocket panel nets on survival with a 78 % rate at the end of the study. Because the suspended system generally makes it possible to work at a given distance from the ground, the pressure of predators on juveniles decreases while the rate of survival increases (Wu & Shin, 1998; Leal-Soto et al., 2011).

Figure 7. Suspension culture trials of *P. nobilis* (Acarli et al., 2011b)

Having collected *P. carnea* individuals, Velasco & Borrero (2004) stocked them at two different concentrations of 10% and 20% in pearl oyster rearing nets and left them in a 9 m depth. By the end of the study, the ones of 10 % and those of 20% concentrations grew to 167.5 mm and to 156.5 mm, respectively. There were no differences in survival rates between the two groups. Unlike other studies, early in the study, predator pressure (Cymattidae, Majidae, Portunidae and Xantidae) was cited and mortalities were encountered due to predation pressure as well as environmental factors. A suspended culture system requires basically higher investment in facilities, sophisticated equipment and labor force, which is of greater cost and thus supposed to be regularly controlled (Leal-Soto et al., 2011).

Bottom Culture

Post-settlement and juvenile mortality in benthic invertebrates are high because of predation (Aucoin & Himnelman, 2011). The ability to perform protected and unprotected systems on the bottom-related cultures is based on the availability of the predator population. The posterior portion of fan mussel species vertically rises over the bottom surface whereas its anterior part is buried into the bottom sediment, which could cause exposure to predators (fish, octopus, crabs, muricid, gastropods, etc.) (Yonge, 1953). Juveniles have relatively thin shells. If there is too much presence of predators likely to harm juveniles in the environment, they should be protected against predation. Kersting & Garcia-March (2017) reported that plastic frame-casing of individuals could result in the reduction of influences of currents and pressure of predators. After fan mussels have been planted into the bottom in the protected systems, net cages of suitable mesh size are placed to cover over in order to prevent predators from reaching there. Unprotected systems just involve the young to be planted into predetermined rearing spots without any protective cages.

A. maura were cultured in the sand bottom of 36 fan mussels/m^2 each in Ensenada Pabellones lagoon system (<10 m deep), Mexico, about which any protective systems were not cited at all. A one-year-culture study showed that the rate of survival was 91.66% with the shell length growing from 28.41±4.69 mm to 218.16±12.41 mm. The study also indicates that the presence of predators in the water column affects the survival rate. In this sense, it is important to choose the area in rearing trials as well. Some authors pointed out that the bottom culture of bivalves had lower growth rates and higher predation loss than the suspended culture Emerson et al., 1994; Barbeau et al., 1996; Lodeiros et al., 2002). However, the pressure of predators can be decreased by choosing an available region or some means to be used for protection. In this sense, it is important to choose the area in rearing trials as well. Some authors pointed out that the bottom culture of bivalves had lower growth rates and higher predation loss than the suspended culture (Emerson et al., 1994; Barbeau et al., 1996; Lodeiros et al., 2002). However, the pressure of predators can be decreased by choosing an available region or some means to be used for protection.

Suspended and Bottom Cultures

Studies on the combination of suspended and bottom cultures for fan mussels to be reared were reported by Miranda-Baeza (1995), and Cardoza-Velasco & Maeda-Martinez (1997). In addition, Leal-Soto et al. (2011) cultured *A. maura* juveniles by combining both production systems. The individuals were first placed into plastic trays covered over with 2 mm plastic mesh and suspended by long line systems. Shell lengths of 1300 juveniles were 15±5 mm early in the trial and kept in the system until they grew to a mean of 32.8±6.7 mm for two months. The survival rate in long line system was 90%. The juveniles were later placed on a location of 20 m^2 present in 4 m depth 100 m off the shore. Prior to the trial, the site bottom was cleared of rocks, pebbles and mosses. 140 individuals/m^2 were manually buried into the bottom and covered over by the cages of 2×1.5×0.2 m framed by a 0.5-inch construction steel bar. The cages were covered with a plastic net of 8mm mesh. The process of ground rearing was continued for 20 months with a mean shell length of 194.6±10.2 mm and a 70% survival rate.

