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Reproductive biology of the rayed pearl oyster (*Pinctada imbricata* radiata, Leach 1814) in Izmir Bay

by

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Abstract

The present study was carried out to determine gonadal stages and quality of pearl oyster meat (*Pinctada imbricata radiata*, Leach, 1814) in Izmir Bay (Turkey). Pearl oyster samples were collected from the study area at a depth of ~5 m between February 2013 and January 2014. The highest and lowest temperature was measured in July and January as 27°C and 14.2°C, respectively. The maximum chlorophyll *a* value of 4.640 µg l^{-1} was calculated in May and the lowest value of 1.009 µg l^{-1} was recorded in April. Individuals reached their first maturity in April. Spawning activity was observed from June to September and the gonad index (GI) was at the highest level during those months. The development was observed from April to February. The overall female to male ratio was 1.32:1 ($p < 0.05$) and it did not affect the GI ($p > 0.05$). There is a strong positive correlation between the GI and temperature ($p < 0.05$). The highest condition index (CI) was recorded in May as 12.31 ± 0.51, whereas the lowest one in January as 7.37 ± 0.22. As a result, this study revealed that the pearl oyster population in the region is characterized by high reproductive activity, especially during the summer months.

Key words: pearl oyster, *Pinctada imbricata radiata*, meat quality, gonad development, gonad index

1. Introduction

The subtropical rayed pearl oyster *P. imbricata radiata* (Leach 1814) is native to the Indo-Pacific region and has been recorded in the eastern Mediterranean Sea since 1892 (Dautzenberg 1895). The pearl oyster *P. imbricata radiata* is a bivalve species belonging to the Pteriidae family. It is a Lessepsian species (Dogan & Nerlovic 2008; Deidun et al. 2014), occurring along the Mediterranean and Aegean coasts, with a significant presence in Tunisia, Sicily (Italy), Malta, Croatia, Portugal, Montenegro, Greece and Turkey (Streftaris et al. 2005; Tlig-Zouari et al. 2010; Antit et al. 2011; Derbali et al. 2011; Lodola et al. 2013; Dogan & Nerlovic 2008; Katsanevakis et al. 2008; Gavrilovic et al. 2017; Petovic & Macic 2017; Theodorou et al. 2019).

Since pearl oyster farming benefits the food and ornamental industries as well as pearl production, it offers significant economic potential due to the valuable species used in rearing (Baqueiro & Castagna 1988; Gervis & Sims 1992; Urban 2000; Sahin et al. 2006; Hernandez-Olalde et al. 2007). The annual market value of pearl culture was 3–5 billion dollars by the year 2000 (Saucedo et al. 2001). The pearl oyster culture industry is not dependent on a large capital (Lodeiros et al. 2002). However, the reason behind the failure in breeding is the lack of knowledge about the species biology and larvae production (Choi & Chang 2003; Gosling 2003; Gribben et al. 2004; Hwang 2007; Wada et al. 1995).

This is the first study on the reproduction activity and the condition index of rayed pearl oysters in this area. The objective of this study is to provide a detailed explanation of the reproductive cycle of pearl oysters in Izmir Bay by analyzing the condition index, meat yield, the gonad index and their correlation with temperature, salinity and chlorophyll *a*.

2. Materials and methods

2.1. Sampling area

The coast of Karantina Island (38°22′44″N, 26°47′12″E), located in the western part of Izmir Bay (Turkey), was selected as a study area between February 2013 and January 2014 (Fig. 1). Pearl oyster samples were collected from sandy, muddy and stony habitats.

2.2. Sampling procedure

Pearl oysters (n = 30) were collected monthly by divers in the sea at a depth ranging from 3 to 5 m.

