



## Comparison of Gonadal Development of Carpet Shell Clam (*Tapes decussatus*, Linnaeus 1758) in Inside and Outside of Çakalburnu Lagoon, Izmir Bay

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### Abstract

A study of the gametogenic cycle of the carpet shell clam, *Tapes decussatus*, was undertaken in the inside and outside of Çakalburnu Lagoon, Izmir Bay, Aegean Sea from August 2006 to July 2007. Histological evidence showed that gametogenesis of *T. decussatus* continued throughout the year and six stages of gonadal development were observed in two sites. In males, except for November in inside and December and January in outside of lagoon ripeness (Stage III) clam was observed during a year. In addition, spawning (Stage IV or Stage V) continued throughout the year in inside and except for February and March in outside. In females, ripe clam (Stage III) was observed similar time with male in two sites. It was observed that Stage 0 (inactive or resting) was only 3.0-5.0% ratio in November, December and January in two sites. The male/female sex ratio for inside and outside of lagoon was 1:1.06 and 1:1.10, respectively ( $P>0.05$ ).

**Keywords:** *Tapes decussatus*, carpet shell clam, gametogenesis, Çakalburnu Lagoon, Aegean Sea.

### İzmir Körfezi, Çakalburnu Dalyan'ında İç ve Dış Bölgelerdeki Akivadeslerin (*Tapes decussatus*, Linnaeus 1758) Gonad Gelişimlerinin Karşılaştırılması

#### Özet

Bu çalışmada İzmir Körfezi, Çakalburnu Dalyan'ında iç ve dış bölgelerden toplanan akivadeslerin (*Tapes decussatus*) gametogenez döngüsü Ağustos 2006–Temmuz 2007 tarihleri arasında incelenmiştir. *T. decussatus*' un gonad gelişiminin yıl boyunca sürdüğünü ve her iki bölgede de altı safhanın bulunduğunu histolojik incelemeler göstermiştir. Erkek bireylerin dalyan içinde kasım ayı haricinde, dalyan dışında ise aralık ve ocak ayları dışında tüm yıl olgun safhada (Safha III) olduğu gözlemlenmiştir. Buna ek olarak döl bırakımı (Safha IV veya V) dalyan içinde yıl boyunca, dalyan dışında ise şubat ve mart ayları haricinde yıl boyunca sürmüştür. Dişi akivadeslerde olgunlaşma (Safha III) her iki bölgede de erkek bireyler ile benzer zamanlarda gerçekleşmiştir. Çalışma alanında safha 0 (dinlenme) sadece %3-5 oranında kasım, aralık, ocak aylarında tespit edilmiştir. Erkek/dişi cinsiyet oranı dalyan içinde 1:1,06 dalyan dışında ise 1:1,10 olarak bulunmuştur ( $P>0,05$ ).

**Anahtar Kelimeler:** *Tapes decussatus*, akivades, gonadal gelişim, Çakalburnu Lagünü, Ege Denizi.

#### Introduction

In bivalves growth, reproduction, condition and biochemical composition are affected internal and external factors (temperature, food supply, salinity and current e.t.c). Temperature is closely associated with geographic location and thus many studies have assessed the importance of geographical locations in defining and controlling gametogenesis (Holland and Chew, 1974; Beninger and Lucas, 1984; Xie and Burnell, 1994; Meneghetti *et al.*, 2004).

In Turkey, *Tapes decussatus* is distributed along the coastline of Aegean, Mediterranean and Marmara

Sea and one of the principal clam species commercially harvested in the Çakalburnu Lagoon, Izmir Bay between September and May. According to fisheries statistic data (TURKSTAT, 2008) carpet shell clam production from Turkish waters were 1,266 t and 1,334 t in 2006 and 2007, respectively. At the same time this clam is a potential species of aquaculture in Turkey. To be able to improve the methods of cultivating this clam, detailed knowledge of the gonadal development and spawning periods is fundamental. Knowledge of gametogenesis will help hatchery managers to determine the best strategy for producing high quality and quantities larvae. In

addition, this information helps to regulate the collecting time, management and protection of natural clam stocks. Unfortunately, there is only preliminary result about carpet shell clam gametogenesis in Çakalburnu Lagoon (Serdar and Lök, 2005) and it needs more information from the area which is very productive. The aim of the current paper is to describe the gametogenic cycle histologically in inside and outside of Çakalburnu Lagoon, Izmir Bay.