Góngora-Gómez et al. (2016b) conducted an experimental study similar to the *A. maura* process, combining suspended and bottom cultures in Isla Los Redos in the southeast of California Gulf, Mexico for 15 months (February 2008-May 2009). Young individuals (n=2500) were subjected to the suspended culture and their mean shell height and total weight varied from 16.20±4.96 mm-and 0.3±0.2 g to 119.66±8.98 mm and 38.68±10.34 g, respectively. The survival rate was found to be 97%. They were then planted into sand bottom within the area surrounded by the protective cover framed with corral or fence and preserved until the harvest process for 11 months with a mean shell height of 220.48±12.41 mm, the total weight of 284.26±54.45 g and a survival rate of 88.72%.

P. nobilis individuals reared by the suspended system were buried into the bottom of 3m depth bottom around Karantina Island, Urla, Izmir in April 2008. Their total height (anterior-posterior) ranged from 125 mm to 185 mm. The survival rate was 100% after 6 months and 1 year (Figure 8) (Unpublished study of the author).

The reason for the combination of the two culture systems is that the survival rate is low based on the fragility of the newly settled individuals. However, their fragility decreases when they have achieved 100 mm length. In other words, the higher the size, the more resistance to predators would be (Velasco & Borrero, 2004). Moreover, juveniles less than 30 mm buried into the bottom could be uprooted by currents and/or easily killed by predators (Leal- Soto et al., 2011). It is generally an important parameter to often clean culture cages used in the suspended process and manually keep predators off in the water in terms of increasing rates of growth and survival. Grown individuals should be vertically buried into the lower layer to maximize their process of nourishment, which is better than suspended culture. Thanks to the above, they could adjust themselves to the process until they have been adults and function their reproduction activity healthily. It seems more appropriate to assess both culture systems in combination for *P. nobilis* to avoid risking any stages of development due to the security of accurate control over the process with minimum mortality in the process.

Transplantation

Transplantation is the method used to sustain and increase the production of endangered or threatened species of commercially valuable species under danger of extinction. The process has been usually performed for bivalve species such as *Crassostrea virginica* (Powell et al., 1997), European oyster *Ostrea edulis* (Çelik et al., 2013) and Gastropod species such as limpet *Patella ferruginea* (Espinosa et al., 2008), green abalone *Haliotis fulgens* (Guzman Del Proo et al., 2004). The conducted activity has also been used for fan mussels such as *Atrina maura* (Mendo et al., 2011), *Pinna bicolor* (Wu & Shin, 1998) and *Pinna rugosa* (Arizpe, 1995) which are of commercial importance and targeted to be cultivated. Achievement of the transplantation process can generally be affected by anthropogenic factors, stock density, size of individuals to be planted, the pressure of predation and environmental parameters (temperature, salinity, sediment structure, nutrient quality and quantity, and currents).

P. nobilis continues to decline where it inhabits. Therefore, transplantation could be performed as one of the remedies for the generation of *P. nobilis* to sustain, for which the first transplantation trial was conducted in the Adriatic Sea by

Table 2. Transplantation studies for *P. nobilis*

Location	Survival rate (%)	N	Depth (m)	Reference
North Eastern Coast of Sardinia	75	18		Caronni et al. (2007)
Le Brusc lagoon, Var, France	100	16		Trigos & Vicente (2016)
Lake Vouliagmeni (Korinthiakos Gulf, Greece)	95.6	45 (11-16 cm)	12	Katsanevakis (2016)
Capo Peloro Lagoon (Central Mediterranean, Italy	83	53	2	Bottari et al. (2017)

