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RESEARCH ARTICLE

Association between the Crab, *Nepinnotheres pinnotheres* (Linnaeus, 1758), and the Endangered Species Fan Mussel, *Pinna nobilis* (Linnaeus, 1758), from the Aegean Sea, Turkey

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ABSTRACT

Pinna nobilis, commonly known as the fan mussel or pen shell, is an endemic species in the Mediterranean Sea. The fan mussel population has recently been significantly endangered along the Mediterranean coast, mainly due to diseases. However, it has a critical role in the ecological system of the throughout the coasts of the Mediterranean. Therefore, it is important to investigate the interactions between *P. nobilis* and their enemies, parasites, symbiont in the ecological environment. Pea crabs are small crustacean and symbionts in a variety of invertebrates. They inhabit the mantle cavities of bivalve. The association between the pea crab, *Nepinnotheres pinnotheres* (Decapoda), and *P. nobilis* (Bivalvia) from the Aegean Sea, Turkey was examined in this study. The crab samples were collected off Urla Karantina Island, Izmir Bay, Aegean Sea. The biometric characteristics of bivalve and crab in this coexistence were analyzed. 80% of *P. nobilis* was occupied by *N. pinnotheres*. The weight of pea crabs was recorded between 0.01 g and 3.87 g.

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Introduction

Commonly known as fan mussel, pen shell, or wings shell, *Pinna nobilis* is the largest bivalve (shell length growing up to 120 cm) and a species endemic to the Mediterranean (Zavodnik et al., 1991). Fan mussel shell length usually varies between 20 and 40 cm (Fischer et al., 1987). They live at depths from 0.5 to 60 m (Zavodnik et al., 1991; Theodorou et al., 2017) and have a long life (27 years) (Galinou-Mitsoudi et al., 2006). It is an infaunal distributed in mud, sandy mud, silt, rocks bottom, and holds on to small gravel or pieces of shells by its byssus (Tebble, 1966; Theodorou et al., 2015). Furthermore, *Posidonia oceanica* meadows can be seen intensively in the habitats of fan mussel (García-March et al., 2006). *P. nobilis* is filter feeding organisms that feed on suspended organic matter, inorganic matter, zooplankton, phytoplankton, bacteria, and viruses from the water column (Gosling, 2003; Davenport et al., 2011). Thus, it contributes to an increase in water quality. Because of this, it has a vital role in the ecological system of the Mediterranean Sea (Natalotto et al., 2015).

The fan mussel population has recently become dramatically reduced on the Mediterranean seashore, particularly in countries such as France, Greece, Spain, Italy, and Croatia (Vicente and Moretau, 1991; Catanese et al.,

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2018). In some regions, the population density has dropped from 17 individuals per 100 m^2 (Richardson et al., 1999) to 1 individual per 100 m^2 (Katsanevakis and Thessalou-Legaki, 2009). Nowadays, in some areas in the western Mediterranean and the Tyrrhenian Sea, Italy, 100% of deaths occurred (Catanese et al., 2018; Carella et al., 2019). Moreover, Katsanevakis et al. (2019) carried out a study about the mass mortalities of the *Pinna nobilis* in the Lesvos Island, northern Aegean Sea. They identified the occurrence of parasite *Haplosporidium pinnae* with 100% prevalence as histopathological and molecular analysis examinations. *P. nobilis* is under threat of extinction due to damage to coastal habitat, increased water pollution, the destruction caused by fishing activities, harvesting for the decorative use, the damage made by dragging anchors of recreational boats, the presence of a haplosporidianum parasite in the digestive tract, and anthropological activities (Vincente and Moreteau, 1991; Ayaz et al., 2006; Hendriks et al., 2013; Deudero et al., 2015; Capó et al., 2015; Dariba, 2017). Due to these causes, *P. nobilis* has been listed as an endangered species in the Mediterranean ecosystem and has been 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 Turkey, *P. nobilis* has been under protection since 1998 when Directorate of Food and Control, Ministry of Food Agriculture and Livestock issued a regulation, circulation No. 32/1, on Marine and Inland Commercial Fisheries.

Pea crabs live either parasitic or in commensal association with an array of marine organisms and are ubiquitous throughout the world. They inhabit the mantle cavity of bivalves (Palmer, 1995) and gastropods (Geiger and Martin, 1999), in the tubes of polychaetous worms (Grove and Woodin, 1996), in the gut cloaca, and respiratory trees of holothurians (Hamel et al., 1999), the oral surface of sea urchins, and pharyngeal cavity of tunicates (Reeves and Brooks, 2001). Pinnotherid crabs are used as a permanent shelter and often as a food source by the breeding female (Becker and Türkay, 2017).

