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Seasonal Changes in the Histological Profile of the Ovary of *Mytilus galloprovincialis* (Bivalvia, Mytilidae) Lamarck, 1819

Mytilus galloprovincialis (Bivalvia, Mytilidae) Lamarck, 1819'in Ovaryum Histolojisindeki Mevsimsel Değişiklikler

Burak Kunduz and Melike Erkan*

Istanbul University, Faculty of Science, Department of Biology, Vezneciler, 34134 Istanbul, Turkey.

Abstract

The seasonal changes in the histological profile of the ovary of *Mytilus galloprovincialis* Lamarck, 1819 were examined at light microscopic level and the alterations in the amount of total protein in the ovary were revealed by biochemical analyses. Samples were monthly collected from designated areas in Yenikapi shores of Sea of Marmara between June 2004 - May 2005. Although reproductive activities of *Mytilus galloprovincialis* populations in these areas decreased in some months, it has been observed that the reproduction activity has continued all the year round. The annual temperature alterations that have been measured were not much effective on the reproductive cycle.

Key words: Mollusca, bivalvia, oogenesis, seasonal changes.

Introduction

The control of reproductive processes plays a critical role in the culture of any species (Saout et al. 1999). Gamet production is ultimately dependent on the energy derived from ingested food, and like other marine bivalves, mytilus exhibit cycles of energy storage and utilization closely linked to annual cycles of gametogenesis (Pazos et al. 1996). Besides, environmental

* Corresponding author: merkan@istanbul.edu.tr

conditions are highly responsible for the changes observed in ovary histology as regards to the species or the populations of the same species (Paulet and Boucher 1991).

For many researchers, temperature is indirectly affective in the regulation of gonad histology and gametogenesis and the proportions of each developmental stage of oocytes to one another change according to seasons (Newell et al. 1982, Paulet and Boucher 1991).

The number of researches carried out on ovaries of various bivalve species is limited. In these researches, the histological structure and biochemistry of ovary in *Pinctada mazatlanica* (Saucedo et al. 2002b); reproduction cycle in *Argopecten ventricosus* and *Mytilus galloprovincialis* (Cáceres-Martínez and Figueras 1998), gametogenesis and the classification of germ cells in *Mytilus edulis, Halotis asinia* (Lowe et al. 1982, Sobhon et al. 1999, Kalinina et al. 2006), the effects of seasonal changes on the ovary histology and biochemical composition of ovary in *Argopecten purpuratus*, *Pecten maximus, Pinctada mazatlanica* (Pazos et al. 1997, Saucedo et al. 2002a); gonadic conditioning in *Pinctada mazatlanica* (Saucedo et al. 2001) were examined. Besides, the researches in comparison to reproduction cycles of different *Mytilus* species (Wilson and Hodgkin 1967); the reproduction biology (Wilson and Seed 1974) and the identity of breeding temperatures of *Mytilus edulis* (Allen 1955), the gametogenic cycle of cultured *Mytilus galloprovincialis* (Villalba 1995) were carried out. *Mytilus galloprovincialis* is consumed as a common food in Turkey. Hence, its production cycle is required to be examined.

Materials and Methods

The sampling was carried out in Yenikapı located at the northern shore of Sea of Marmara annually between September 2004 and August 2005. 10 adult individuals (the length of their shells at least 10 cm) (Cáceres-Martínez and Figueras 1998), were gathered from the 1-5 meter depths. The water temperature was measured in the sampling area. *Mytilus galloprovincialis* species were transferred to laboratory alive in sea water. The ovaries were dissected quickly and fixed in Bouin's solution for 12 hours at 20 °C. 5-6 µm sections taken from those tissues were stained with Haematoxylin and Eosin following the method described by Humason (1972).

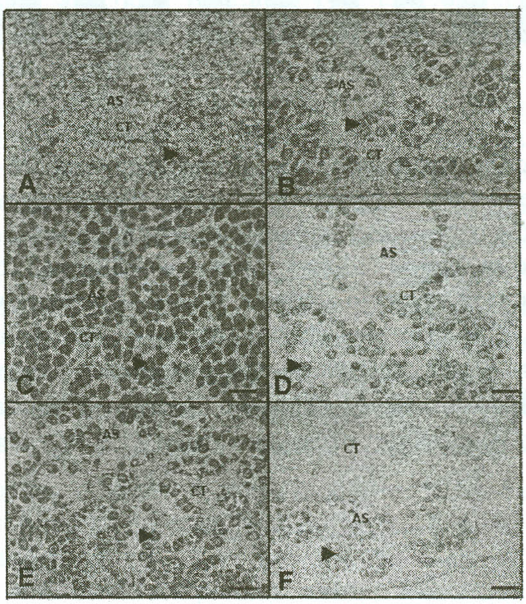
The biochemical components of the ovary were determined spectrophotometrically using the Lowry method for proteins (Yang et al. 2002). SPSS 10.0 Statistic Package Program was use for the statistical evaluations. Comparison of parameters between months was carried out by Kruskal Wallis's one-way variant analysis ($p>0.05$).