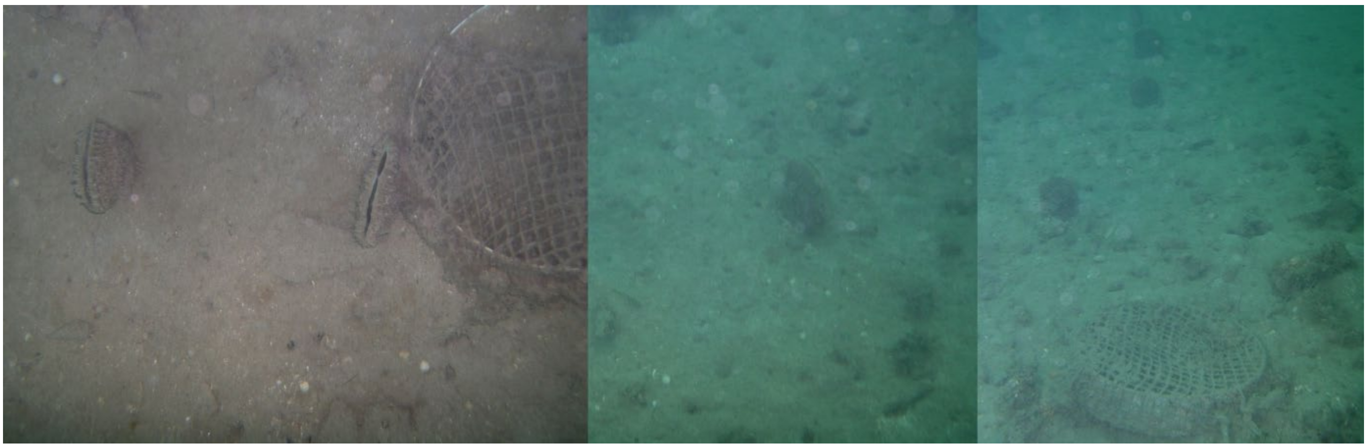

Figure 8. Individuals planted into bottom after rearing trial in the Karantina Island (İzmir Bay, Aegean Sea) in December 2006

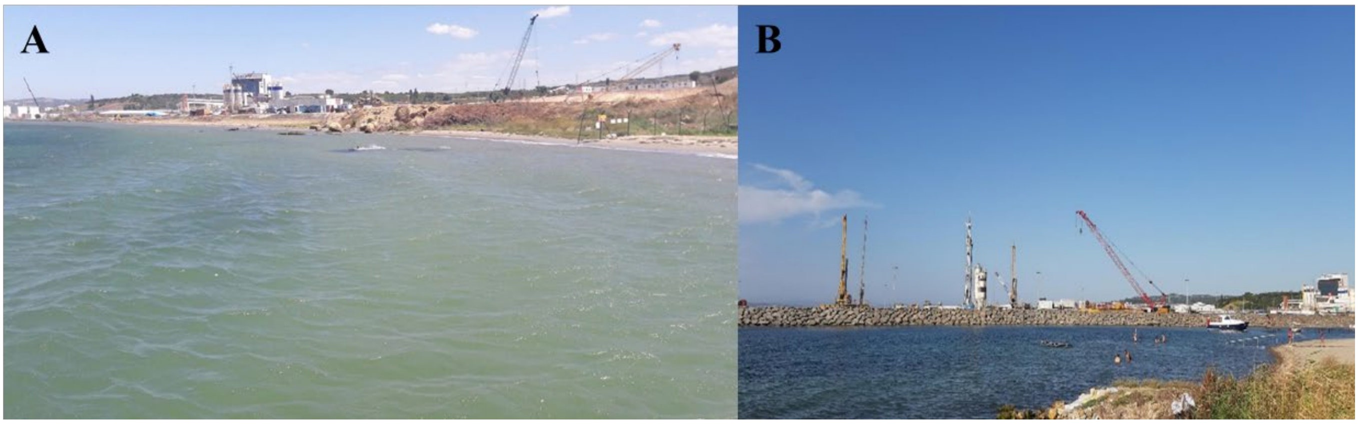

Figure 9. The location where *P. nobilis* collected from the Çanakkale Strait. Before (A) and after (B) the construction of the 1915 Çanakkale Bridge

Mihailinović (1955) for commercial purposes such as growing to market its shell, meat and byssus (Mihailinović, 1955 as cited in Trigos & Vicente, 2016), followed by the fact that Vicente et al. (1980), Hignette (1983). de Gaulejac & Vicente (1990) designed transplantation processes and performed small scale experiments. The importance of transplantation processes has further increased in the consequence of intense mortalities of *P. nobilis* species in several sections of the Mediterranean Sea (Table 2), which is therefore of additional emphasis for the rehabilitation of affected stocks. The related process is of additional importance in determining, monitoring and protecting surviving individuals in infected populations in order to pave the way for the studies to be made later. In other words, strong individuals are necessary to be able to survive. It would be a crucial issue to transplant generations that could be obtained from individuals able to survive and thus become resistant to diseases in natural and culture-based conditions where they could be rehabilitated and cultured in terms of their sustainability.