Nepinnotheres pinnotheres (described as *Cancer pinnotheres* Linnaeus, 1758) is known as pinna pea crab with brown color. Carapace average length is 7 mm for male and 12 mm for female (Hayward and Ryland, 1995). This species is parasitic and found in the mantle cavity of the *P. nobilis* and also in ascidians (Becker and Türkay, 2017). It is thought that *N. pinnotheres* live in *P. nobilis* as an impermanent refuge. Therefore, more than one male is encountered in the same *P. nobilis* (Rabaoui et al., 2008).

At present, the population of *P. nobilis* is under significant threat on the Mediterranean coasts. Moreover, little information on the behavior, morphology, ecology, enemies, and diseases of *P. nobilis* is known and there is need of detailed investigations. This study examines the association between the fan mussel, *P. nobilis* and the pea crab, *N. pinnotheres*.

Materials and Methods

This study was carried out with individuals sampled during the investigation of reproductive cycle biochemical composition of *P. nobilis* between March 2008 and February 2009 by Acarlı et al. (2018). The materials were obtained monthly and a total of one hundred and fifteen individuals of fan mussels were examined throughout the study. The study area is shallow coastal water with a depth from 5 to 20 m. The bottom is primarily composed of muddy sand and covered with meadows (Figure 1).

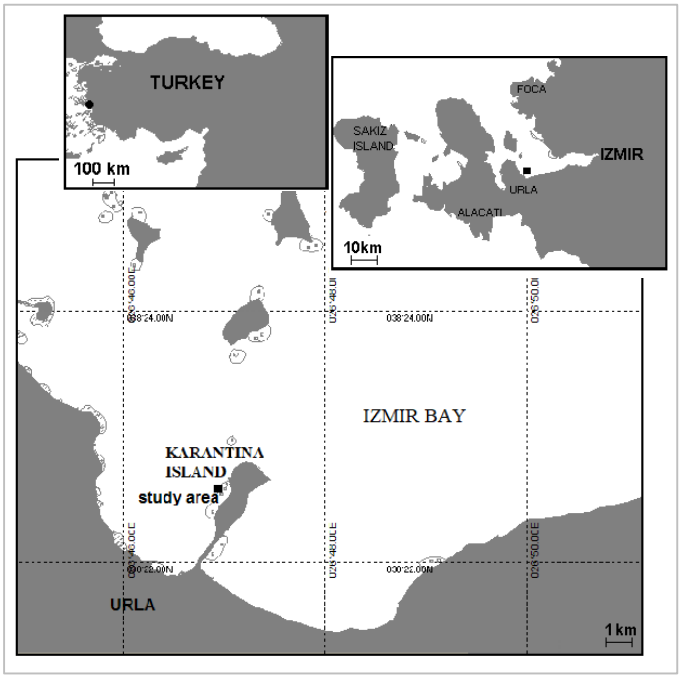

Figure 1. Map showing the study area

Fan mussels were transported to the Shellfish Laboratory of Fisheries Faculty (Ege University, Izmir, Turkey). Biofouling and other adherence were entirely removed from samples that then were for biological parameters such as shell length and total weight. Total weight and shell length were measured with a caliper and an electronic scale, respectively. Anterior and posterior adductors of *P. nobilis* were cut by a knife, and then its shells were opened. Careful examination for pea crabs was made inside fan mussels, and species were determined using the key of Silas and Alagarswami (1965). The monthly distribution of infested individuals was identified during the study period. Each pea crab was weighed during the study period. The Kolmogorov-Smirnov Test was applied to determine the normality of the data. According to the results of this test, Pearson's correlation analysis was used to assess the degree of association between host size and weight of pea crab. Data were analyzed using SPSS 13.0 for Windows.

Results and Discussion

The shell length of *P. nobilis* from the Aegean Sea was recorded as 51.45 cm (min. 36.5- max. 73.5), and width as 17.36 cm (min.12.5- max.24.5). The weight and thickness were recorded as 2076.4 g (min.864.9- max.4533.9) and 6.11 cm (min.3.9- max.12), respectively.

The crab samples were found in the mantle of the fan mussel (Figure 2). A total of 93 pea crabs were found in 84 *P. nobilis* individuals. 73% of the population was infected.

