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Determination of the minimum reproductive size of mussels *Mytilus galloprovincialis* (Lamarck, 1819) in the Eastern Mediterranean

Ali KIRTIK

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Ege University, Faculty of Fisheries, Department of Aquaculture, 35040, Izmir, Türkiye

ORCID IDs of the author(s):

A.K. 0000-0002-5004-1391

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Correspondence:
Ali KIRTIK
E-mail: ali.kirtik@ege.edu.tr

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ABSTRACT

The minimum shell length of sexual activity of the Mediterranean mussel (*Mytilus galloprovincialis*, Lamarck 1819) was studied at three different stations in the Aegean Sea in November 2020. Individuals ranging between 7mm and 30mm in length were collected, and gonads were histologically observed in November, which is the initial sexually active month of mussels annually for this region. Water temperature was monitored in situ at the stations, and environmental parameters, chlorophyll-*a* and particulate matter were determined. Gonad activity was first observed in a male individual measuring 10 mm in length at Site I. First appearance of oogonia in females was observed at 14mm length in the shell at all Sites, while spawning was observed at 19mm. A statistically significant difference in the size at the onset of sexual maturity was found among stations ($p<0.05$).

Keywords: Mediterranean mussel, Reproduction size, Minimum gametogenesis size

Introduction

Mediterranean mussel (*Mytilus galloprovincialis*, Lamarck 1819) is a very economically important species due to its practical culture methods and its worldwide distribution (Branch & Stephanni, 2004). Alongside their economic value, mussels have been used as indicator species in ecological studies for a long time (Banni et al., 2007; Viarengo et al., 2007; Dailianis, 2010; Okaniwa et al., 2010). Although it plays an economic and scientific role, the Mediterranean mussel is listed among the "World's worst 100 invasive species" (Lowe et al., 2000; Shinen et al., 2009).

The world's major mussel producers are France, Norway, Germany, the USA, Canada, the UK, the Netherlands, Denmark and Spain (Araújo et al., 2020). Global *Mytilus sp.* production was 1 925 902 tonnes in 2022 (FAO, 2024). In Turkey, production of *M. galloprovincialis* is increasing, and new mussel farms are being founded recently. The total mussel production in Türkiye was 8738 tonnes (TÜİK, 2024).

There are many studies on the reproductive cycle of the Mediterranean mussel (Bhaby et al., 2014; Eads et al., 2016; Villalba, 1995; Caceres-Martinez & Figueras, 1998; Okaniwa et al., 2010; Rouabhi et al., 2019; Smart et al., 2021). While the minimum reproductive size of some other bivalve species has been studied previously (Nakaoka, 1994; Franz, 1996; De Souza et al., 2019; Suchanec, 1981), limited knowledge exists about the minimum reproductive size of the Mediterranean mussel, especially in the Aegean Sea, where this study was conducted.

Identifying the minimum reproductive size is critical for sustainable fisheries management and setting regulatory harvest limits to protect mussel populations (Camacho–Mondragon et al, 2012). This study aims to determine the minimum reproductive size of mussels and the difference in this size across sites.

Materials and Methods

The Mediterranean mussels were collected from three different sites. Site I was a sea cage farm that produced sea bass and sea bream in Balıklıova, and the mussels were gathered from the buoys of the sea cages (38°27'0.23"N; 26°37'9.66"E). Site II was a shore with a rocky substrate of a natural mussel bed (38°25'25.94"N; 26°35'19.43"E) in Balıklıova. Site III was another natural mussel bed (38°24'47.40"N; 27° 3'7.14" E), which is the inner part of Izmir Bay, Türkiye (Fig. 1).