Results

The ovaries of *Mytilus galloprovincialis* were observed throughout the year by taking considerations of connective tissue and acinus contents of the ovary from June 2004 to May 2005. The seasonal changes occuring in the histology of *Mytilus galloprovincialis* ovary can be evaluated in five stages: 1) Beginning to gametogenesis, 2) Development, 3) Maturation, 4) Spawning and 5) Acinus regression. The proportion of adipogranular connective tissue and proportion of vesicular connective tissue were affected by seasonal changes in the ovary (Figure 1).

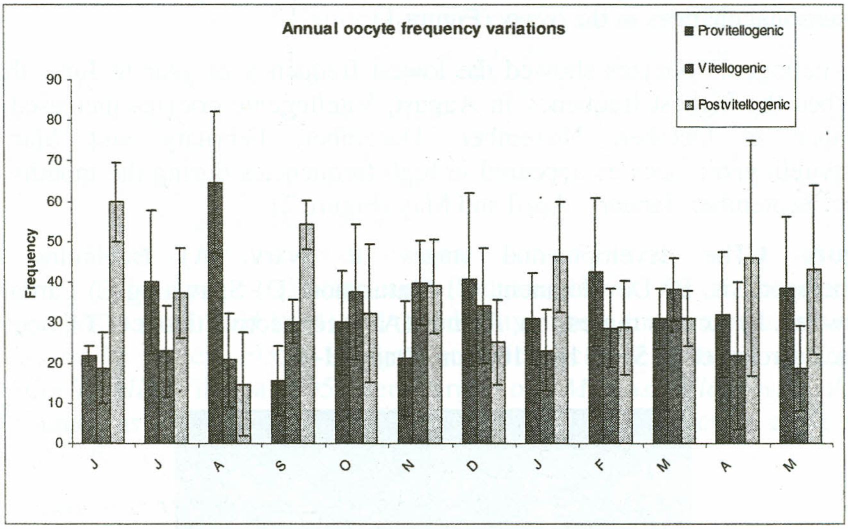
Previtellogenic oocytes showed the lowest frequency of year in June, they reached the highest frequency in August. Vitellogenic oocytes increased in number in October, November, December, February and March. Postvitellogenic oocytes appeared in high frequencies during the months of June, September, January, April and May (Figure 2).

Figure 1. The developmental stages of ovary. A) Beginning to gametogenesis, B) Development, C) Maturation, D) Spawning E) Partially spawned, F) Acinus regression. Acinus (AS), connective tissue (CT), oocyte (black arrow head). Scale bar: 100 µm. Stain: H+E.



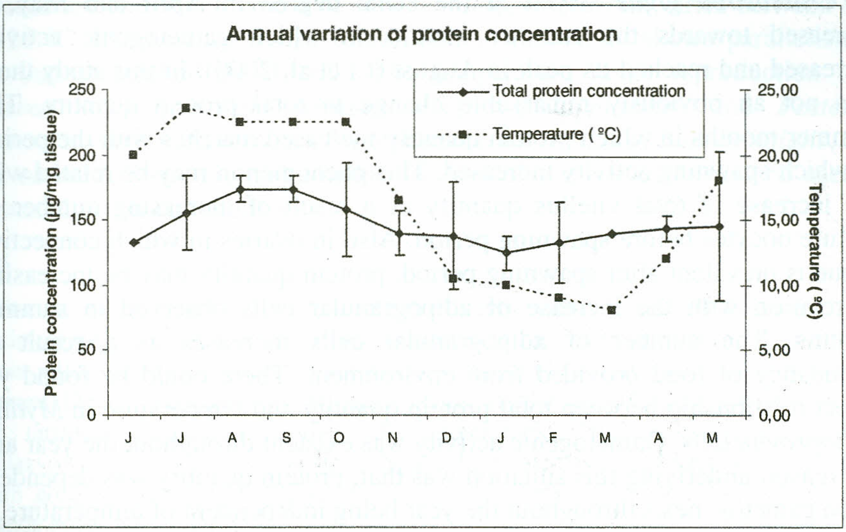
The individuals in the stage of beginning to gametogenesis came into sight in November and April. On the other hand, the individuals in the development stage came into sight in all of the months except August, November, January and May. In February and March, the early and late phases of development were fulfilled at the same time. The individuals that have mature ovaries came into sight in September, November, January and February. The individuals that were in the stage of spawning appeared in June, August, November and May. The individuals showing the last stage, acinus regression, appeared in December, January and May.

Figure 2. Annual oocyte frequency variations.