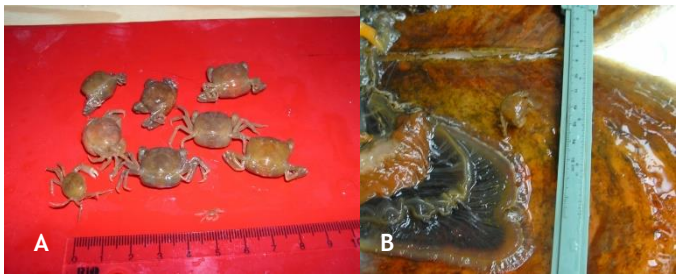

Figure 2. Photographs showing pea crabs (A) and pea crab on the *P. nobilis* shell (B)

Seventy-eight fan mussels were occupied by one crab, one fan mussel by three crabs, four fan mussels by two crabs, and one fan mussel by four crabs. The weight of pea crabs was recorded as between 0.01 g and 3.87 g (Figure 3). The shell length of infested fan mussels ranged between 38 cm and 73.5 cm (Figure 4). When the association rate of *N. pinnotheres* and *P. nobilis* seasonally is examined, infested fan mussels were observed throughout the year, reaching the highest value with %100 in April. The lowest value was found to be in October with 70% infestation (Figure 5). No correlation was found between the weight of the pea crab and host size (P>0.05) (Figure 6).

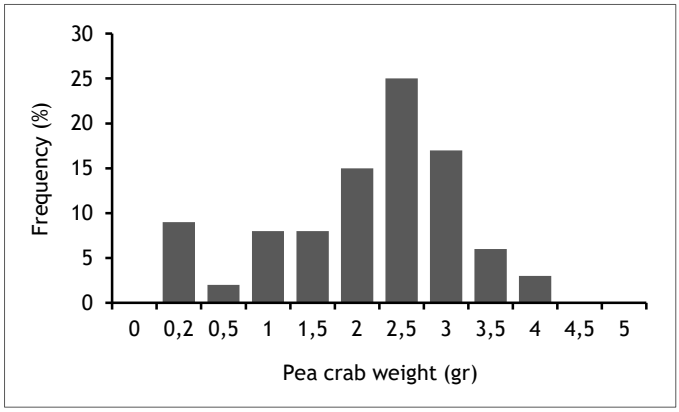

Figure 3. Weight frequency of pea crab, *Nepinnotheres pinnotheres*

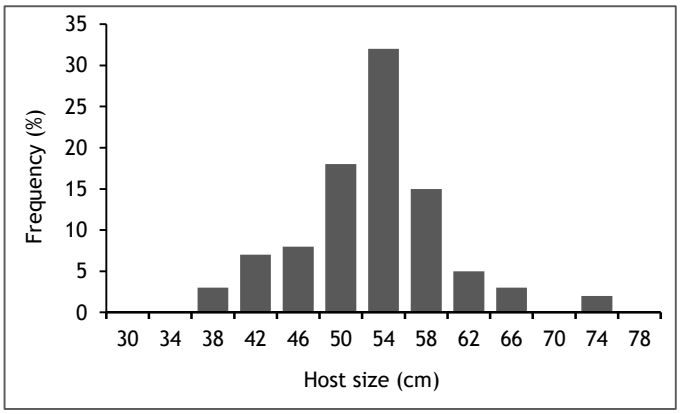

Figure 4. Length frequency of infested *Pinna nobilis* population of Urla Karantina Island

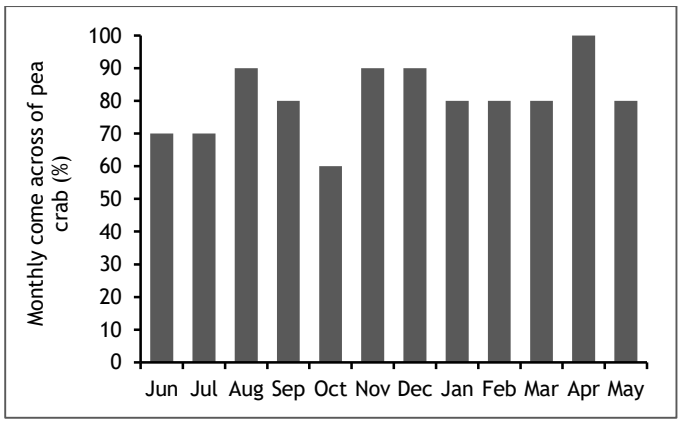

Figure 5. Monthly variation of infested *Pinna nobilis* (%)