## Materials and Methods

### Study Site and Sampling

Çakalburnu Lagoon is located at the south part of Izmir Bay, Aegean Sea (Figure 1). The lagoon covers an area of 150 ha acreage and depth of inside lagoon is between 0.5 and 1.0 m. A widely area on the outside of lagoon has a depth of 1.0 m and fisherman collect carpet shell clam easily with both diving and sieving in this area. Monthly samples of about 50 clams from the natural clam bed were collected from inside (38°24'79"N and 27°02'96"E) and outside (38°24'70" N and 27°03'06"E) of Çakalburnu Lagoon between August 2006 and July 2007. Shell length of clams (anterior –posterior axis) ranged from 35.0 to 57.0 mm for two sites.

### Environmental Parameters

The gametogenic cycle can be influenced by a number of environmental conditions such as temperature, salinity and levels of nutrition (Drummond *et al.*, 2006; Dridi *et al.*, 2007; Serdar and Lök, 2009). Water temperatures were measured using a mercury-in-glass thermometer (-10 to 50±0.5°C). Salinity values were determined by the Mohr-Knudsen titration method. Seawater samples for seston (Total Particulate Matter, TPM), Particulate Organic Matter (POM), Particulate Inorganic Matter

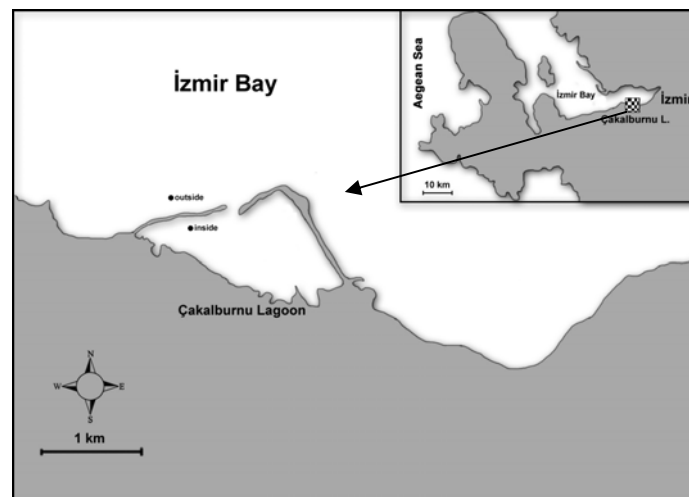
(PIM) and chlorophyll a analysis were collected at the two sites using three replicated 1.0-2.5 L bottles and stored in ice chest for carrying to the laboratory. To determine the chlorophyll a concentration, each seawater sample was filtered onto Whatman GF/C filter and frozen at -20°C until analysis. Seston and chlorophyll a concentrations were determined using the protocols of Strickland and Parsons (1972). All measurements for determining environmental conditions were taken at monthly intervals between August 2006 and July 2007.

### Histological Techniques

The soft tissue of each clam was removed from the shell using a scalpel and then a part of gonad sample was taken. Gonad tissue was fixed in Davidson fluid and embedded in paraffin. Histological sections 4 µm in thickness were stained with Harris's haematoxylin and eosine. The prepared slides were observed under the light microscope (Olympus BX-51 Model) for both sex determination and stage of gametogenesis. Gametogenic stages of clam were categorised into six stages (Stage 0: Inactive, Stage I: Early active, Stage II: Late active, Stage III: Ripe, Stage IV: Partially Spent, Stage V: Spent) according to Xie and Burnell (1994) and Drummond *et al.* (2006). When more than one developmental stage was evident within a single individual, the clam was assigned to the reproductive stage that was observed in the majority of follicles.

### Data Analysis

In this study, all statistical analyses were performed according to the methods of Zar (1996). A Chi-square test ( $\chi^2$ ) was used to analyse sex ratios. The t-test was used to ascertain the differences between salinity, temperature, Chlorophyll a and TPM value at the two sites. Statistic analyses were



**Figure 1.** Map of Çakalburnu Lagoon in Izmir Bay, Aegean Sea, showing sampling sites.

carried out with the aid of the SPSS computer software programme.