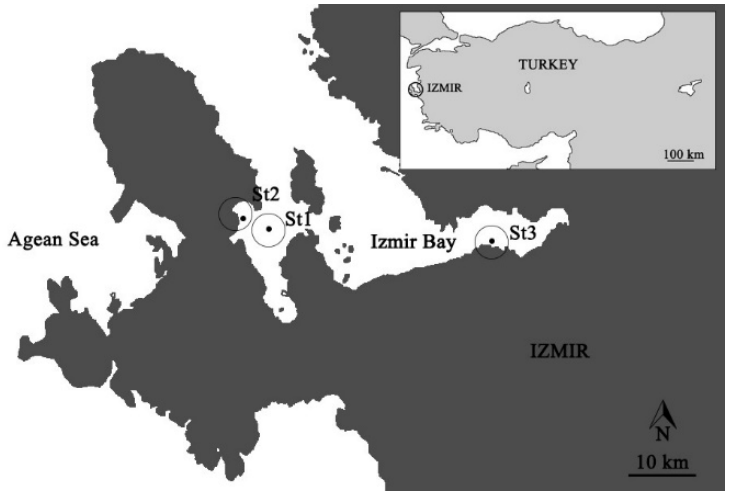


Figure 1. Location of stations at the study site

It was reported that adult mussels are sexually active in November in this region (Kırtık, 2014; Kurtay & Lök, 2023). In November 2020, when spawning peaked, 1000 mussels were collected from each site by free diving and transferred to the laboratory in ice containers. Shell lengths of mussels were measured with a digital calliper (Mitutoyo Absolute) and classified by size. Shell lengths which were between 7.00 and 7.99mm were named as "7", shell lengths which were between 8.00 and 8.99 were named as "8" and continued in this manner until between 30.00 and 30.99mm, so that 24 size groups were made up. Every size group is organised into 30 individuals. Mussels smaller than 7mm in length were not included in the study due to physical limitations regarding the dissection process.

Water samples were also collected to determine environmental parameters, including temperature, salinity, chlorophyll a, total particulate matter (TPM), particulate inorganic matter (PIM), and particulate organic matter (POM). Temperature was measured using a mercury-in-glass thermometer, and chlorophyll a, TPM, PIM and POM were determined by filtering through GF/C fibreglass filters according to the spectrophotometric method of Strickland and Parsons (1972).

A total of 2160 mussel gonads, 30 in each of 23 size groups between 7mm and 30mm, organised from mussels taken at every 3 stations, were dissected. Since the gonads of the most petite sizes were uncertain, all soft tissue was taken under a histological process.

Mussel gonads and whole soft tissue of small individuals were fixed in Davidson's solution for 48 h (Shaw & Battle, 1957). Gonadal tissues were dehydrated with alcohol and xy-

lene series, embedded in paraffin (Histowax, Leica), and sectioned to a thickness of 6 µm with a Thermo Shandon Finesse 325 microtome. Cross-sections of samples were stained with haematoxylin and 0.5% eosin (Howard et al.,1983). The reproductive stages were determined as resting, development, maturation, and spawning, according to Seed (1976).

The differences in reproductive sizes between sites were determined using the nonparametric Kruskal-Wallis test, and the Mann-Whitney U test was applied for the dual comparison between sites. The significance level used for the test was p = 0.05. Statistical analysis was performed using SPSS software.

Results and Discussion

In November, the highest temperature was 18.70 ±0.20 °C at the İnciraltı station, while the lowest was 16.53 ±0.67 °C at the natural mussel bed in Balıklıova. Chlorophyll-*a* values at Site I, Site II and Site III were 1.47 ±0.21, 1.03 ±0.31 and 1.77 ±0.16 µg/L, respectively. TPM, PIM, and POM values were much higher at Site III than at the other stations (Table 1).

The flesh of small mussels was dissected carefully from the shell and histologically examined with several sections to ensure the observation of the whole body in terms of not missing out possible gonad tissue (Figure 2). Sex could not have been determined in mussels that are sexually inactive due to the absence of gamete cells. Histological cross-section photographs of mussels 7mm and 9mm in size are shown in Figure 2A and 2B, respectively.

Spawning was observed in mussels 10mm in length, but formed follicles were very rare (Figure 3A). A developing gonad was observed in only one male individual, measuring 11mm in shell length (Figure 3B). The gonad structure, which consists of follicles containing oocyte samples at various stages of development, is seen in the female individual (Figure 3C). In the 28mm size group, the gonad tissue is covered with follicles in the mature male individual (Figure 3D). Although follicles are not fully developed in the mature female, mature egg sizes have reached their maximum (Figure 3E). In the 19 mm length group, the spawning stage was detected, although only a small number of individuals were observed (Figure 3F).

