

REPRODUCTIVE CYCLE OF *DONAX TRUNCULUS* (MOLLUSCA: BIVALVIA) IN PORT SAID, MEDITERRANEAN COAST, EGYPT.

Ali, E.M¹; Mohammed, S.H²; Mohamed, S.Z¹ and Aamer, M.A¹.

¹Department of marine Biology, Faculty of Sciences, Suez Canal University

²Department of Zoology, Faculty of Sciences, Suez Canal University

ABSTRACT

Seasonal variations in reproductive cycle of the clam *Donax trunculus* were studied between December 2006 and January 2008 at two sites in Port Said, Mediterranean coast Egypt. Gametogenic activity declined at the beginning of winter. Spawning took place in spring, when seawater temperature began to increase and performed a good peak in summer, when temperature reading at its highest level. At the end of autumn the specimens were found to be in the resting stage. Both males and females showed a simple synchronization in gonadal development and spawning. The clams reached maturity during the first year of its life at 15.5 mm. Sex ratio was generally 1: 1 but sometimes found to be in favor of males.

INTRODUCTION

The carpet shell clam *Donax trunculus* is important component of the shallow bottom macrofauna of exposed sandy beaches of tropical and temperate coasts [Ansell, (1983)]. It is found in Mediterranean, in the black sea [Bayed & Guillou, (1995)] and in the Sea of Marmara in Turkey [Özden, *et al.*, (2009)]. Donacidae are suspension feeders bivalves, feed on phytoplankton [Moužza & Chessel, (1976)] and suspended particulate organic matter [Wade, (1964)]. In the essentially tidless Mediterranean Sea, the clams occupy a relatively narrow zone in the shallow sub littoral in depths of a few centimeters to 2m [Salas-Casonova, (1987)] and its distribution is limited to moderately exposed beaches of well graded fine sands [Mazè & Laborda, (1988)]. This species can inhabit the high energy environments on sandy beaches, where it is exposed to tidal rhythm, intense wave action and sediment instability [Ansell, (1983)].

The reproductive cycle has been analyzed by [Gasper & Monterio (1998)]. [Moužza & Frenkiel-Renault (1973) and Ansell & Lagardère (1980)] recorded that spawning of this species take place in summer. [Badino & Marchionni (1972), Lucas (1965), Poli (1972), Gaspar *et al.*; (1998), Manca Zeichen *et al.*; (2001) and Bayed (1990)] also stated that *Donax trunculus* was spawned in spring and summer. [Ansell & Bodoy (1979)] reported long spawning period extend from April to September.

The present work deals with reproductive cycle of *Donax trunculus* to elucidate gametogenesis, maturity stages, spawning period, Synchronism between males and females and sex ratio.

MATERIAL AND METHODS

Study area:

Port Said located on the Mediterranean coast **fig. (1)** at northern of Suez Canal (Egypt) ($32^{\circ} 18' E$, $31^{\circ} 16' N$). The total surface area of beach is about $55m^2$. The maximum depth in Mediterranean Sea is a bout 300 km.

Sampling Methods:

Samples were monthly collected between December 2006 and January 2008 from two sites.

The first site is El Macabre lies at the northern west of Port Said ($32^{\circ} 16' 26.4'' E$, $31^{\circ} 16' 37.7'' N$). This site does not expose to human activity and consider as control one.

The second site is fishing club **fig. (2)** lies at the northern east of Port Said ($32^{\circ} 19' 02.7'' E$, $31^{\circ} 16' 23.2'' N$). This site is exposed to extensive clam fishing and other human activities as tourisms and extensive fishing.

Specimens were collected by simple harvesting tool (hand dredge).

Water temperature measured by a simple bucket thermometer graduated to 0.1° and salinity of water were monthly measured by a calibrated digital refractometer.

Clams shell length was measured with a Vernier caliper. (Up to 100 mm accuracy).

The data were categorized into size group based on 5 mm size intervals. The percentage of each class interval was then calculated.

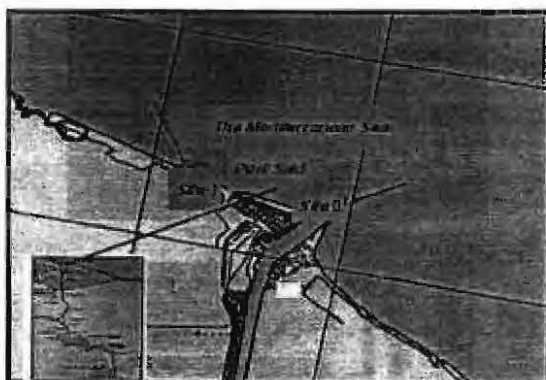


Fig.(2): Sampling sites and study areas at Port Said Beach.