## Results

### Environmental Parameters

Seawater temperature was shown similar pattern both inside and outside of Çakalburnu Lagoon (Figure 2). The minimum value in inside and outside of the lagoon were recorded 13.2°C in December and 13°C in December and January, respectively. The

maximum temperature was measured 28.6°C and 27.6°C in August in inside and outside of lagoon, respectively ( $P>0.05$ ). Salinity remained relatively stable throughout the year for two sites and these values were determined between 36.1‰ and 38.5‰ in inside and between 35.68‰ and 38.0‰ in outside of lagoon ( $P>0.05$ ) (Figure 3).

Chlorophyll-a value in inside was generally greater than in outside (Figure 4). The mean chlorophyll a concentration in inside of Çakalburnu Lagoon was 12.59 µg/L, with a minimum of 5.29 µg/L in April and a maximum of 24.02 µg/L in

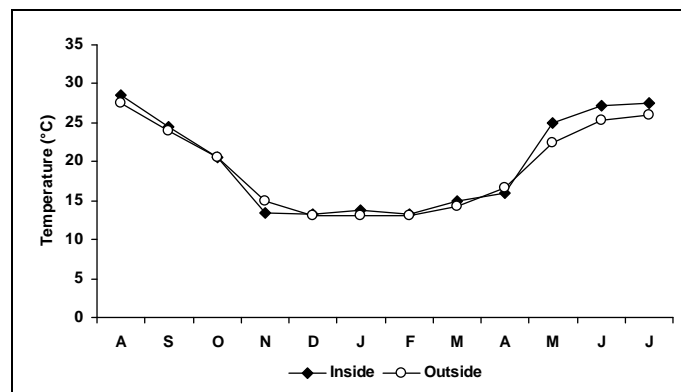


Figure 2. Variation of seawater temperature in two sites from August 2006 to July 2007.

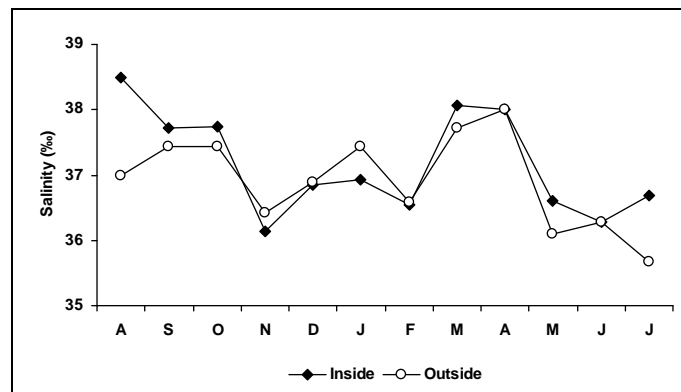


Figure 3. Salinity values in inside and outside of Çakalburnu Lagoon during the study.

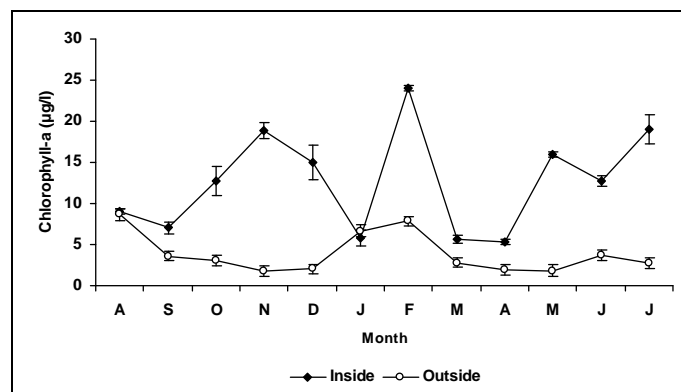


Figure 4. Chlorophyll-a concentration of two sites ( $\pm$ SD).