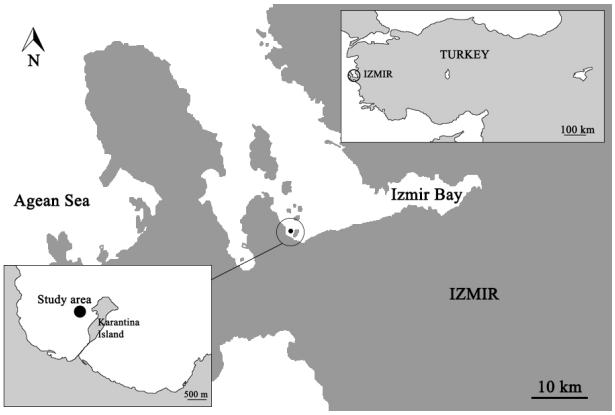

Figure 1

Geographical location of the sampling area (Izmir Bay, Karantina Island, Turkey)

The length of each individual was measured along its dorsoventral axis. The length of shells was measured with a digital caliper (sensitivity of 0.01 mm). The total fresh weight was measured with an accuracy of 0.001 g.

2.3. Hydrological parameters

Temperature, salinity and chlorophyll *a* profiles were measured. Temperature was measured using a mercury thermometer. Salinity was measured using a refractometer. Chlorophyll *a* was determined at the laboratory of Ege University by filtering through GF/C filters according to Strickland and Persons (1972).

2.4. Reproductive analysis

Oyster shells were opened using a shell opener and the gonad section was separated with a scalpel. The gonadal tissue was fixed in Davidson's solution for 24 h (Shaw & Battle 1957), and then stored in 10% formalin (Lillie 1965). Samples were subsequently dehydrated with alcohol and xylene series, embedded in paraffin (Histowax, Leica), sectioned to a thickness of 5 µm with a microtome and stained with haematoxylin and 0.5% eosin (Howard & Smith 1983). The obtained sections were examined under a light microscope. The gonadal stages were determined by modifying the number of gametogenic cycles specified by Tranter (1958).

Inactive Female and male reproductive cells are not visible. Only connective tissue is observed (Fig. 2a).

Development The first stage in males, where spermatogonia, spermatocytes and spermatids are placed in the follicle. It represents early

spermatogenesis. The volume of the follicle decreases and spermatogonia turn into spermatocytes (Fig. 2b). In females, early oogenesis is observed. Previtellogenic and vitellogenic oocytes are located on the follicle wall. At this stage, vitellogenic oocytes grow along the follicle wall (Fig. 2c).

Mature At this stage in males, centralized spermatids are transformed into spermatozoa, filling the center of the follicle. This is followed by a reduction in the number of spermatogonia around the walls of the follicle (Fig. 2d). In females, the volume of vitellogenic oocytes adhering to the follicle wall increases. Mature oocytes accumulate in the follicle lumen (Fig. 2e).

Spawning The number of spermatozoa increases in male individuals. Follicles expand. The distance between the follicles decreases (Fig. 2f). The number of mature oocytes in females increases. The follicle volume expands. Follicle walls adhere to each other (Fig. 2g).

Spent At this stage in males, the follicle is almost empty. Few residual spermatozoa are visible. The cytolysis is observed (Fig. 2h). In females, few residual oogonia and cytolysis are visible (Fig. 2i).

2.5. Gonad Index (GI)

The gonad index (GI) was estimated using a numerical grading system based on the maturity stages of pearl oysters (Soria et al. 2002). Based on the gonadal development, three category scores (CS) were defined as follows:

1 = Inactive (I) + Spent (VI),

2 = Development (II),

3 = Mature (III) + Spawning (IV).

The total number of individuals in each reproductive stage is multiplied by the category score and the sum is calculated. The result is divided by the total number of individuals.

