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Reproductive strategy of the black lip pearl oyster, *Pinctada margaritifera* (Linnaeus, 1758) in Red Sea, Egyptian Waters

Mohamed M.A. Zeid[2]; Fatma A. Abdel Razek[1]; El Sayed A. E. Hamed[1] and Somaya M. Taha[1].

[1]National Institute of Oceanography and Fisheries, Alexandria, Egypt
[2] Faculty of Science, Al-Azhar University, Cairo, Egypt
E-mail: fatma_abdelrazek@hotmail.com

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Abstract

The reproductive conditions of the pearl oyster *Pinctada margaritifera* (Linnaeus) were examined based on 240 specimens, collected north and south coast of Red Sea of Egypt during the period from September, 2006 to August, 2007. Histological observations on gonadal development indicated that *Pinctada margaritifera* females had one spawning period a year in summer season in both of the areas. The spawning continued till early autumn months, however, by December all the collected samples were in the spent condition and some of them were at the resting stage with synchronization between both males and females. Spawning of southern area population starts as early as April at which the water temperature is normally higher by about five degrees in south part than that of the north part. This indicates that the onset of reproduction appears to be regulated by sea surface temperature. The results of this study can be applied to the induction of spawning and the production of seeds in the aquaculture system of this pearl oyster species in Egypt.

Keywords: Pinctada margaritifera; Gameto gensis; gonadal development; spawning; Red Sea; Egypt.

1. Introduction

The pearl oyster, *Pinctada margaritifera* (Linnaeus) inhabit temperate, subtropical and tropical coral reefs, and are widely distributed in the Indo-Pacific area. In Egypt, *P. margaritifera* are distributed in Red Sea coast, El Sayed (2009). Pearl oysters draw research attention because their use in the commercial culturing of pearls can potentially provide economic benefits to the countries where these resources are available.

The reproductive biology of the pearl oyster is important for the pearl culture industry in order to understand gonadal development and population dynamics of wild stock. This knowledge can be used for the development of hatchery techniques and the importance of nucleus insertion into the gonads for pearl culture. Gametogensis and breeding cycles of pearl oysters have been well documented in various species of the family Pteriidae (Tranter, 1958, a,b,c,d; and 1959; El-Eisawy, 1974; Wada, 1984; Yassien, 1998 and Abou Zeid, 1991).

In Egypt, although a diversity of pearl oysters of the family Pteriidae have been found (Gabal, 1982, GEF, 1998 and Mohamed & Yassien, 2008), little is known about the biological aspects of the wild stocks of these species.

Therefore, this study investigates the reproductive biology of the wild stocks of *P. margaritifera* in the Egyptian Waters of the Red Sea. The results obtained in this study can provide useful informations for pearl culture in the future.

2. Materials and Methods

A total of 240 living pearl oyster *P. margaritifera*, were collected regularly on monthly visits during the period from September 2006 to August, 2007 from two areas near Hurghada city, Red Sea as a northern area and the other southern of Shalatein as a southern area Abdel Razek *et al.* (2010). Each living specimen of the pearl were cleaned from the extraneous bio-fouling organisms as external parasites by scraping it from the ventral and dorsal shell.

2.1. Macroscopic examination of the gonads

The shell height and total weight of each collected oyster were recorded. The soft body was removed from the shell and its wet weight was determined to the nearest 0.01 g. The mantle and gills were then folded back to expose the body of the oyster adjacent to the foot to permit a macroscopic assessment of the reproductive condition. Information on gonad development, sex and colour of gonad was recorded. Gonad development was estimated by taking a small sample of tissue from an area next to the urogentital

pore using a clean glass pipette and smearing the sample on a slide examined and photographed on a zeiss photomicroscope II.