February. In outside of lagoon, the mean chlorophyll a value was 3.89 µg/L and changed between 1.79 µg/L in November and 8.63 µg/L in August (P<0.05). The average of TPM in inside and outside was 14.20 mg/L and 6.20 mg/L, with a peak in July (41.33 mg/L) and June (12.86 mg/L), respectively (P<0.05) (Figure 5). Both maximum POM and PIM values in inside of lagoon were shown similar pattern with TPM but in outside the maximum POM value was recorded in January (8.6 mg/L) instead of June and the highest PIM value (5.13 mg/L) was obtained same month with TPM value. Except for some months POM values were higher than PIM concentrations throughout the study.

**Gametogenesis**

**Female**

At the start of the study in august, females were in late active (Stage II), ripe (Stage III) and partially spawning (Stage IV) in inside. In addition, females were in ripe (Stage III), partially spent (Stage IV) and spent (Stage V) in outside. Except for november, ripe female was determined in inside of lagoon in each month. However, in outside ripe female was observed in each month aside from november, december and january. Moreover, all of female in december were Stage V (spent) in outside of lagoon. In february and

march females in inside were in Stage I (46.67% and 63.15%), Stage II (46.67% and 31.58%) and Stage III (6.66% and 5.27%). In outside of lagoon females were in Stage I (35.72%), Stage II (21.42%) and Stage V (42.86%) in january and females were in Stage I, Stage II and Stage III in february and march (Figure 6).

**Male**

In males, throughout the study except for november, Stage III was observed in inside of lagoon in each month. However, in outside of lagoon, Stage III was determined in each sampling except for december and march. At the start of the study, in August 2006, 17.65% and 82.35% of male were in Stage III and Stage IV in inside of lagoon, respectively. Similarly, in outside 27.78% and 72.22% of male were in Stage III and Stage IV, respectively. In september, same stages except for Stage V (11.12%) in inside were observed in two sites. In november, 90% and 10% males were in Stage IV and Stage V in inside of lagoon, respectively. In outside of lagoon, males were determined in Stage III (6.67%), Stage IV (73.33%) and Stage V (20%). Although any male in Stage I was not detected in february, it was observed in march in inside. Similarly, in january, there were no male clams in Stage I but in february (25%) and march (16.67%)

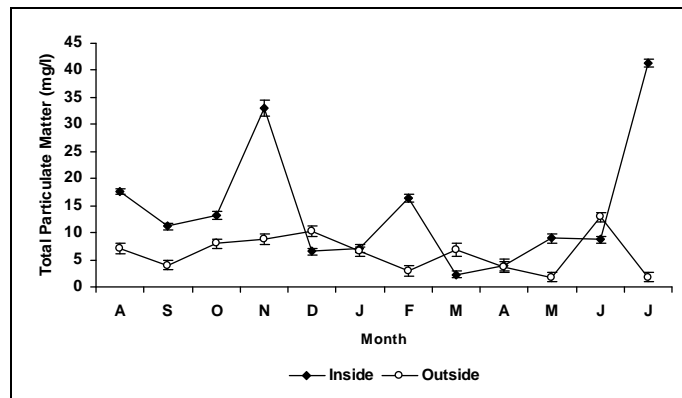


Figure 5. Change of TPM in inside and outside of lagoon (±SD).

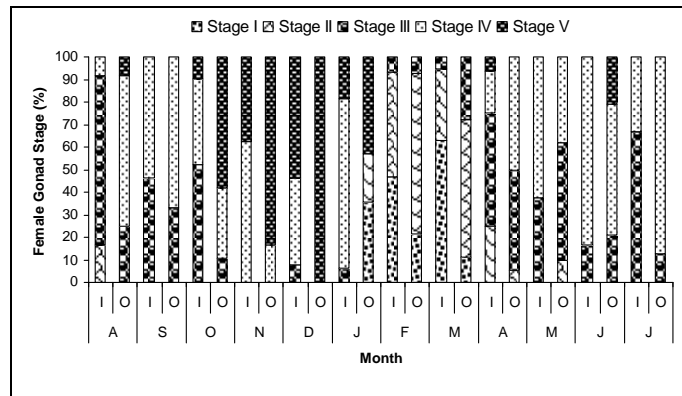


Figure 6. Gametogenic stages of female clam in inside (I) and outside (O) of lagoon.

males were found in this stage in outside. Stage I and Stage II in males were observed in a short time in two sites (Figure 7). In april, all of males were in Stage III and except for april, spawning (Stage IV or Stage V) occurred in inside in each month. On the other hand, apart from february and march spawning was appeared in outside throughout the study.