Microscopic Studies:

It includes two types of examinations:-

Gonadal Smears:

A total of 1400 specimens of *Donax trunculus* were sexually differentiated. Small portions of gonads were spread over clean microscopic slides after adding a drop of water and examined under a light microscope.

Histological Preparation:

A total of 140 specimens of clams of different size were monthly collected. The shell was removed and small cub of tissue (2 to 3 mm³) was dissected from the proximal region of foot (where the gonad intertwines with the intestine) by a sharp scalpel. The specimens were fixed for 48 hours in Bouin's solution, dehydrated through an ascending series of ethyl alcohol (an hour for each change), cleaned in terpineol for 2-5 days and embedded in paraffin wax (three changes, each for half an hour). Sections of 6μ were stained with Ehrlich's hematoxylin and Eosin and mounted by Canada balsam. Photographs were taken with a compact digital camera (Canon pc 1200) fixed with Axiostar plus corl zerss Microscope.

Phases of gonadal maturation:

The gonadal histological sections were examined by light microscope to assess the relative reproductive maturity and development. The degree of gonadal development was classified according to [Gasper & Monterio (1998)] into six phases for each sex. These categories are inactive, early active, late active, ripe, partially spawned and spent. Determination of the gonadal phase based on the external appearance of the gonads and the percentage occurrence of mature follicles, as more than one phase may occur simultaneously within the gonad. Monthly percentages of maturation phases were calculated and graphed for males and females separately.

RESULTS

Hydrographic parameters:

Temperature readings showed monthly variations at both sites throughout the study period. The maximum temperature (27 °C) was recorded in August 2007, there after; it gradually decreased to reach its minimum value (14 °C) in January 2008. Salinity showed small variation between the two sites. It ranged between 36 ‰ (January 2008) and 38 ‰ (July 2007). There was no significant difference in temperatures (P= 0.92) (T.test) or salinities (P= 0.49) (T.test) between the two sites.

Gametogenic phases:

The reproductive cycle of both sexes of clams were followed up and described. The amount of connective tissue between the follicles play an important role in the

classification of its gametogenic phase as it decline with the gonadal ripening in both sexes.

Female Categories:

Reproductive cycle of females is classified, into six phases. This classification is mainly depending on the gonad shape and size, follicle size, abundance of different stages of oogenesis within the follicle and amount of connective tissue.

1-Inactive phase:

The ovary appears very small, flaccid and white in color. The foicles of the ovary mainly composed of oogonia. The connective tissue is evident and spread between follicles in a great amount **plate (1)**.

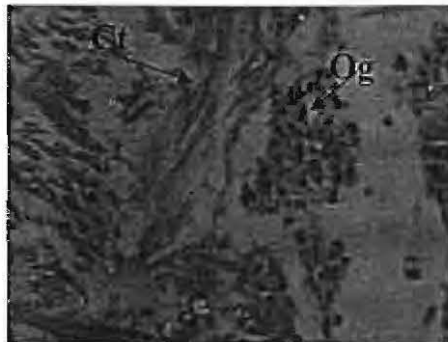


Plate (1): In active phase of female *Donax trunculus*, showing the oogonia (og) with large amount of connective tissue (ct).

(H.&E. × 200)

2-Early active phase:

In this phase, the ovary is very small and pink in color. Microscopically, the ovary contains a small amount of connective tissue, which enclosed small follicles mostly occupied with oogonia and early oocytes **Plate (2)**.

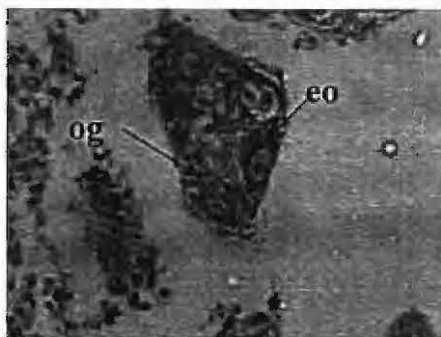


Plate (2): Early active phase of female *Donax trunculus* showing that oogonia (og) and early oocyte (eo) mainly occupy each follicle.