2.2. Histological examination of the gonads

A total of 120 individuals of the studied oyster species were used for gonad histological examinations. The gonads were separated from the tissue and fixed in Bouin's fluid (75 ml of picric acid, saturated in filtered sea water; 25 ml formalin:- 40 HCHO; 5 ml glicial acetic acid) at room temperature for 24 hours prior to dehydration through a series of alcohols:- 30%, 50% and 70%, allowing 2 hours between each change. Samples were then preserved in 70% alcohol for 1 to 2 weeks and kept for further treatment for histological preparations.
Samples were then dehydrated through a graded series of alcohol, each cleared in xylene and then embedded in paraffin. Sectioning was done through different regions of the gonads of each specimen at 5 – 7 micron thickness. Sections were stained with Haris hematoxylin – eosin, and with Eosin as a counter stain.
After staining, sections were mounted using canda balsam and stored to dry at temperature 25°C for about 24 hours.
Samples were examined microscopically to assess gonad condition and photographed using Nikon microscope equipped with Digital camera.
The scheme used for gonadal development was according to Hwang (2007) depending on the size and density of various germ cells, which are formed from stem cells of gametogensis in the gonad.

3. Results

3.1. Gonadal development:

The gonadal development of *P. margaritifera* can be divided into six stages according to Hwang (2007) in males Figure 1 (A – F) as well as in females Figure 2 (A – F). During the examination of the gonadal tissues of all samples of the different populations no hermaphrodite individuals were recorded from *P. margaritifera*.
The sequences of spermatogensis in males and oogensis in females based on histological preparations of gonads. The histological changes in the gonad condition were, ingeneral, supportive of the macroscopic observations. However, macroscopic observations were unable to distinguish clearly between ripe gonads and those that had entered the first regressive stages.
The description of the different developmental stages are as follows:-

3.1.1. Stage I: Early developmental stage

Figure (1-A) illustrate the early spermatogensis of stage I in males of *P. margaritifera* where stem cells and spermatogonia (SPG) reside in the follicle wall (FW). Here spermatogonia, as well as a few spermatocytes (spc) could be observed.
Figure (2 – A) in females, show the follicle walls to be thick. Small primary oogonia (og) could be seen as well as developing oocytes (OC) were attached to the follicle wall.

3.1.2. Stage 2: Developing stage

Figure (1 – B) in males the lumen of the follicle is gradually filled up and spermatogonia which will develop to spermatocytes (spc). Similarly, spermatocytes and spermatids (spt) filled the follicle lumen, while the male follicle walls become thinner. Spermatocytes are condensed in a dark band beneath the layer of spermatogonia and attached to the follicle walls.
Figure (2 – B) illustrates an advanced stage in females where oogonia growing along the wall of the follicle and are pear – shaped.

3.1.3. Stage 3: Mature stage

Figure (1 – C) shows actively developing testis of the previous stage where spermatides (spt) are transformed into spermatozoa with free active tails-spermatozoa which are observed in the center of the lumen central. With a larger quantities of mature spermatids occurring at this stage.
Figure (2 – C) in females, mature free oocytes accumulate in the lumen of the follicle are resident. This stage is an actively developing ovary. Also in this stage, the follicle wall is swollen.

3.1.4. Stage 4: spawning stage

Figure (1 – D) illustrates that follicles are enlarged as the spermatozoa continuosly increase in number and this gives rise to spawning – ripe condition. A few resorptive phagocytic cells have appeared. Part of spermatozoa had been released, the central lumen appear to be more or less empty.
In females Figure (2 – D) free oocytes are gradually filling the lumen of the follicle and the follicle wall is broken. With a crorresponding reduction in the numbers of earlier stage of oogonia.
A characteristic feature of the ova at this stage is their polygonal shape which results from the pressure exerted by the follicle wall.

3.1.5. Stage 5: spent stage

Figure (1 – E) shows some gametes remain unreleased in the lumen. Although phagocytes have cleared unreleased spermatids and residual mass, the sex can still be identified as a male by the presence of the unresorbed gametes. Thereafter, the follicle has shrunken with isolated pockets of spermatids.

In case of female as in Figure (2 – E) the volume of the follicle has reduced and regressed or collapsed but not all are empty, because some free oocytes which are small or not well developed remained. The sex can still be identified by the presence of the unresorbed gametes. Follicle walls are shrunken and broken into debris. Presence of some phagocytes with unreleased oocytes are observed.