The GI was calculated according to the formula presented below (Soria et al. 2002):

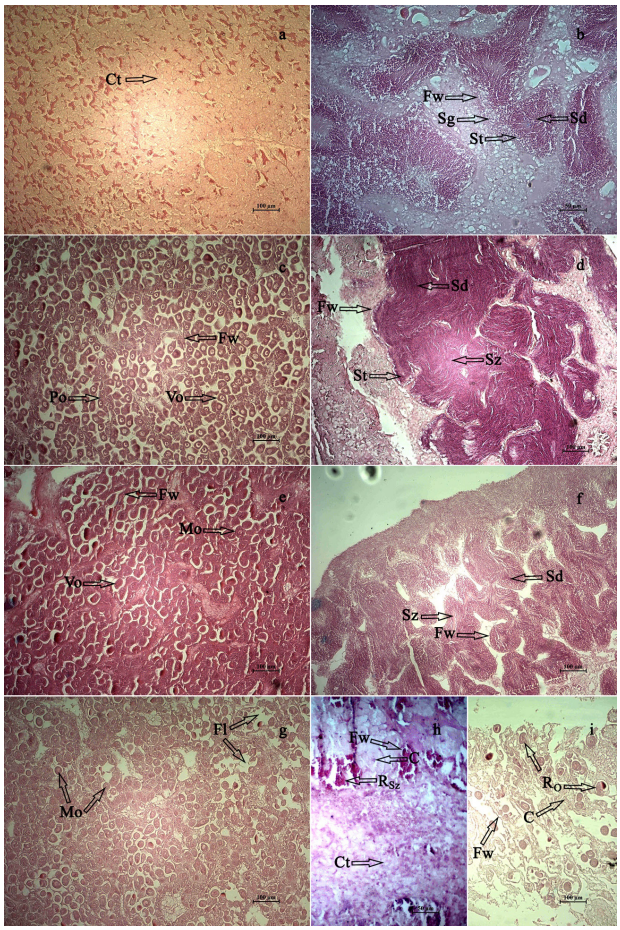

Figure 2

Gonadal stages of *Pinctada radiata*

a – Stage 0: Inactive; b – Stage 1: Development in male individuals (Fw: Follicular wall, Sd: Spermatid, Sg: Spermatogonia, St: Spermatocyte; c – Stage 1: Development in female individuals (Po: Previtellogenic oocyte, Vo: Vitellogenic oocyte); d – Stage 2: Maturity in male individuals (St: Spermatocyte, Sd: Spermatid, Sz: Spermatozoa, Fw: Follicular wall), e – Stage 2: Maturity in female individuals (Mo: Mature oocyte, Vo: Vitellogenic oocyte, Fw: Follicular wall); f – Stage 3: Spawning in male individuals (Sd: Spermatid, Sz: Spermatozoa, Fw: Follicular wall); g – Stage 3: Spawning in female individuals (Mo: Mature oocyte, Fl: Follicular lumen); h – Stage 4: Spent in males (Fw: Follicular wall, Rsz: Residual spermatozoa, C: Cytolysis, Ct: Connective tissue); i – Stage 4: Spent in females (Fw: Follicular wall, Ro: Residual oogonia, C: Cytolysis)

$$GI = \frac{\left[n\,\text{stage}(I+VI)\times CS\right]+\left[n\,\text{stage}(II)\times CS\right]+\left[n\,\text{stage}(III+IV)\times CS\right]}{\text{Number of animals in the sample}} \quad (1)$$

2.6. Analysis of Condition Index (CI) and Meat Yield (MY)

To analyze the CI and MY, 30 specimens of pearl oysters were processed monthly. The accompanying parameters were measured for each pearl oyster in grams (g): dry meat weight (DMW) and dry shell weight (DSW) for the condition index (CI), and wet meat weight (WMW) and total weight (TW) for meat yield (MY). The calculation of CI and MY (Walne 1976; Crosby & Gale 1990) was based on the following formulas:

$$CI = \frac{DMW}{DSW} \times 100 \quad (2)$$

$$MY = \frac{MWW}{TW} \times 100 \quad (3)$$

2.7. Statistical analysis

The distribution of data was tested to determine their normality using the Kolmogorov–Smirnov test. Pearson's correlation analysis was applied to determine the degree of association between the environmental parameters and the gonad index, the condition index, and the meat yield. ANOVA followed by Tukey's post hoc test (Zar 1996) was used to confirm differences in the monthly condition index and meat yield. The sex ratio was analyzed using chi-square (χ^2). The results were presented as means (standard deviation) and the significance level used for the test was $p = 0.05$. Statistical analysis was performed using SPSS software.