(H.&E. × 200)

3-Late active phase :

The ovary contains little amount of connective tissue. The follicles become larger than the previous phase, containing more early oocytes. Growing oocytes are represented with few ova (o) in this phase Plate (3).

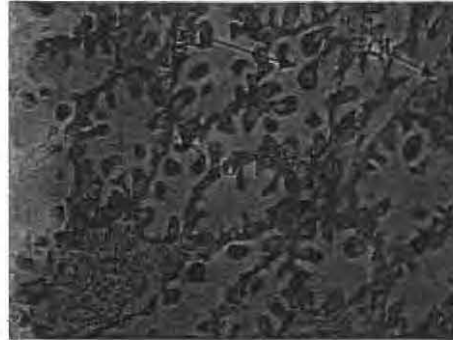


Plate (3): Late active phase of female *Donax trunculus* showing the distribution of follicle within few amount of connective tissue (ct).The follicle is occupied by early oocyte (eo), growing oocyte (go) and few ova(o) .
(H.&E. × 200)

4-Ripe phase :

The ovary is very large, reddish in color and hides entirely the visceral mass.

The follicles become larger with scarcely amount of connective tissue occurred in the ovary. The follicle is mainly composed of mature ova Plate (4).

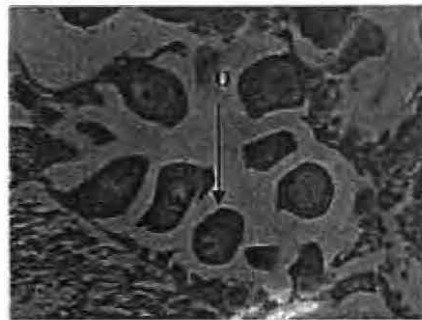


Plate (4): Rip phase of female *Donax trunculus* showing that large follicles and little amount of connective tissue (ct) occlude the gonad. The follicle is mainly composed of ripe ova (o).

(H.&E. × 200)

5-Partially spawned phase :

In spite of the large size of the ovary at this at this phase, its rigidity is less than the former phase. The ova are round in shape. Some parts of the follicles are empty due to the partial releasing of ripe ova. Oogenesis increased gradually at the periphery of the follicle at this stage **Plate (5)**.

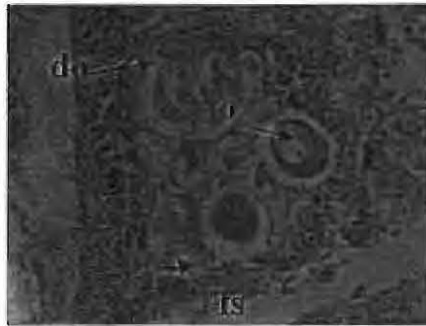


Plate (5): Partially spawned phase of female *Donax trunculus* showing large follicles occupied by rip ova (o),degenerated ova (do) and empty follicular sac (fs).
(H.&E. × 200)

6- Spent phase:

The spent phase is characterized by regeneration of the connective tissue and tearing of the follicle. Degenerated ova are detected in the follicular lumen. Redeveloped of oogonia and early oocytes began to appear **Plate (6)**.



Plate (6): Spent phase of female *Donax trunculus* showing redeveloping connective tissue (ct),degenerated ova (do) , redeveloped stages of oogonia and early oocytes .
(H.&E. × 200)

Male Categories

Gonadal development of males were divided into six phases according to gonadal shape, size, color and the percentage of testicular fullness, the follicle shape and an abundance of the connective tissue.

1- Inactive phase:

The testis is very small and white in color. It has indistinct follicles mainly composed of spermatogonia (sg) which embedded in a large amount of connective tissue **Plate (7).**

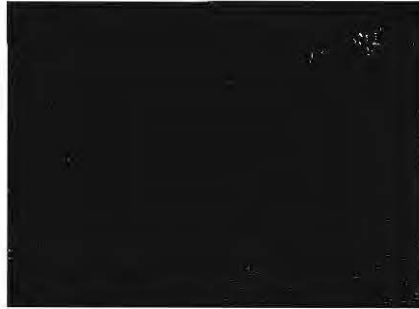


Plate (7): In active phase of male *Donx trunculus* showing indistinct male Follicles mainly composed of spermatogonia (sg).