Table 1. Water conditions of the study sites

a	Aqua Farm Balıklıova	Natural Bed Balıklıova	Natural Bed Inciraltı
Temperature (ºC)	17.07 ±0.25	16.53 ±0.67	18.70 ±0.20
Chlorophyll-*a* (µg/L)	1.47 ±0.21	1.03 ±0.31	1.77 ±0.16
TPM (mg/L)	7.77 ±0.45	8.83 ±0.50	19.83 ±2.62
PIM (mg/L)	6.37 ±0.83	7.87 ±0.35	15.40 ±1.57
POM (mg/L)	1.40 ±0.44	0.97 ±0.38	4.43 ±1.19

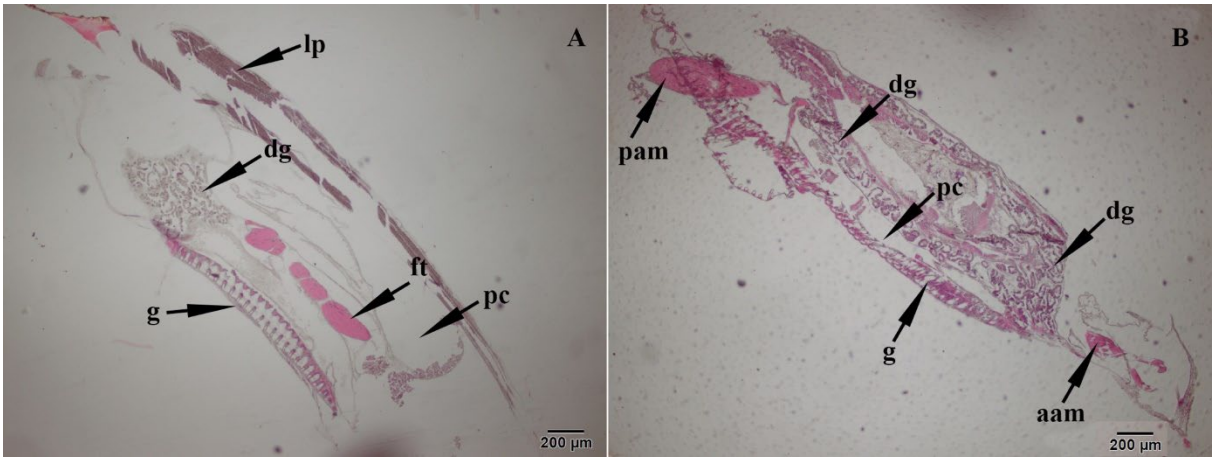
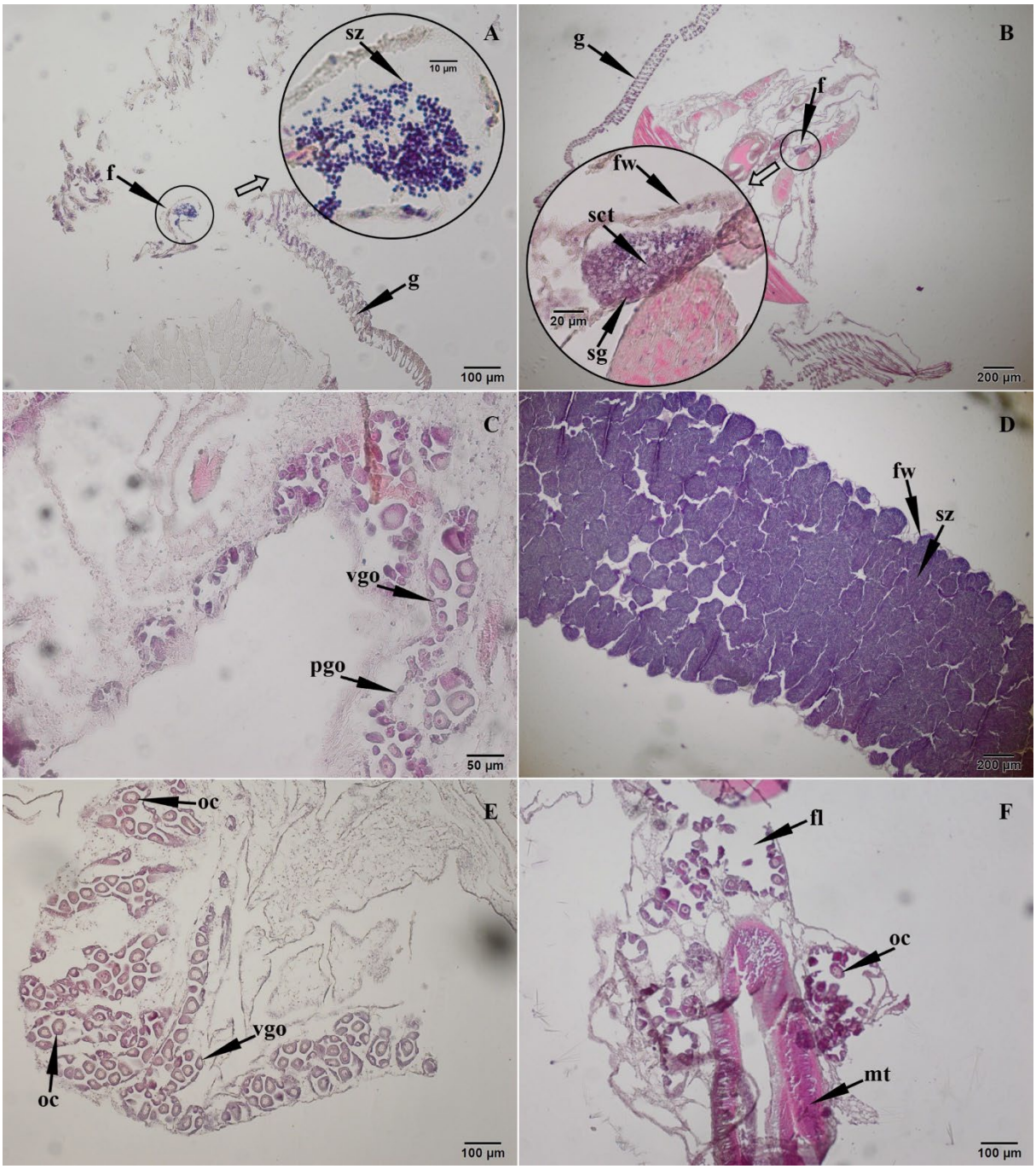