**Sex Ratio**

The gametogenic cycle was determined using 1200 clams (600 clams for inside and 600 clams for outside). Inside and outside of lagoon, the number of females was 306 (52%) and 312 (51%), while males were 288 (47%) and 282 (48%), respectively. Undifferentiated (Stage 0) specimens were 6 (1%) in each site. The male/female sex ratio for inside and outside of the lagoon was 1:1.06 and 1:1.10, respectively. The male/female sex ratio did not show a significant deviation during the study ( $P>0.05$ ).

**Comparative Gametogenic and Spawning Patterns in Two Sites**

The percentage of the different stages of the gonadal cycle in carpet shell clams were observed in the two sites (inside and outside) of Çakalburnu Lagoon between August 2006 and July 2007 and it

was shown in Figure 8. It was observed that Stage 0 (inactive or resting) was only 3.0-5.0% ratio in november, december and january in two sites. Ripe clams were determined in inside and outside during the study except for november and december, respectively. Clams of spawning stage (Stage IV and Stage V) were observed in inside of the lagoon throughout the year and in outside they were found each month apart from february and march. Moreover, in january, all five stages (Stage 0, I, II, III and IV) of gametogenesis were appeared in outside.

**Discussion**

Among environmental factors, water temperature and food supply are mainly and salinity and photoperiod are secondarily effect on gametogenesis (Pouvreau *et al.*, 2000). However some authors mentioned that seawater temperature is the one of the most important factor for gonadal development in bivalve (Sastry, 1975; Daou and Gouletquer, 1988; Xie and Burnell, 1994; Delgado and Pérez-Camacho, 2007). The lower temperature limit for gonadal activity reported to be 8-12°C for gamete ripening and 14°C for spawning (Holland and Chew, 1974; Xie and Burnell, 1994). In this study, the lowest temperature was measured 13.0°C in outside

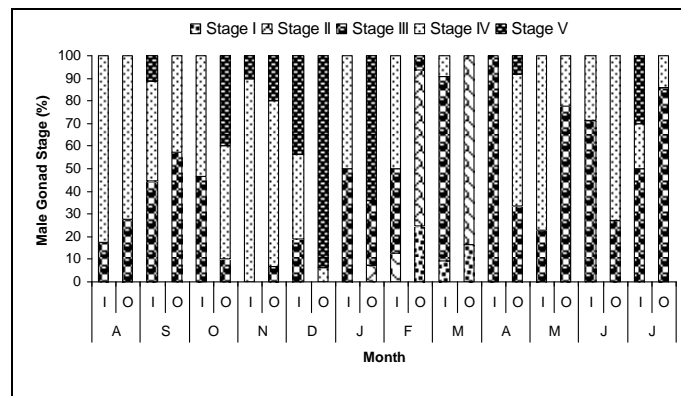


Figure 7. Gametogenic stages of male in inside (I) and outside (O) of lagoon.

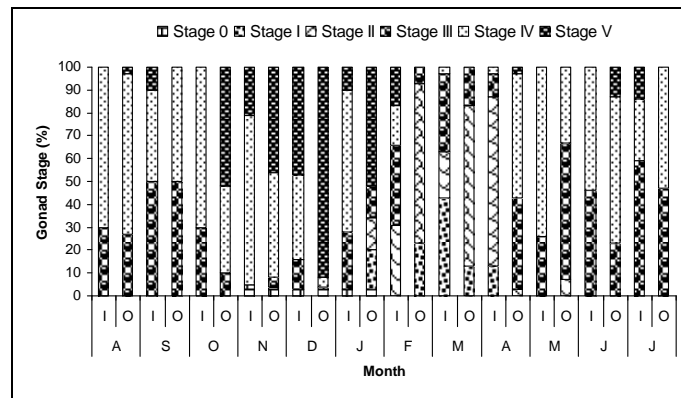


Figure 8. Gonadal development of clam in inside (I) and outside (O) of lagoon.

and 13.2°C in inside and these values were not significantly different in two sites during the study ( $P>0.05$ ). However, water temperature never decreased 8.0°C so histological section showed that gamete ripening and spawning continued throughout the year in the study area. Delgado and Perez-Camacho (2007) reported that the ability of *R. philippinarum* and *R. decussatus* to produce mature oocytes at 14°C and partial spawning occur at this temperature in the laboratory conditions. The present study carried out in the natural environment and our results show similar pattern with natural environment (Xie and Burnell, 1994, Sbrenna and Campioni, 1994; Drummond et al., 2006) and laboratory conditions (Delgado and Perez-Camacho, 2007).