(H.&E. × 200)

2-Early active phase:

The testis is flaccid; white in color and numerous indistinct follicles. Most of them are filled with spermatogonia, primary spermatocytes and secondary spermatocytes **Plate (8).**

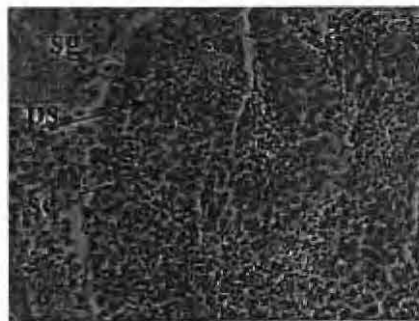


Plate (8): Early active phase of male *Donx trunculus* showing indistinct male follicles mainly composed of spermatogonia (sg), primary spermatocyte (ps) and secondary spermatocyte (sc) .

(H.&E. × 200)

3-Late active phase:

The follicles elongated and increase in number. Spermatids and a few sperms appear in this phase in addition to spermatogonia and spermatocytes. This phase is characterized by clustering the spermatids in radiating forms Plate (9).

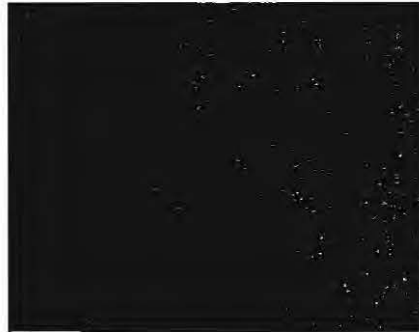


Plate (9): Late active phase of male *Donx trunculus* showing spermatids (sd) and few sperms (sz) in the follicles.

(H.&E. × 200)

4-Ripe phase:

The testis becomes rigid, yellowish white in color. The follicles become lobulated and increased in size. Although, early stages of spermatogenesis are found at the periphery of the follicle, large numbers of sperms in the center of most follicles were seen Plate (10).



Plate (10): Rip phase of male *Donx trunculus* showing lobulated follicles containing numerous spermatozoa (sz) in its lumen.

(H.&E. × 200)

5-Partially spawned phase:

In this phase, the follicles are partially empty and fragmented with large lumen
Plate (11).



Plate (11):Partially spawned phase of male *Donax trunculus* showing fragmented follicle with lumen.

(H.&E. × 200)

6-Spent phase

The spent phase is characterized by tearing off the follicles and regeneration of connective tissue **Plate (12).**

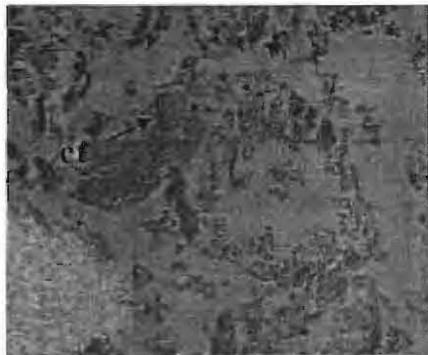


Plate (12): Spent phase of male *Donax trunculus* showing fragmented follicle and redeveloping connective tissue (ct).

(H.&E. × 200)

Reproductive Cycle:

A- Female:

Annual reproductive cycle of female is summarized in fig.(3A). The gametogenic cycle began in December 2006 as 63% of individuals were in the early active stage and the remaining was in the late active stage which dominated the samples in the following 2 months (January and February, 2007). Ripe stage reached its peak in February 2007, after which partially spawning began (March 2007). Spawning activity continued till July 2007, after that most individuals (62%) were in spent phase. The entire populations were in an inactive stage from September to October 2007. At the end of November 2007, a new gametogenic cycle began.

B- Male:

Fig.(3B) illustrates monthly changes in the percentage of males in each maturity stage. The gametogenic cycle started to appear before December 2006 where only few individuals (20%) were in the early active stage while the remaining (80%) were in the late active stage. Ripe males were recorded in the following three months. Meanwhile, partially spawning was observed and lasted to August, at which most of individuals (70%) were in spent phase. In September 2007 and October 2007 the entire population was in an inactive stage. At the end of November 2007 a new gametogenic cycle began.