Transplantation Studies in Turkey

Present in the area of construction of 1915 Çanakkale Bridge was started in 2018 between the European and Asian sides of the Çanakkale Strait, live transportation of *P. nobilis* population was transported to a secure location (Figure 9) 14 *P. nobilis* individuals were found to exist in the construction of the European side of the strait and carried to the area 500 m away from where they would eventually be buried into a 1-1.5 m depth of the sea bed. 1040 individuals from the construction on the Asian side were transplanted to the area 300-350 m afar into which to bury them 4-4.5 m depth. Two months after the transplantation, submarine observation indicated 1% mortality. One year later, however, *P. nobilis* population was seen to be healthy with good adaptation to the transplantation site. Observatory scuba diving performed in 2020 showed high rates of mortality where transplantation was performed as well as in other sections of the strait. The fact that young individuals in the transplantation area on the Asian side are those estimated to be within the age range of 1-1.5 showed that the population completed the process of adaptation with the managed stress and resultant reproduction activity. However, the area may be currently exposed to the influence of the disease *H. pinnae.*

Recommendations for the Transplantation Operation of P. nobilis

The parameters to be considered during the transplantation can be listed as below;

- Depths, water conditions, individual density and sediment structure of the areas where fan mussels are distributed are to be taken into account.
- "Specific Protection Areas" have to be constituted in transplantation sites with the exception of those where fisheries activities are forbidden and anthropogenic factors are negligible,
- "Specific Sheltered Area" is supposed to be designed to decrease or eliminate the pressure of predation similar to Subaquatic Park Systems during the studies of rehabilitation,
- Caution and care should be exerted lest byssal threads could be broken off while removing *P. nobilis* individuals off the sea bed.
- Individuals ripped off the bottom should be carried without being shaken in seawater to the transplantation area,
- Individuals should be planted as vertically as possible in the plantation process.
- Individuals should be planted considering the previous depth traces of mud and sand or unburned depth.

Conclusion and Recommendations

P. nobilis and *P. rudis* were used as human consumption for meat and their shells and byssal threads for decorative purposes until 1992 and 1996, respectively. However, due to overfishing and the destruction of their habitats, their populations have been recorded to be significantly reduced. Considering the damage to the population of *P. nobilis*, the status of the species was established to be at critical levels. Accordingly, new plans of action should be constituted in terms of its sustainability.

By determining active disease agents (*Mycobacterium spp*. and *H. pinnae*), the interaction between pathogenesis and epizootiology namely, between host, parasite and environment should be explained, which is thus vital in determining methods to be followed for regeneration of the stocks and preventing diseases from spreading.

The rapid flow of information could be provided thanks to the formation of a network system involving local administrations, people, diving clubs and commercial and sportive fishermen along marine regions in terms of mapping distribution of *P. nobilis*. Following the establishment of healthy populations, in particular, the constituents within the network system above should be allowed to assume missions on information, protection and control. Therefore, a program of protecting and rehabilitating *P. nobilis* could be instituted within such an integrated system.

Surveys of field scanning helped to record masses of mortalities in most regions from Western Mediterranean shores to Aegean Sea. Similarly, a 100% rate of mortality was seen at the Aegean mouth of the strait whereas the entrance of the Marmara Sea exhibited a 9.2% rate of mortality, where there have yet to be any cases of the diseases. The disease-stricken populations are assumed to need a long time to recover, that is, the ability of resistant individuals to function in reproduction activity. It is therefore of great importance to determine, monitor and protect such populations in view of sustainability.

It is to be aimed those spats to be picked up by collectors from healthy populations in the Marmara Sea should be exposed to rearing and planting into new sediments when they have become 15 cm in length and thus forming new populations. However, it is quite necessary to keep the area of the newly planted individuals under protection and follow them up in terms of constituting the new population. Reviving mostly or completely damaged populations by replanting processes should be applied as a mere solution.

Knowledge of the reproductive biology of the species is very important. Development of programs for adult maturation is needed. What reduces the survival rate of larva, in particular, is its high hydrophobicity, to put clearly, its sticking onto water surface and walls of the tanks, which requires an emphasis on specific techniques. It seems very important that individuals preserved in the incubator under culture conditions until the phase of spats should be taken to progressive sheltered culture systems and reared on the surface and the bottom in terms of regeneration.

The related references should be taken into consideration in prospective research concerning the studies aimed at understanding comparative evolution, comparative genomics or genetic variation of *P. nobilis* to develop effective protective plans of the genome.

Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

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

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