3. Results

3.1. Sampling measurements

The average shell length and the total flesh weight of the examined pearl oysters are shown in Table 1. The length of shells measured in this study ranges from 47.79 mm to 100.85 mm (mean lengths 73.21 ± 9.46 mm). The weight of the oysters ranged between 14.02 g and 135.02 g (mean weight 54.09 ± 22.60 g).

3.2. Hydrological parameters

The maximum and minimum water temperature in the region was measured as 27°C in July 2013 and 14.2°C in January 2014, respectively. The lowest salinity value of 35 PSU was recorded in May and the highest in September – 38 PSU (Fig. 3a). The highest and the lowest values of chlorophyll *a* were determined as 4.64 µg l^{-1} in May and 1.009 µg l^{-1} in April, respectively (Fig. 3b).

Table 1

Mean length and total fresh weight of pearl oyster samples used in the study

Months	Pearl oyster samples	Mean length	Mean weight
Feb.	30	73.99 ± 10.67	52.72 ± 23.08
Mar.		75.42 ± 8.99	60.02 ± 19.30
Apr.		75.22 ± 9.54	61.25 ± 22.23
May		72.03 ± 9.28	51.85 ± 23.03
June		73.42 ± 11.26	57.03 ± 29.62
July		71.10 ± 9.60	54.40 ± 25.43
Aug.		72.87 ± 9.71	56.33 ± 24.46
Sept.		74.57 ± 11.19	60.45 ± 29.50
Oct.		73.87 ± 6.54	53.41 ± 13.59
Nov.		75.22 ± 9.54	55.60 ± 21.98
Dec.		72.52 ± 5.91	49.39 ± 13.58
Jan.		68.25 ± 6.36	41.38 ± 12.37

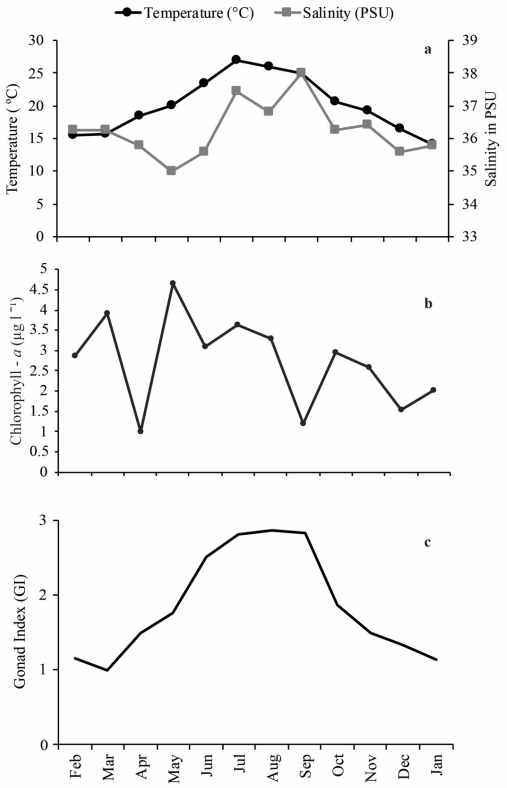

Figure 3

Environmental parameters in the study area: a) temperature and salinity; b) chlorophyll *a*; c) gonad index from February 2013 to January 2014

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3.3. Reproductive analysis

Five stages of reproduction were determined according to the cycles of gametes on histological images: inactive, development, mature, spawning, spent.

According to the evaluation of histological sections, *P. imbricata radiata* spawns almost every month except March, but the peak of spawning was observed from June to September (Fig. 4). The development, maturity and spawning in female and male individuals were first observed in April (Fig. 4a,b). When histological sections were examined by sex, it was found that the spawning rate in April was higher in male individuals than in females (Fig. 4b).