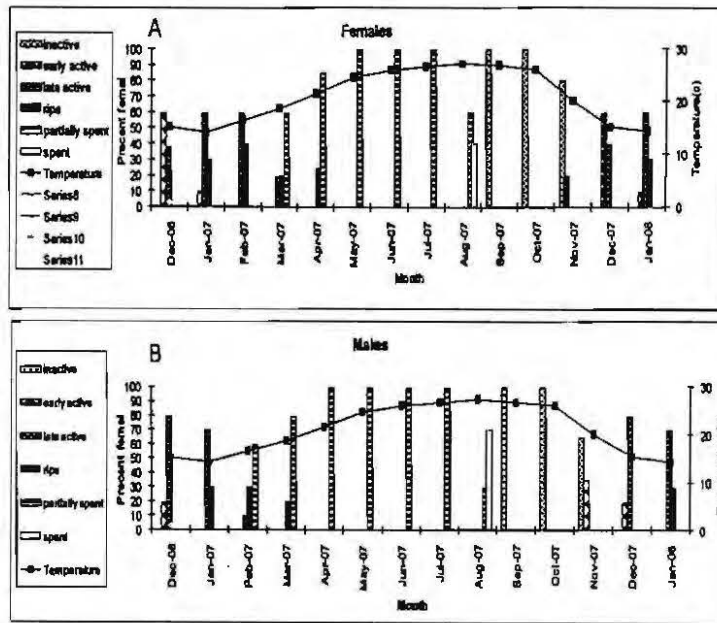


Fig. (3): Monthly variations in gonadal developmental stages of females (A) and males (B) *Donax trunculus* with temperature.

Synchronization in reproductive phases:

Fig. (4) shows simple synchronization in the inactive phase then male preceded females in the late active phase. Synchronization becomes more evident in the partially spawning specially between May and July 2007.

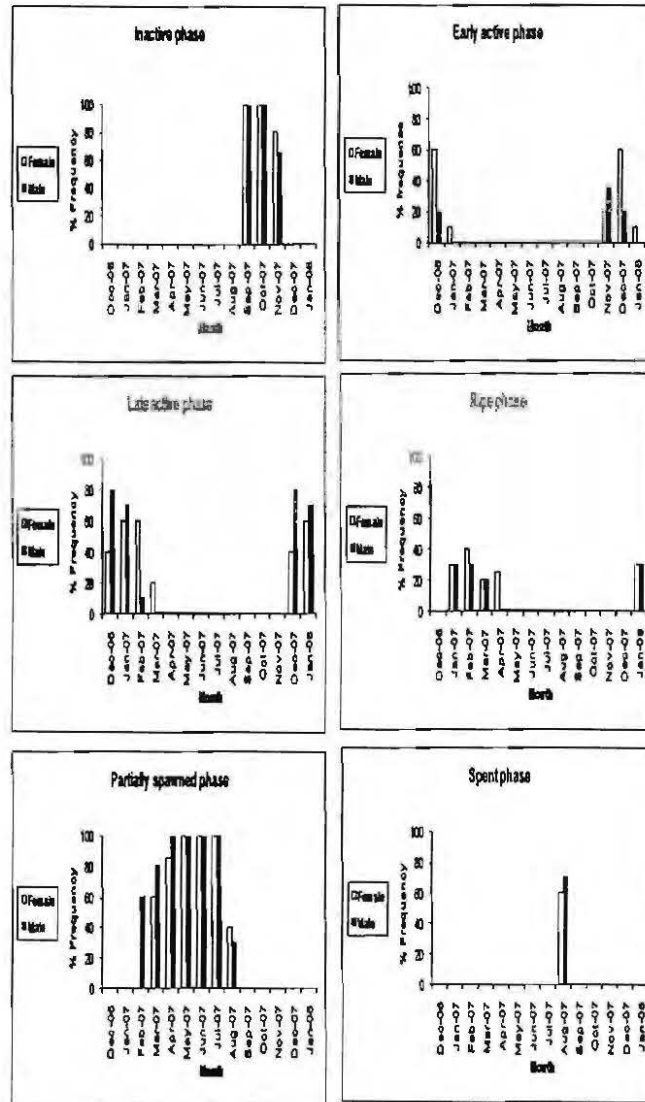


Fig. (4): Synchronization between males and females *Donax trunculus* for different reproductive phases.