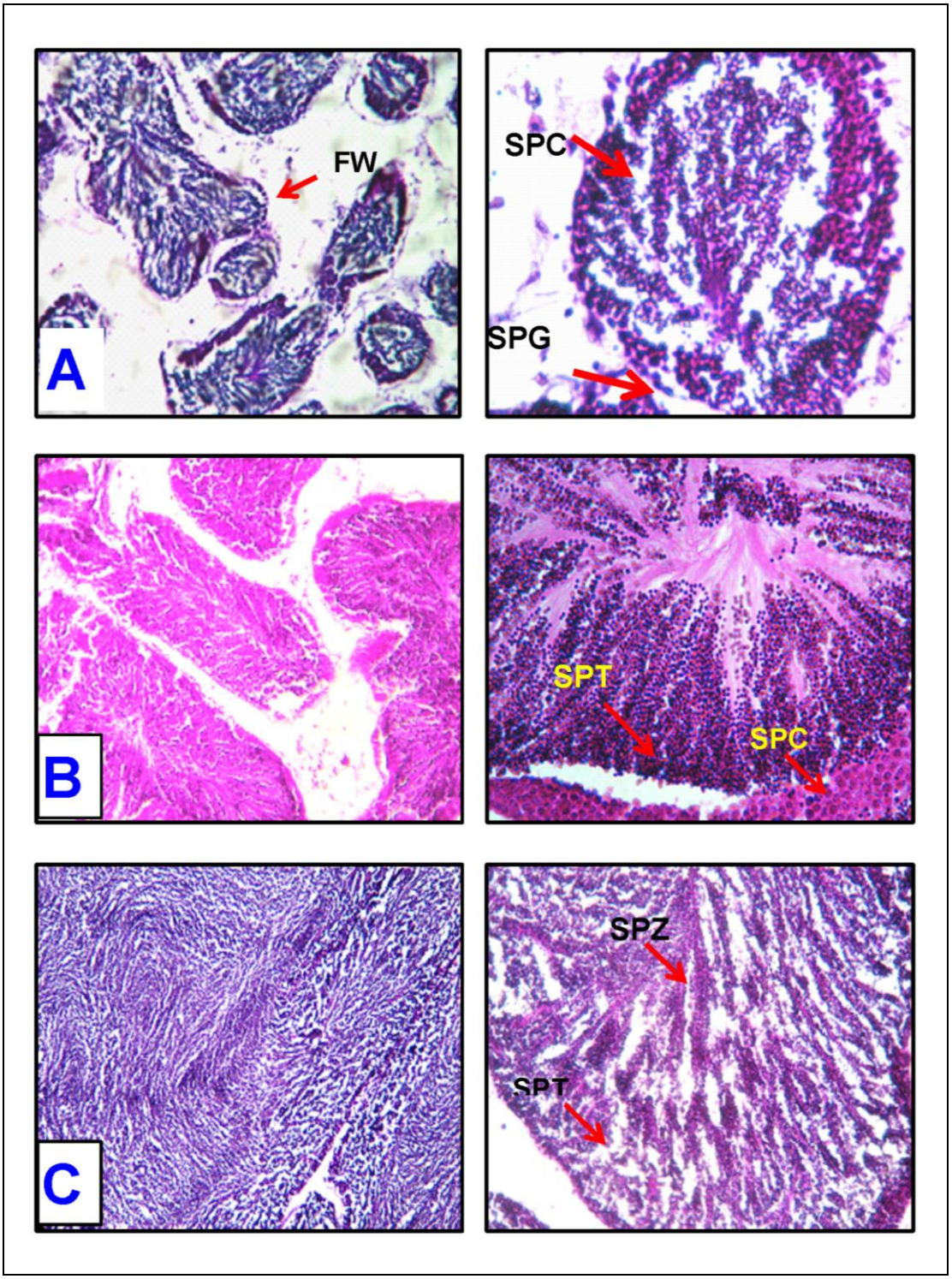


Figure (1 A, B, C): Microphotographs of gonadal development stages of male P. margaritifera. (A) Early developing; (B) developing; (C) mature, Hx&E ;(left X 200, right X 1000).
FW= follicle walls SPC= spermatocytes SPG= Spermatogonia
SPZ= spermatozoon SPT=Spermatids