Figure 2. Inactive stage in 7 mm (A) and 10 mm (B) mussels (lp: Labial palp, dg: digestive gland, g: gill, ft: foot, pc: pallial cavity, pam: posterior adductor muscle, aam: anterior adductor muscle)



(Sz: Spermatozoa, g: gills, Sg: Spermatogonia, Sct: Spermatocyte, f: follicule, fw: follicular wall, g: gills, Pgo: Previtellogenic oocyte, Vgo: Vitellogenic oocyte, sz: spermatozoa, fw: follicular wall, oc: mature oocyte, vgo: vitellogenic oocyte, fl: follicular) lumen

Figure 3. Gonadal stages of *Mytilus galloprovincialis*: (A) Spawning in male, (B) Development in male, (C) Development in female, (D) Ripe male, (E) Spawning in female, (F) Spent in female

Site I

Sexual activity was first observed in 10 mm in length mussels. All mussels under this size were sexually inactive at Site I. Mussels with a shell length of 25mm or over were totally sexually active, and the highest spawning rate was observed in group "26", as 43% (Figure 4). The difference between the size groups in terms of gonad development stages was statistically significant ($p<0.05$).

Sex could not be determined under the 10mm group due to sexual inactivity. Only males were defined in 10-13 size groups. Of the 23 individuals, 3 males and 4 females were sexually inactive in the 14mm group. Sex was defined for 19mm and all oversized groups. The highest number of males was found as 21 in the 27mm group, and the highest number of females was 15 in the 29mm group (Figure 5). The difference in sex between size groups was statistically significant ($p<0.05$).

The sex rate of mussels was 31% male, 20% female and 49% inactive in all size groups at Site I throughout the study (Figure 6).