Sex Ratio:

The proportion of male to female in *Donax trunculus* varied monthly between the two sites. Males usually made up a slightly higher proportion but with no significant difference from a sex ratio of 1:1. Male: female ratio ranged between 71.5 : 28.5 % (Dec 2006) and 69 : 31 % (Jan 2008) in site 1. It varied between 75: 25% (Dec 2006) and 65: 32% (Jan 2008) fig. (5).

For all size classes, Males outnumbered females except at size class 30.1- 35 mm at site 1 fig. (6).

Male dominated female significant difference was found between male and female in all months. (P: 0.00) (Chi- square test).

Donax trunculus began to differentiate into males and females after 15 mm in length fig. (6).

Fig. (6) illustrates Males also dominated females in the first and second year of life. This variation was significant (P = 0.00). (Chi- square test).

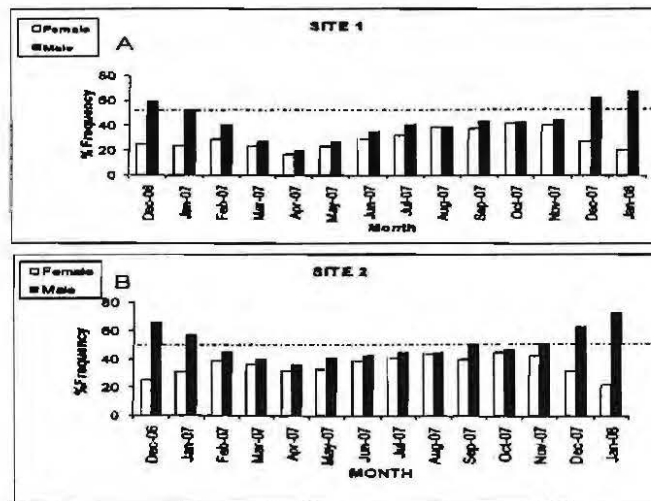


Fig. (5): *Donax trunculus*. Monthly percentage occurrence of males and females at site 1(A) and site 2(B) along study period.

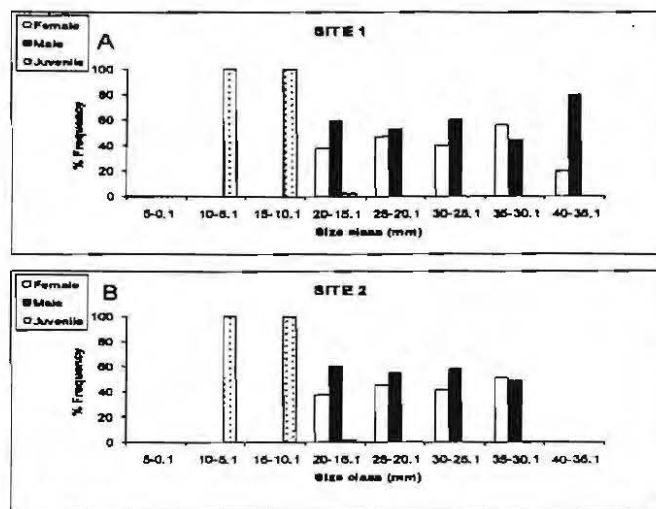


Fig.(6): *Donax trunculus*. Percentage occurrence of both sexes at site 1(A) and site for 2(B) for different shell lengths (5 mm Size

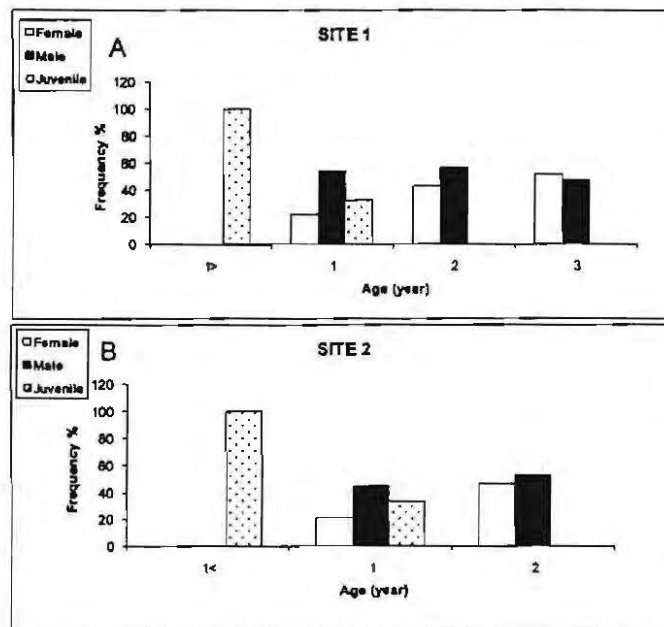


Fig. (7): *Donax trunculus*. Percentage occurrence of both sexes at site 1(A) site 2 (B) for different ages.