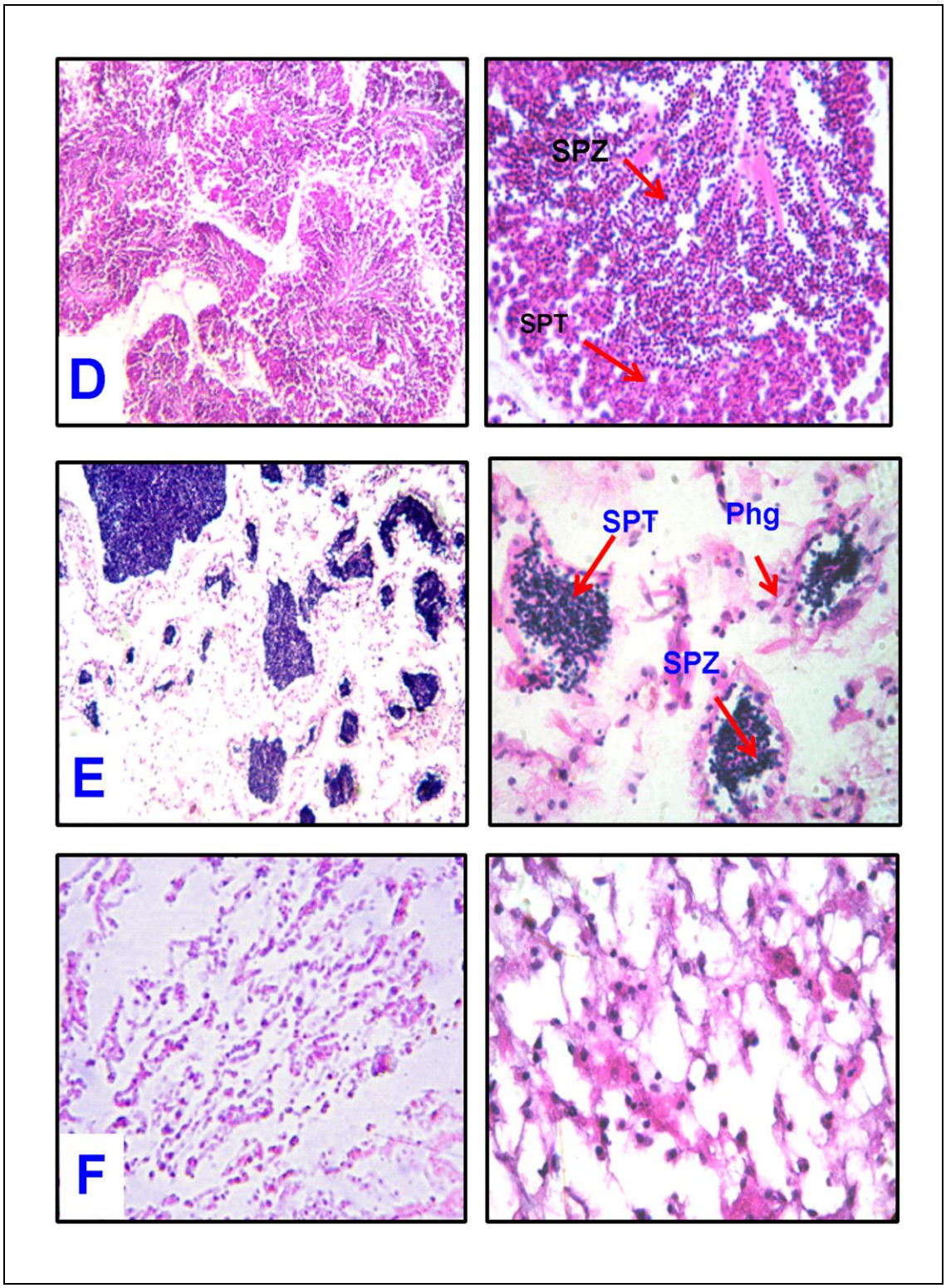


Figure (1 D, E, F): Microphotographs of gonadal development stages of male P. margaritifera (D) spawning; (E) spent; (F) resting.
Hx&E; (Left X 200, right X 1000),
Phg= phagocytes