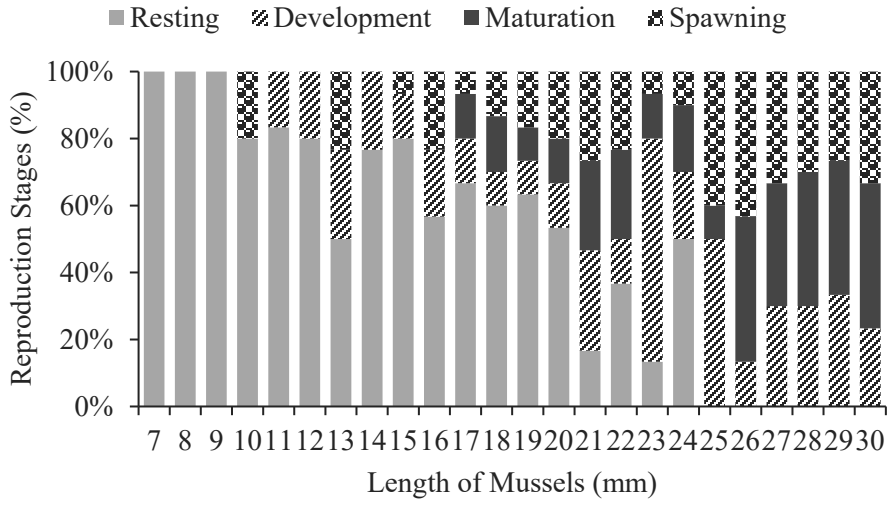


Figure 4. Reproductive stages according to size groups of mussels at Site I

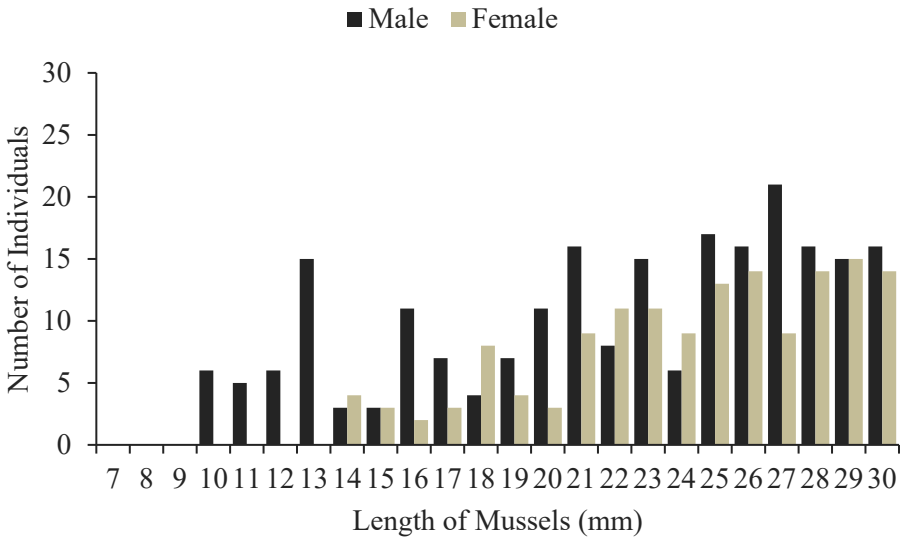


Figure 5. Number of males and females at Site I

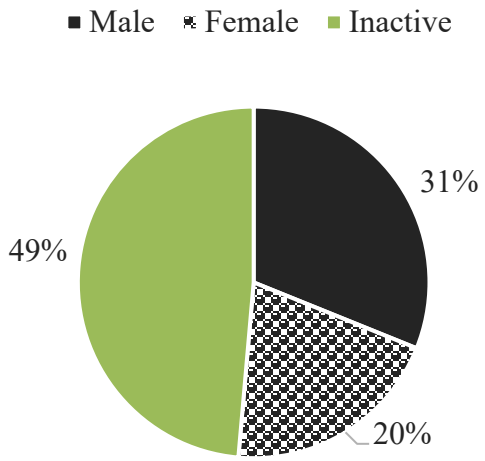


Figure 6. Sex ratio of mussels at Site I

Site II

Mussels in the 7-10mm size group were sexually inactive at Site II. Sexual activity was first observed in 11 mm-sized group mussels, and 23% of the group were in the development stage. All mussels which belong to groups 25mm and over were sexually active. It was observed that mussels in 28 and 29-mm sizes were spawning at Site II with a rate of 57% (Figure 7). The difference in sex between size groups was statistically significant ($p<0.05$).

Sex was not determined for the 11mm and under size groups due to sexual inactivity. All individuals in groups 11 to 13 were male, and both sexes were observed beginning at 14mm and above. The highest number of males was found as 19 in the 25mm group, and the highest number of females was 15 in the 28mm group (Figure 8). The difference in sex between size groups was statistically significant ($p<0.05$).

The sex rate of mussels was 32% male, 25% female and 43% inactive in all size groups at Site II throughout the study (Figure 9).

Site III

Mussels in the 7-9mm size group were not sexually active at Site III. In contrast to the other two stations, mussels in the development (17%) and maturation (3%) stages were observed in the 10mm size group. All mussels from the 25 mm and over groups were sexually active. The highest spawning rate was 60% in the 30mm size group of mussels (Figure 10). The difference in sex between size groups was statistically significant ($p<0.05$).

Sex was not determined for the 10mm and under size groups due to sexual inactivity at Site III. Mussels belonging to groups 10-13 were only male. Both sexes were observed from 14mm onward, and oversized groups were observed. The highest number of males was 15 individuals in the 21mm and 25mm groups. At the same time, the highest number of females was 18 individuals in the 30mm size group (Figure 11). The difference in sex between size groups was statistically significant ($p<0.05$).

The sex rate of mussels was 35% male, 31% female and 34% inactive in all size groups at Site III throughout the study (Figure 12).

A total of 2160 mussels were observed across three sites, and no hermaphrodite individuals were found throughout the study.