DISCUSSION

Donax trunculus is gonochoric, no hermaphroditism or sex reversal was encountered. In this study both females and males showed a synchronism in the gametogenic stages and spawning throughout the period studied. [Gasper et al; (1998)].

The present study revealed that clams reached its maturity during the first year of life at 15.5 mm. [Mouëza & Frenkiel-Renault (1973)] on the Algerian coast, recorded that *Donax trunculus* reached maturity during the first year and at 16mm shell length. [Manca Zeichen, et al; (2001)] on the south Adriatic coast (Italy) founded that *Donax trunculus* reached maturity during the second year of life at 18 mm shell length.

Sex ratio of *Donax trunculus* in the present study was found to be in favor of males in most of the years. This variation was significant. This was agree with the studies of [Lucas (1965), Badino & Marchionni (1973), and Mouëza & Frenkiel-Renault (1973)] who mentioned that males of *Donax trunculus* usually made up a slightly higher proportion but with no significant difference from 1:1. Males outnumbered females in most size classes and differential mortality probably as a result of spawning effort [Gasper et al; (1998)].

Gametogenic cycle of *Donax trunculus* began in December 2006 and spawning lasted from spring to summer. Similar observation on the reproductive cycle of *Donax trunculus* were observed by [Anseel & Bodoy (1979)]. They reported that spawning period of the clam *Donax trunculus* started from April to September, in two phase spring and summer. [Badino & Marchionni (1972)], for the Mediterranean, [Lucas (1965) and Poli (1972)] for French Atlantic coast, [Bayed (1990)] for Al media, Moroccan Atlantic coast, [Gasper, et al; (1998)] for [faro& southern Portugal & Manca Zeichen, et al; (2001)] for the south Adriatic coast (Italy) also observed that *Donax trunculus* spawned between March and August, with also two peaks (spring and summer).

However [Mouëza & Frenkiel-Renault (1973) and Ansell (1980)] mentioned that spawning occurred in summer. Sexual resting period spanned from summer to autumn.

The environmental factors are likely to play a crucial role affecting the variability of the gonadal maturity period [Manca Zeichen, et al; (2001)]. Gametogenic activity started with decreasing temperature at the beginning of winter. Spawning took place in spring, when sea water temperature began to increase and ended in summer (August 2007), when temperature declines at the end of autumn, [Gasper, et al; (1999)] recorded same results in Vilamoura, southern Portugal on the sub tidal white clam *Spisula solida* and attributed the role of temperature in the gametogenesis of this bivalve.

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الملخص العربي

الدورة التنكاثرية في محار أم الخلول (نوات المصراعين)
بمدينة بورسعيد على شواطئ البحر الابيض المتوسط (مصر)

مقدم من إيمان مصطفى على القلبان¹ و د.سلمية صيون محمد²
و أ. د.مسعد زكريا محمد¹ و د.ماهر عبدالعزيز أعر¹

¹ قسم علوم البحار.كلية العلوم. جامعة قناة السويس .

² قسم علم الحيوان.كلية العلوم. جامعة قناة السويس .

تم دراسة التغيرات الموسمية في الدورة التنكاثرية في محار أم الخلول ما بين ديسمبر ٢٠٠٦ و يناير ٢٠٠٨ في موقعين مختلفين في مدينة بورسعيد على شواطئ البحر الابيض المتوسط. وكان نضوج المناسل قليل في بداية الشتاء وحدث تفريغ الامشاج في الربيع عندما بدلت درجة الحرارة في الزيادة وكانت أعلى نسبة لها في الصيف عندما وصلت درجة الحرارة الي أعلى درجاتها. وقد تم رصد حالة السكون الجنسي في نهاية الخريف. ووجد تزامن بسيط لكل من الذكور والاناث في حالتها نضوج المناسل وتفريغ المناسل. ووصل محار أم الخلول الى النضوج الجنسي أثناء عامه الاول عند طول ١٥,٥ ملليمتر. وكانت نسبة الإناث للذكور ١:١ مع زيادة نسبة الذكور في بعض الاحيان.