Vesicular connective tissue appeared almost every month throughout the year in a variable density. Adipogranular connective tissue cells increased in number throughout spring and summer began to decrease in autumn and sharply decreased in winter and early spring. Total protein quantity slightly changed throughout the year. In summer, it increased slightly in comparison to other seasons. This increase was not significant statistically ($p>0.05$). In sample station, the temperature values ranged between 8.0 and 23.5 °C. The temperature values below 11 °C were measured between December and March. The highest temperature value (23.5 °C) was measured in July (Figure 3).

Figure 3. Annual variation of total protein concentration.



Discussion

In this study, the samples in the spawning period were observed in the months of June, August, September, November, January, February, March, April and May. However, except these months the individuals which had undergone their spawning periods recently were also observed. The spawning occurred not only in hot months of the year, but also cold months of the year. The ovaries which were in different stages of development appeared at the same time, so it may be suggested that gametogenesis and spawning activity of *Mytilus galloprovincialis* populations were not affected by temperature changes measured during the time period in which this study was carried out. Drawing the same development graphic of *Mytilus galloprovincialis* with the findings of Caceres-Martines and Figureaŝ (1998), Villalba (1995) differs from their findings them by revealing that the spawning activity takes place in winter and not goes on till spring. This situation proves that it is natural to come across individuals beginning to and going on gametogenic activity throughout a year. In other words, gametogenic activity goes on throughout the year in *Mytilus galloprovincialis*.

The protein content of ovary is also a crucial factor on the development of the oocyte according to different studies (Saout et al. 1999, Saucedo et al. 2002a and Kalinina et al. 2006). Besides, the seasonal increase of total quantity of protein, the ovary proteins related with vitellus increase in line with oocyte maturation process. Whereas protein quantity stored in ovaries of *Crassostrea gigas* stayed at the same degree in April and May, it increased towards the summer months in which gametogenic activity increased and reached its peak in August (Li et al. 2000). In this study there was not an obviously remarkable change in total protein quantity. The summer months in which protein quantity increased matches with the period in which spawning activity increased. This phenomenon may be related with the increase of total vitellus quantity as a result of increasing number of mature oocytes before spawning period. Also in ovaries in which connective tissue is prevalent after spawning period, protein quantity may be increasing in relation with the increase of adipogranular cells observed in summer months. The number of adipogranular cells increases as a result of abundancy of food provided from environment. There could be found yet direct relationship between total protein quantity and temperature in *Mytilus galloprovincialis*. Gametogenic activity was existent throughout the year and the reason underlying this situation was that, protein quantity was dependent upon gametogenesis throughout the year being independent of temperature.

Among these factors, as one of the most significant factors affecting gametogenesis, water temperature was analyzed by many researchers (Wilson and Hodgkin 1967, Caceres-Martines and Figuearas 1998, Saout et al. 1999, Morriconi et al. 2002, Saucedo et al. 2002a, Fernández-Reiriz, 2007).

In their study including five different Mytilid species, Wilson and Hodgkin (1967) reported that the important factor is temperature in regulation of reproductive cycles and proper timing of spawning period in reproduction season.

There are studies in which it is pointed out that the maximum and minimum temperature threshold of gametogenesis takes place on different scales for different species (Sastry and Blake 1971, Saout et al. 1999). Loosanoff (1942) pointed out that the shallow water oysters (*Ostrea virginica*) complete their spawning activity earlier than the oysters living in deeper water. As another finding related with *Aequipectan irradians*, gameotogenic cycle follows a different route in connection with latitudes. Spawning activity takes place in autumn in populations settled on South latitudes (Barber and Blake 1983). It is clear under the light of these studies that reproduction cycle of bivalves takes place on temperature degrees changing with species. Moreover, as they reach their ideal temperature degrees in different months, populations of same species living on different regions may enter into their reproduction period in different periods of time.

In Bilecik's opinion (1989), ideal water temperature for the nutrition, reproduction and development of *Mytilus galloprovincialis* varies between 8 and 26 °C. Temperature range established in this study shows parallelism with that mentioned by Bilecik. The temperature range reproduction is observed in *Aequipectan irridians*, *Tapes philippinarum* and *Pecten maximus* is narrow. In respect of reproduction of *Mytilus galloprovincialis*, a large active temperature range makes us think that this species may be used for the culture in our country.

Özet

Mytilus galloprovincialis Lamarck, 1819 ovaryumunun histolojik yapısındaki mevsimsel değişiklikler ışık mikroskobu düzeyinde incelendi ve ovaryumdaki total protein miktarındaki değişimler biyokimyasal analizlerle ortaya çıkarıldı. Örnekler, Marmara Denizi Yenikapı kıyılarındaki belirli alanlardan, Haziran 2004- Mayıs 2005 tarihleri arasında toplandı. Bu bölgelerdeki *Mytilus galloprovincialis* popülasyonlarında üreme aktivitesinin, bazı aylarda artmasına rağmen, yıl boyunca devam ettiği görülmüştür. Ölçülen yıllık sıcaklık değişimleri ile üreme döngüsü üzerinde etkisi görülmemiştir.

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