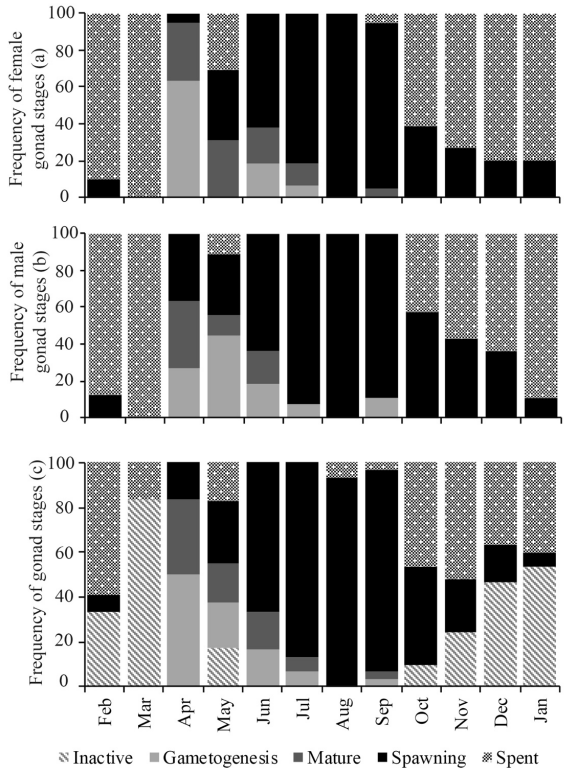

Figure 4

Frequency of gonad development stages in *P. radiata* in Izmir Bay from February 2013 to January 2014 (a – frequency of female gonad stages, b – frequency of male gonad stages, c – frequency of gonad stages)

The overall female to male ratio was 1.32:1 (χ^2 test $p < 0.05$). A hermaphrodite was found in 0.27% (n = 1) of pearl oysters. When the sexes were assessed by length, the male ratio was found to decrease with increasing length (Fig. 5).

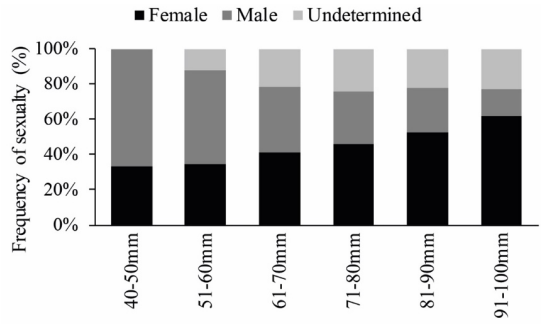

Figure 5

Changes in the sex proportion (%) in *P. imbricata* radiata in the study area

3.4. Gonad Index

The GI value was calculated as 1 in March when all individuals were inactive and spent. The highest values of the GI (above 2.5) were calculated in the months of peak reproductive activity of this species (June, July, August and September): 2.8 in July, 2.9 in August and 2.8 in September (Fig. 2c). There is a strong positive correlation between the GI and temperature ($p < 0.05$; $r = 0.968$).

3.5. Meat Yield and Condition Index

The MY and CI were calculated for pearl oysters, whose average length and weight were 73.21 ± 0.47 mm and 54.09 ± 22.60 g, respectively. The minimum MY was 19.11 ± 0.50 in October, while the maximum MY was 24.8 ± 0.66 in April (Fig. 6). The difference between the monthly mean values of the MY values was statistically significant ($df = 11$; $F = 24.577$; $p < 0.05$). The CI was 7.61 ± 0.37 in August 2013 and the highest value of 12.31 ± 0.51 was recorded in May 2013, while the minimum value of 7.37 ± 0.22 in January 2014 (Fig. 6). There was a significant difference in the CI between the months ($df = 11$; $F = 14.675$; $p < 0.05$).