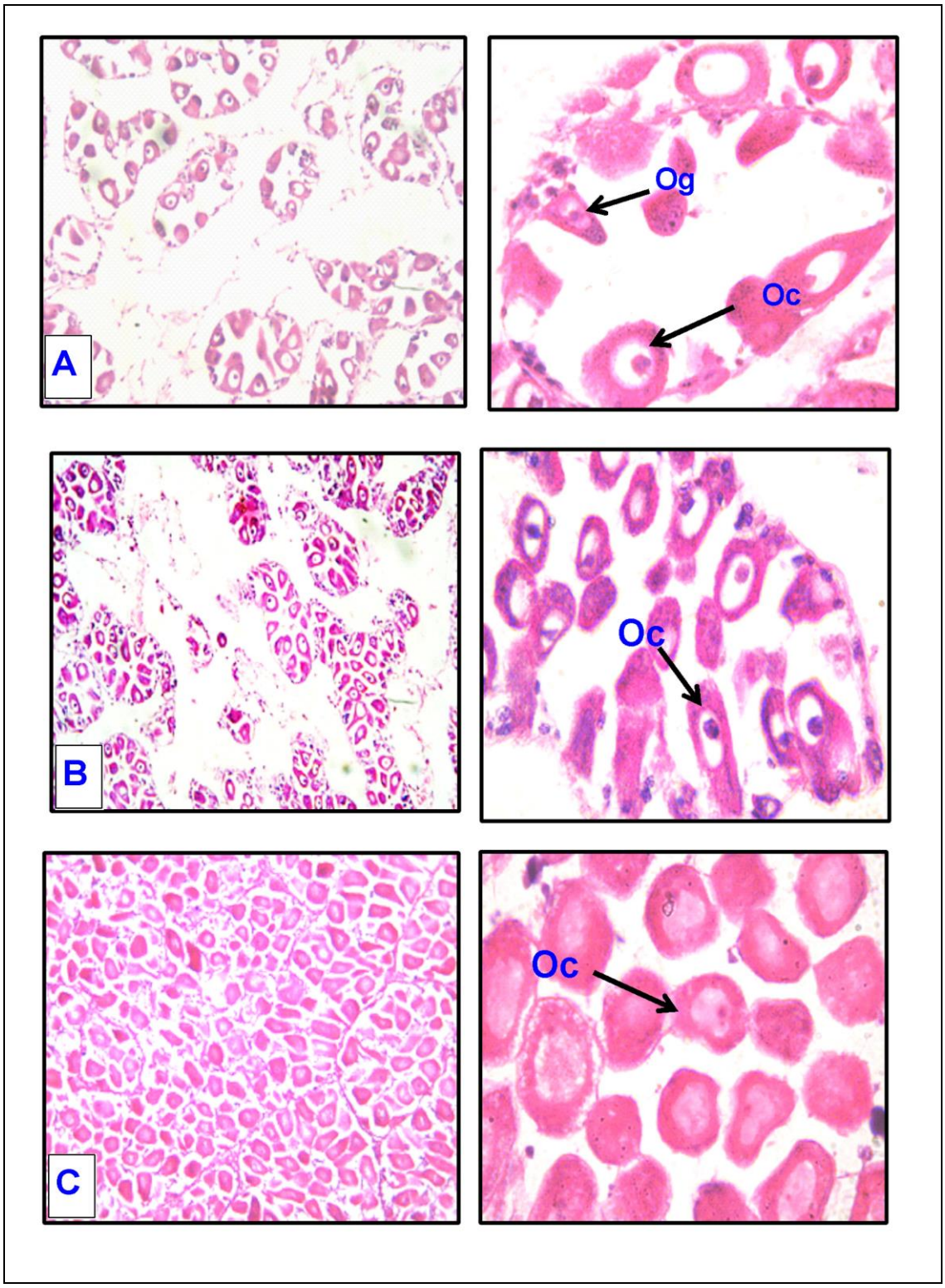


Figure (2 A, B, C). Microphotographs of gonadal development stages of female *P. margaritifera*. (A) Early developing; (B) developing; (C) mature; Hx&E; (Left X 200, right X 1000), Fw = follicle walls
Oc = oocytes Og= oogonia