Feeding is another determining factor for gonadal development in bivalves and if the abundance of food is enough in the water, gametogenesis begins and sexual maturing continues (Pérez Camacho et al., 2003). In the present study, average Chlorophyll-a was 12.59 µg/L in inside and 3.89 µg/L in outside and mean TPM value in inside and outside were 14.20 mg/L and 6.20 mg/L. There was a significant difference in Chlorophyll-a and TPM between outside and inside of lagoon ( $P<0.05$ ). However, gametogenic cycle showed similar pattern between two sites. There is a direct relationship between food availability and acceleration of the reproductive process, provided that temperature is favourable (Delgado and Perez-Camacho, 2005). Toba and Miyama (1995), reported that the gonads of *R. philippinarum* develop in a range of temperature of 10-27°C under sufficient food supply. Although Chlorophyll-a and TPM value in outside of lagoon was lower than inside, gametogenic process (ripening and spawning) was parallel between two sites. It can be explained that in Çakalburnu Lagoon both inside and outside nutritional value is high and seawater temperature is suitable for gametogenesis and proliferation activity continue throughout the year.

In present study, ripe clams were observed throughout the year except for November in inside and December in outside. Some authors reported that ripe carpet shell clam observed between June and September in Ireland (Xie and Burnell, 1994), in Çardak Lagoon, Turkey (Gözler and Tarkan, 2000) and between April and June in Galicia, Spain (Ojea et al., 2004), Sufa Lagoon, Turkey (Serdar and Lök, 2009). These results showed that ripe gamet was observed for a longer period in Çakalburnu Lagoon than Ireland, Çardak Lagoon, Galicia and Sufa Lagoon. Xie and Burnell (1994) reported that the gametogenic cycle of the carpet shell clam showed one spawning period and spawning occurred between August and September. In our study spawning was observed almost a year. Moreover, Serdar and Lök (2009) mentioned that spawning period was between July and October in the Sufa Lagoon, Izmir Bay. Geographical location is important in the timing and duration of gametogenesis and spawning (Sarasquete

et al., 1990; Urrutia et al., 1999). Clam population reach ripe and spawning period in Mediterranean earlier than Northern population (Rodriguez-Moscoso et al., 1992; Xie and Burnell, 1994). Although Çakalburnu Lagoon and Sufa Lagoon located in the same bay (Izmir Bay), resting, ripening and spawning period of clam differ from each other. Meanwhile, in Çakalburnu Lagoon both inside and outside, ratio of resting (inactive) period was only 3.0-5.0% between November and January. Resting stage of carpet shell clam was 15-70% in Çardak Lagoon (Gözler and Tarkan, 2000), 40-60% in Galicia (Ojea et al., 2004) and 70-100% in Sufa Lagoon (Serdar and Lök, 2009). This situation showed that if the seawater conditions such as temperature, food availability and salinity e.t.c. were suitable for gametogenesis, gamete maturation and spawning of clam continued throughout the year and resting stage was too short period.

The male/female ratio of *R. decussatus* was 1:1.23 in Çardak Lagoon (Gözler and Tarkan, 2000) and 1:1.36 in Sufa Lagoon (Serdar and Lök, 2009). However in this study sex ratio for inside and outside of the lagoon was 1:1.06 and 1:1.10, respectively ( $P>0.05$ ). The male/female ratio in this study was similar to other studies.

In conclusion, both inside and outside of the Çakalburnu Lagoon, gametogenic activity has continued throughout the year and this area has been very productive for carpet shell clam which has been collected and exported. The results of this study have pointed out that knowledge of gonadal development of *T. decussatus* has been important for sustainable management of the wild stocks as well as the future aquaculture progress with this species.

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