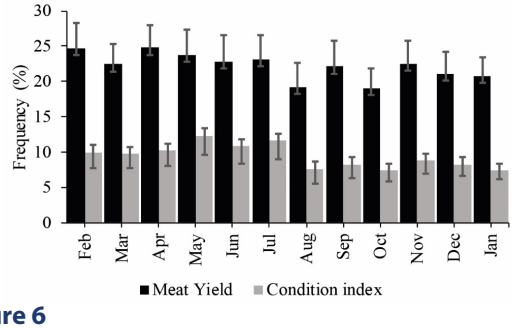

Figure 6

Meat yield (%) and the condition index of pearl oysters in the study area

4. Discussion

Gonad development is defined as changes in gonads during inactive reproductive periods, which are complex processes that occur with seasonal changes when appropriate biological and physical conditions are provided (Quintana 2005). Many studies have attempted to explain the effect of environmental parameters on gonadal development and reproduction of different species of bivalve mollusks. Some researchers reported that temperature, as an environmental parameter, has a major effect on reproduction (Behzadi et al. 1997; Choi & Chang 2003; Delgado & Camacho 2007; Wada et al. 1995; Sastry 1979). Wada et al. (1995) reported that various factors such as temperature, salinity and availability of food in the environment can affect the gametogenic cycle of bivalves, and this applies in particular to temperature.

When water temperature increased in April to 18.4°C, the reproductive cells developed rapidly and the maturation process began. During that period, spermatocytes and spermatids from reproductive cells were observed in large numbers in male individuals, while oocytes and oogonia were observed in female individuals. It was found that the spawning phase began as the water temperature increased to 20°C in May. Choi & Chang (2003) reported that oocytes develop and mature as water temperature rises and the reproductive activity of pearl oysters was observed in water with temperature above 15°C. Mature oocytes were released by pearl oysters when water temperature began to rise to 20.1°C. Choi & Chang (2003) reported that the main breeding peak activity of *Pinctada fucata martensii* (= *P. radiata*) occurred in summer and fertilization continued until September (25°C; July maximum temperature of 28.3°C). The spawning of *P. mazatlantica* in Mexico was associated with high water temperature (27°C to 30°C; Saucedo & Monteforte 1997). Behzadi et al. (1997) reported that the beginning of *P. fucata* breeding in the Persian Gulf was observed when the temperature started to rise in spring and summer, i.e. at 27.6°C in April and 31.7°C in June. On the other hand, the breeding period continued for a considerable part of autumn at temperatures ranging from 32°C in October to 24°C in December. In a similar study on *P. radiata* conducted in Tunisia, it was found that the development started in March when the water temperature reached 17°C. The first spawning period occurred when the seawater temperature rose from 27°C in May to 32°C in August. The second spawning period occurred when the water temperature dropped from 30°C in September to 20°C in November (Lassoued et al. 2018). It was concluded that high temperatures affect the release of gametes and a decrease in temperature enabled the gonads to mature quickly in a short time (Lassoued et al. 2018). In our study, the reproduction activity of pearl oysters decreased with decreasing water temperature. Suarez et al. (2005) stated that high and low temperatures had different effects on oogenesis and spermatogenesis in their studies. Derbali et al. (2009) reported that the first increase in breeding activity in Tunisia was associated with the seawater temperature rising from 26.7°C in May to 29.7°C in June, while the second spawning occurred when the temperature dropped from 25.5°C in September to 20.6°C in October. Another study on *P. radiata* in Tunisia reported that a breeding peak occurred at about 25°C (Zouari & Zaouali 1994). Khamdan (1998) reported two peak periods for *P. radiata* in Bahrain (August–September and November–December) between 20°C and 30°C. A study from Kenya reported that *P. radiata* at two different locations matured at a temperature of 26°C to 32°C throughout the year, with a breeding peak at a temperature of 26°C to 29°C (Kimani et al. 2006).