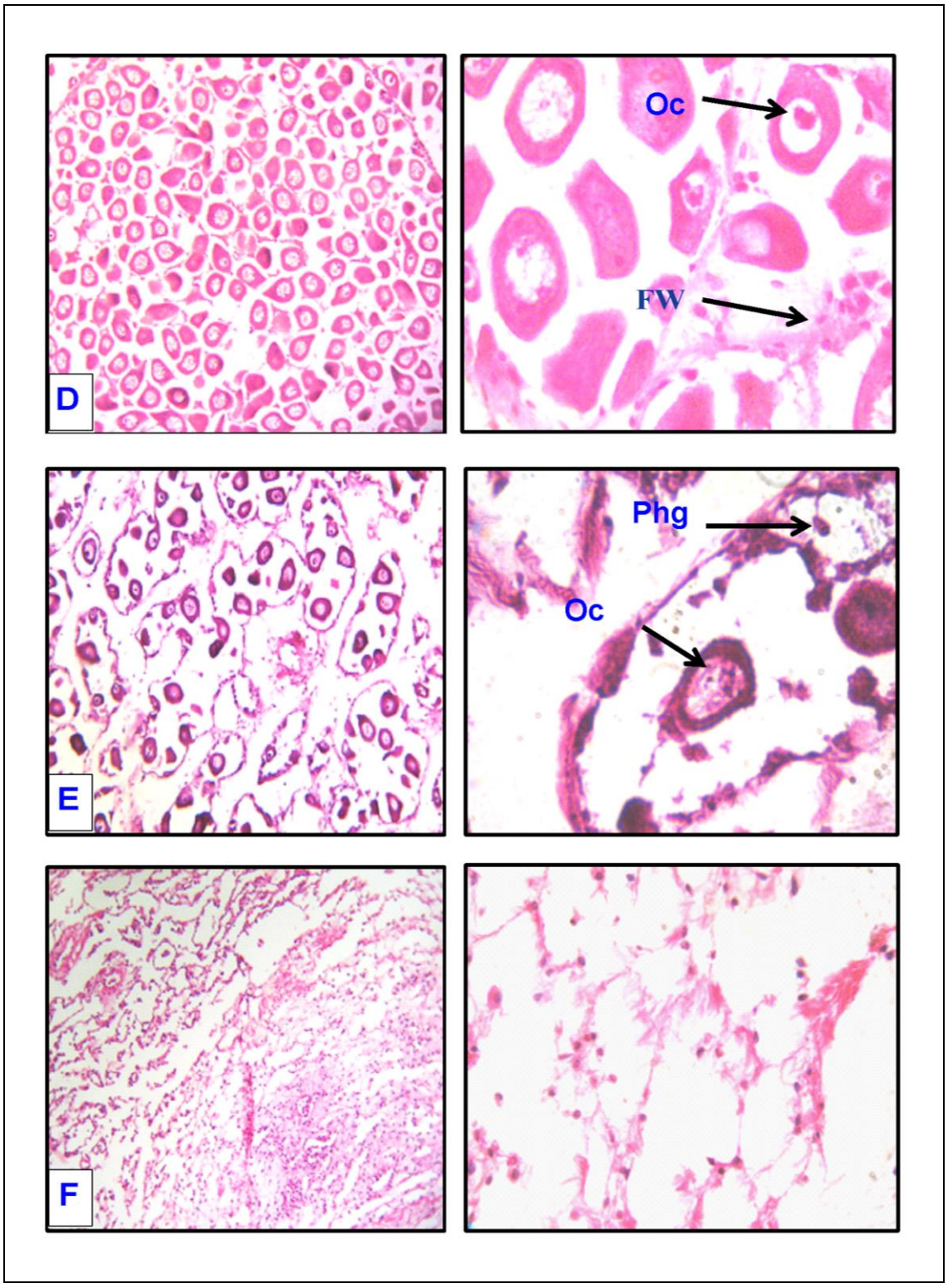
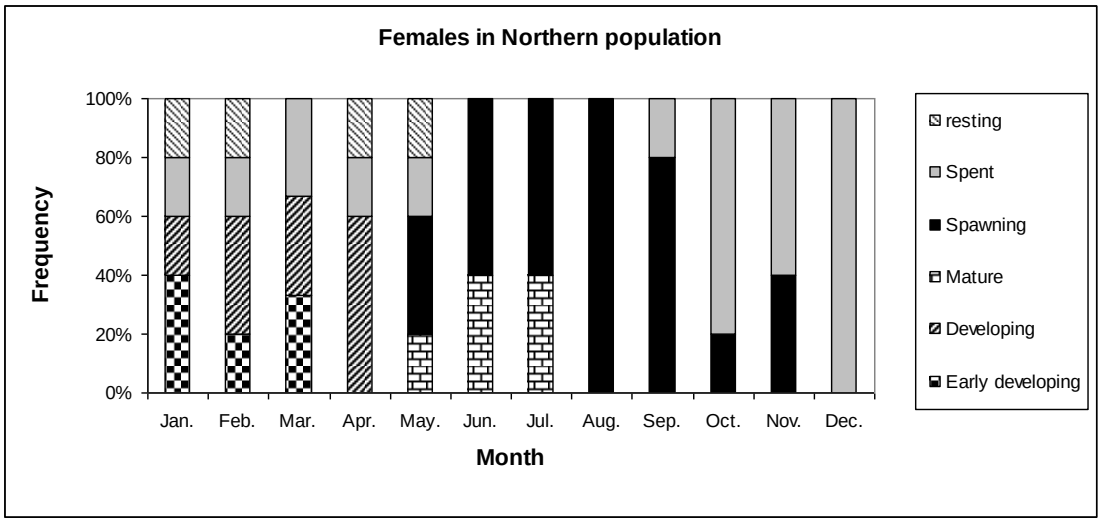