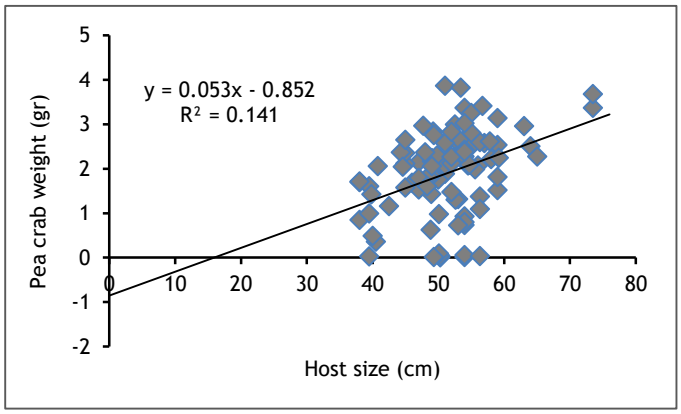

Figure 6. Relationship between infested *Pinna nobilis* (cm) and pea crab, *Nepinnotheres pinnotheres*

N. pinnotheres were reported especially on fan mussel *Pinna nobilis* (Rabaoui et al., 2008; Becker and Türkay, 2010; Cabanellas-Reboredo et al., 2010; Akyol and Ulaş, 2015; Trigos and Vicente, 2018), *Atrina pectinata* (Delongueville and Scaillet, 2002), *Perna canalicus, Mytilus edulis, Mactra ocata* (Stevens, 1990), and ascidians such as *Halocynthiapapillosa*, *Microcosmus* spp. (Becker and Türkay, 2017), and *Ascidia mentula* (Lutzen, 1967; Becker and Türkay 2017).

There is no detailed study on the association between the crab, *N. pinnotheres* and *P. nobilis*. Only, Rabaoui et al. (2008) found an association of 56.7% between *N. pinnotheres* and *P. nobilis* from StahJaber. Cabanellas-Reboredo et al. (2010) identified *N. pinnotheres* in 8.3% of the fan mussel samples they examined. The association rate of *N. pinnotheres* in *P. nobilis* in the Aegean Sea is found to be higher than those reported in those studies. This difference possibly may be explained with environmental factors such as tide, flow, sediment structure, amount of nutrition, mollusk status (length of buried shell and age of mussel), or amount of fan mussel examined by researchers.

There are several studies about epibionts (Giacobbe, 2002, Cosentino and Giacobbe 2007, Rabaoui et al., 2009, Addis et al., 2009) and commensals (Richardson et al., 1997; Kennedy et al., 2001, Calafiore et al., 1991, Laganà et al., 2007; Rabaoui et al., 2008, Rada and Milat, 2009; Cabanellas-Reboredo et al., 2010, Akyol and Ulaş, 2015) of fan mussel. Acarlı et al. (2010) and Basso et al. (2015) stated that fan

mussel has a rich biodiversity that is composed of macrobenthos, epibionts, and commensal species.

Va´zquez-Luis et al. (2015) examined the mass mortalities of the bivalve *P. nobilis* with mortality rates up to 100% in the Spanish Mediterranean Sea. Their histological examinations indicated that a haplosporidian-like parasite living in the digestive gland is responsible for these mortalities. Darriba (2017) reported that a parasite belonging to the haplosporidian group infected *P. nobilis* and caused mortality in Alicante (Spain, Western Mediterranean). On the Spanish coasts of the Western Mediterranean Sea, *Haplosporidium pinnae* are most likely responsible for the mass mortality of *P. nobilis* (Catanese et al., 2018). In Campania and Sicily, large scale mortality of *P. nobilis* population was noticed pen shella of all sizes because of mycobacterial diseases (Carella et al., 2019).

Trottier (2012) indicated that pea crab *Nepinnotheres novaezelandiae* (Filhol, 1885) caused a significant loss of production on aquaculture in New Zealand green-lipped mussels, *Perna canaliculus*. Pea crabs lead to a variety of negative effects such as gill damage, shell distortion, nodule formation, and decrease in condition index to their host (Trottier, 2014). Becker and Türkay (2017) indicated that larger hosts were probably preferred due to more significant food resources. Consequently, it might be expected that *P. nobilis* is a highly infested host. There was a relationship between damage to hosts and host size. Thus, small individuals infested with pinnotherids may be more severely affected than older individuals.

Conclusion

In this study, the damage to the tissue of the infested fan mussel was inconspicuous during the whole study period. We think that *N. pinnotheres* is not directly effective on the healthy *P. nobilis* population. On the other hand, with adverse environmental conditions or the spread of infectious diseases of bivalve such as a mycobacterial and a haplosporidian parasite, it may threaten its physical condition.

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