As an environmental factor, salinity is often considered to a lesser extent in terms of its effects on the survival and distribution of marine organisms and their reproductive strategies (Steel & Steel 1991). Gervis and Sims (1992) reported that ovulation in pearl oysters was usually triggered by a change in environmental conditions, such as an increase or decrease in water temperature or salinity. In this study, the lowest salinity of 35 PSU was recorded in May. Derbali et al. (2009) reported that the spawning season of *P. radiata* was probably associated with changes in water temperature accompanied by fluctuations in salinity, and salinity was a factor to be considered. In addition to the effect of environmental parameters on reproduction, the availability and abundance of food in the natural environment play an important role in the reproductive cycle (Delgado & Camacho 2007; Gervis & Sims 1992). Urban (2000) reported that the spawning of *P. imbricata* in the Caribbean occurred during the upwelling season, when food availability was higher. The phytoplankton concentration was generally higher in spring and early autumn compared to summer and winter (Gómez & Gorsky 2003; Valiela & Cebrián 1999). In this study, chlorophyll *a* reached the highest level with a value of 4.645 µg l^{-1} in May. It appears that the peak of nutrient abundance in water triggered pearl oysters to start spawning. Chlorophyll *a* values were determined as 3.633 µg l^{-1} and 3.293 µg l^{-1} between July and August (spawning peak months), respectively, and decreased to 1.192 µg l^{-1} in September.

In this study, spawning of *P. imbricata radiata* was observed in all months from April to February except

for March. The spawning peak was observed in July and August, but June and September should be mentioned too as the GI was above 2.5. In the studies on spat collection conducted in the same region, it was found that *P. radiata* spats were mostly collected in July, August and September (Yigitkurt et al. 2017; Yigitkurt et al. 2020). However, in a similar study conducted on *P. radiata* in Tunisia, the reproduction period continued between May and December, and two different breeding peaks were observed in July and November (Table 2; Derbali et al. 2009). It was also reported that the spawning peak of *P. radiata* in Tunisia was in June (Zouari & Zaouali 1994). Another study conducted in Tunisia reported that *P. radiata* reproduced in June, September and November, and the spawning peak occurred in June (Lassoued et al. 2018). The study on the reproduction of *P. radiata* conducted in Iran reported that the mature stage was observed between February and April, and the spawning peak was in summer (Karami et al. 2014). In Bahrain, reproduction of *P. radiata* was observed between May and November, and the spawning peak was observed from January to March, with another breeding peak occurring in October (Khamdan 1998). In a similar study conducted in Kenya, it was determined that the gonadal activity of *P. radiata* was relatively continuous and its reproduction peak was in July and October (Kimani et al. 2006). Reproduction of *P. fucata martensii* in South Korea was observed from April to August, and the spawning peak in June and July (Choi & Chang 2003). In the study conducted on *P. imbricata* in Colombia, reproduction was reported from December to June and October, with the spawning peak in March and October (Urban 2000). A similar study conducted in Australia reported that the reproduction period of *P. imbricata* varied from year to year, so in the first year of the study it was observed from October to April, and in subsequent years from May to August and from April to July, while the spawning peaks were observed from December to January and from March to May (O'Connor & Lowler 2004).

In the study on the growth and gonad development of the pearl oyster (*P. margaritifera*) in the Gulf of Aden, the female to male ratio was determined as 1:1.2 (Aideed et al. 2014). In the study carried out in Gazi Bay, the female to male ratio for pearl oysters *P. imbricata* was 0.76:1 (Kimani et al. 2006). In this study, 44.44% of the individuals were determined as females and 33.59% as males, while 21.69% of the individuals could not be identified due to inactive gonads and 0.27% of individuals were hermaphroditic (Fig. 6). The female to male ratio was 1.32:1 and, in contrast to the above-mentioned studies, females dominated in the population. A positive correlation was found between the female to male ratio and temperature ($p < 0.05$). Only one individual in the studied population was hermaphroditic, which indicates that hermaphroditism is a very rare condition for this species.