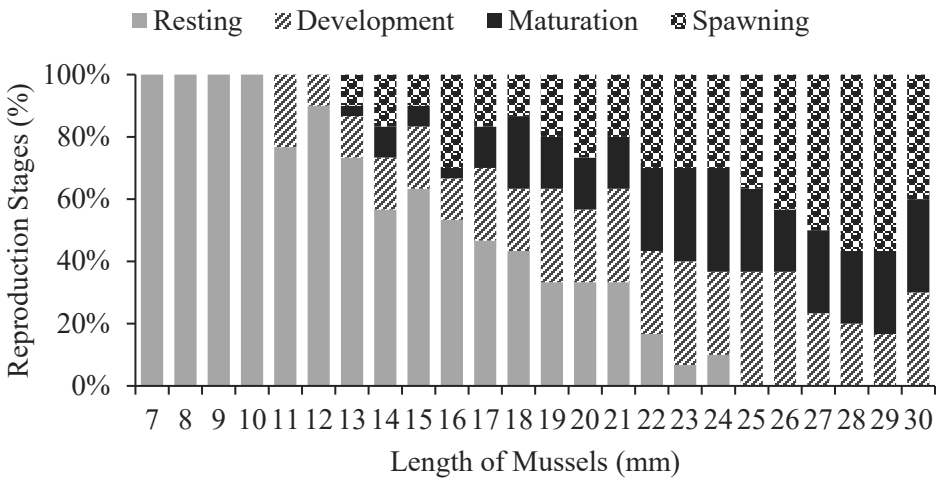


Figure 7. Reproductive stages according to size groups of mussels at Site II

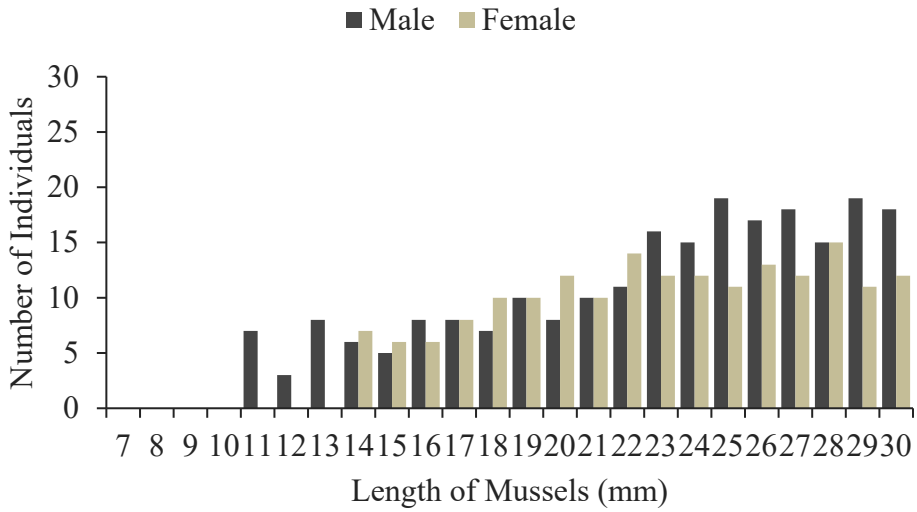


Figure 8. Number of males and females at Site II

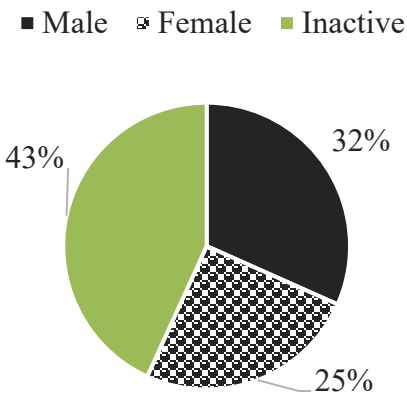


Figure 9. Sex ratio of the mussel at Site II

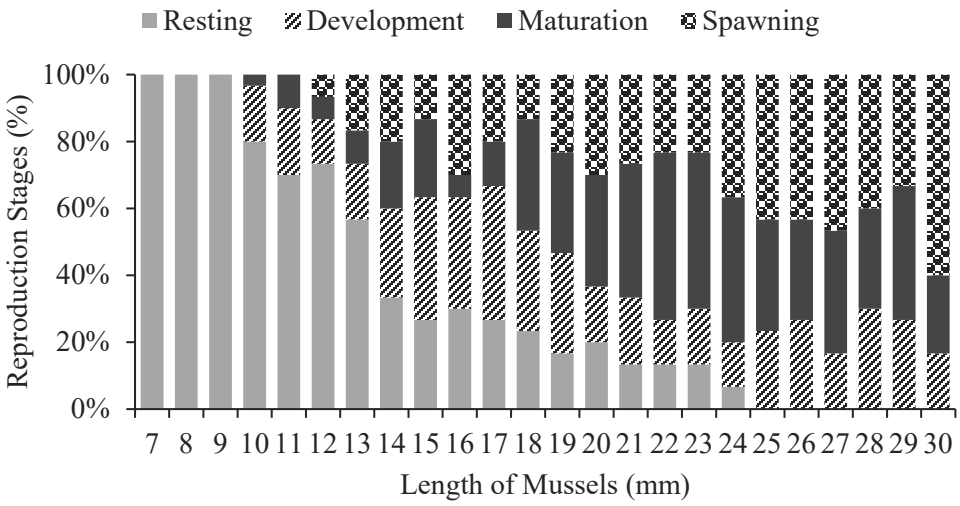


Figure 10. Reproductive stages according to size groups of mussels at Site III

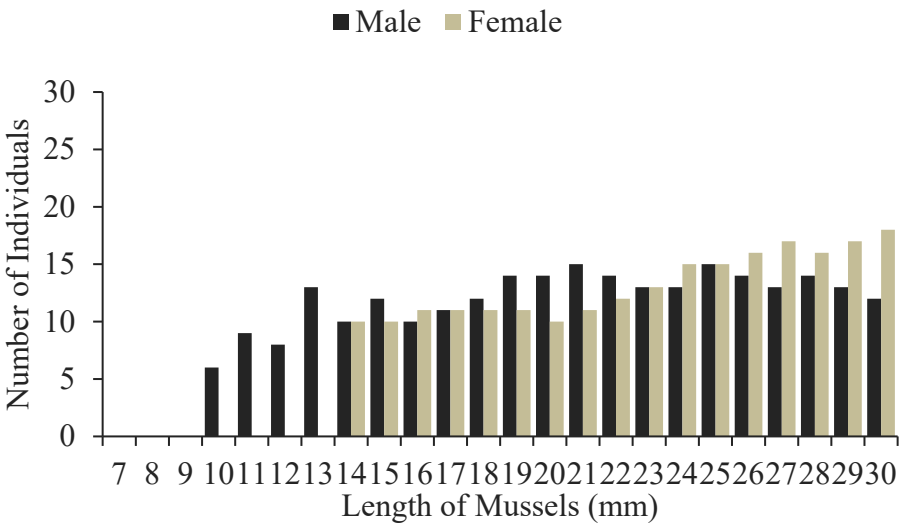


Figure 11. Number of males and females at Site III.

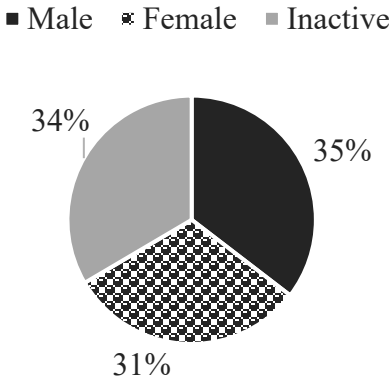


Figure 12. Sex ratio of the mussel at Site III

Spawning of mussel populations in the Southern Aegean Sea was reported as 3 times a year in October-November, January-February, and April (Kırtık, 2014). In Balıklıova, where the mussels were collected for this study, spawning occurs between October and May (Kurtay & Lök, 2023). Mussel samples in this study were collected in November to ensure the population was in the spawning period, as reported in previous studies. Okaniwa et al. (2010) reported that in Tokyo Bay, the gonads of *Mytilus galloprovincialis* developed when the length reached 20mm, and an extended spawning period was observed between November and March. *Mytilus edulis* is sexually active in the first year of their life span with small sizes (Seed and Brown, 1977). In North America, the first reproductive size of *M. edulis* and *M. californianus* was observed at 15-20 mm and 25-30 mm, respectively (Suchanec, 1981). Sunila (1981) reported that there was no sexual maturity in *M. edulis* until 5-10 mm in shell length. In 1996, Franz reported that the first sexual activity occurs in *Geukensia demissa* (Mytilidae), measuring 15-20 mm in length, in the New York Salt Marsh. First sexual maturity was observed in *Perna perna* mussels at 20.70 mm for males and 21.90 mm for females (De Souza et al., 2019). Minimum sexual maturity of mussels was observed in male individuals when the shell length was 10mm in Site I and Site III, while 11mm in Site II in this study.