It was found that the number of females in pearl oysters increases with their length and age, as this is linked to protandric hermaphroditism and the availability of suitable food in the environment (Aideed et al. 2014; Kimani et al. 2006; Peharda et al. 2006; Acarli et al. 2018). Similarly, in this study, 33.33% of the individuals in the 40 to 50 mm length range

Table 2

Observations of the reproductive status and temperature range in pearl oysters in different areas

Study area	Species	Temperature (min.–max °C)	Spawning periods	Spawning peak periods	Authors
South Korea	*P. fucata martensii*	13.5–28.3	April–August	June–July	Choi & Chang 2003
Tunisia	*P.radiata*	12–30	May–December	July–November	Derbali et al. 2009
Iran	*P.radiata*	–	February–April	summer	Karami et al. 2014
Tunisia	*P.radiata*	12–27	–	June	Zouari & Zaouali 1994
Columbia	*P. imbricata*	24.7–27.5	December–June and October	January–March and October	Urban 2000
Bahrain	*P.radiata*	15–30	May–December	August–September and November–December	Khamdan 1998
Tunisia	*P. radiata*	17–32	June, September and November	June	Lassoued et al. 2018
Kenya	*P. radiata*	26–32	July, September–February, April–May	July (site2) and October (site1)	Kimani et al. 2006
Australia	*P. imbricata*	15–25	October–April and May–August and April–July (two years)	December–January and March–May	O'Connor and Lowler 2004
Turkey	*P. imbricata radiata*	14.2–27	April–January	June–September	this study

were females, while 61.53% of the individuals in the length range between 91 and 100 mm were identified as females, and the number of female individuals clearly increased with increasing length of individuals, because *P. radiata* is a protandric successive hermaphrodite species.

The GI is used to determine the reproductive status of individuals (Karami et al. 2014; Raleigh & Keegan 2006; Le Moullac et al. 2009). The highest GI values were recorded in the summer months when spawning peaked – 2.8 in July and September and 2.9 in August. The GI value (2.5) recorded in June was also very close to the highest value. The GI was 1 in March when there was no reproductive activity. In a similar study from Tunisia on the development of gonads in *P. radiata*, the GI was referred to as "Mature Index", with the highest value in June (4.2) and the lowest in March (1.3; Lassoued et al. 2018). These reported values were almost consistent with our study. The GI results reflect the identified gonad development stages. The increasing index indicates that the gonads are developing, while the decreasing index indicates the continuation of ovulation (Raleigh & Keegan 2006). A very strong correlation between the GI and the water temperature was found in this study ($p < 0.05$).

Fluctuations in the CI were associated with the nutritional status and reproduction of mollusks (Searcy-Bernal 1984). Le Moullac et al. (2012) reported that the CI was an effective indicator of reproductive events in pearl oysters. The CI and MY in Bivalvia show seasonal variations, which was strongly related to water temperature, food availability and reproductive cycle (Fernandez-Reiriz et al. 1996). Okumus & Stirling (1998) found that the CI and MY in bivalves are affected by various external and internal factors such as water temperature and salinity, food availability and the gametogenic cycle of the animals. The main purpose of determining the CI and MY in this study was to compare seasonal differences in reproductive activity in relation to different gonad stages identified in histological examinations. The maximum CI (12.31) recorded in May started to decrease in July, with the spawning peak in August and September, which was determined as 8.23 ± 1.85 in September. Similarly, MY started to decrease with spawning. A decrease in CI and MY values during the peak of the spawning season is expected due to the release of gametes. The CI values were low due to partial fertilization in the period from October to February. In the study conducted in Tunisia, the lowest CI values for *P. radiata* were recorded in August–September and December (Lassoued et al. 2018). Le Moullac et al. (2012) associated changes in the CI with weight loss caused by gamete oscillations, demonstrating a certain degree of consistency in histological analysis of the population.

It was the first study to determine the reproduction characteristics of the rayed pearl oyster *P. imbricata radiata* in Izmir Bay, Turkey. As a result of histological examinations, it was concluded that July, August and September were the months when reproduction was most intense and a single peak period was determined in the study area. The reproductive activity was observed throughout the year except for March and April. It was revealed that the condition index and meat yield values decreased in the months when the reproductive activity increased. The reproduction and condition index were correlated with water temperature, salinity and chlorophyll *a* values. These results were the first for the region and will serve as a reference for further studies.

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