Temperature and chlorophyll *a* are the most important environmental factors that trigger the reproduction (Yiğitkurt et al., 2020). Okaniwa et al. (2010) reported that the lowest chlorophyll a values were recorded in November-December, with a mean of <3 g/L. That water temperature was between 12 and 14 °C during the same period in Tokyo Bay. Similar results were observed in temperature and chlorophyll *a* in this study. Sea water temperatures ranged from 16.53 to 18.70 °C, while chlorophyll a was <2 µg/L in November. Compared to Okaniwa et al.'s (2010) results, lower chlorophyll a values did not affect reproduction in this study; in addition, seawater temperature was higher, and it was conceived that higher water temperatures can support mussels' reproduction in smaller sizes.

It is known that gametogenesis can occur in mussels even at 0 °C (Grey et al., 1997). In a previous study, the average annual water temperature was reported as 19.46 ±4.41 °C (min 14 °C in February; max 26.2 °C in August), and in November, water temperature was between 16 and 17 °C in Balıklıova, which includes Sites I and II (Kurtay & Lök, 2023). During a study conducted on the Inciraltı coast, including Site III, on bivalve reproduction, it was reported that water temperature ranged from 13.5 °C to 28 °C annually, with 19 °C in November (Kırtık, 2014). In a study on the reproductive cycle of *Mytilus galloprovincialis* in Italy, it was reported that sexual activity was not observed in the summer months when the temperature increased and gametogenesis began to be seen because of the end of the summer season and the temperature falling below 20°C (Da Ros et al., 1985). In this study, water temperature was measured at 17.07 ±0.25 °C and 16.53 ±0.67 °C at Sites I and II, respectively. Consistent with previous studies, reproductive activity was continuous at water temperatures below 20 °C.

Kurtay and Lök (2023) reported the lowest chlorophyll a values in Balıklıova (Site I and Site II in this study) as 0.2 µg/L in July and 1.91 µg/L in December, while the lowest TPM values in the same region were 6.69 mg/L in November and the highest in October reported as 15.45 mg/L. On the Balıklıova coast, the highest PIM value was 10.56 mg/L in October and the lowest 5.1 mg/L in June, while the highest POM value was 5.9 mg/L in October, and the lowest 0.73 mg/L in November and February (Kurtay & Lök, 2023). In the mussel breeding study conducted on the coasts of İncialtı, chlorophyll a values were reported as 1.31 g/L in December (the lowest), 41.21 g/L in June (the highest), and 1.52 µg/L in November (Kırtik, 2014). The amount of feed in the environment is considered a limiting factor in mussel gonad development (Grey et al., 1997). In his study, Kırtık (2014) reported that the year-round average TPM data for İnciraltı was 17.27 7.87 mg/L, while TPM was 21.84 mg/L (PIM=15.07 mg/L, POM=6.76 mg/L) in November. Annual water changes were examined alongside similar studies conducted in the same region and compared with the November data in this study. The results reported in the studies are consistent with the water data we obtained. The smallest size in which gametogenesis was observed in mussels obtained from İnciraltı Coasts, where water parameters are at higher values, was determined as 10 mm. In the Balıklıova farm region, both the development phase and the maturation phase were observed in mussels of this size. Higher POM and chlorophyll a levels may accelerate gametogenesis in smaller mussels, potentially triggering earlier reproductive maturity.

Hermaphroditism is very rare in the *Mytilus* spp. (Seed, 1976). Bhaby (2015) reported that 345 females, 400 males, and 2 hermaphrodites were detected among 750 individuals of *M. galloprovincialis* on the Moroccan Coast. Sunila (1981) reported that hermaphroditism was observed in only 2 of 1832 individuals of M. edulis in the Gulf of Finland. Supportive of the Seed 1976's determination, hermaphroditism was not found in any of 2160 individuals in *M. galloprovincialis* in this study.

Conclusion

Our results, unlike those of other studies, showed that gametogenesis began in mussels at much smaller sizes; however, there were slight differences between stations in the region. It has been determined that sexual maturity occurs faster in smaller mussels, especially in regions with higher nutritional value, and male mussels reach sexual maturity earlier than female individuals.

Compliance with Ethical Standards

Conflict of interest: The author declares no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: Ethics committee approval is not required for this article.

Data availability: The data will be made available upon request from the author.

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Disclosure: -

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