Figure (2 D, E, F). Microphotographs of gonadal development stages of female *P. margaritifera*. (D) Spawning; (E) spent; (F) Resting; Hx&E; (Left X 200, right X 1000), Phg= phagocytes

a-



b-

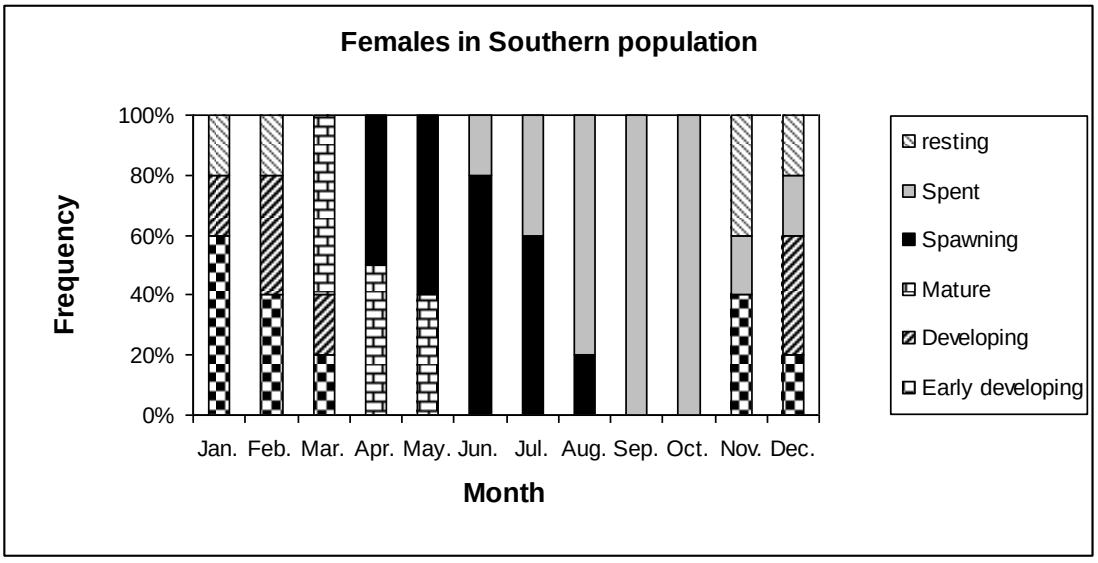


Figure (3 a&b): Monthly percentage distribution of females maturity stages in different populations of *P. margaritifera* in Egyptian coast of Res Sea.

Table (1 a&b): monthly distribution of different maturity stages of *P. margaritifera* of Northern (a) & Southern (b) populations of the Egyptian coast of Red Sea during 2006/2007.

a-

Month	males						females					
	Early developing	Developing	Mature	Spawning	Spent	resting	Early developing	Developing	Mature	Spawning	Spent	resting
Jan.	2	1			1	1	2	1			1	1
Feb.	1	2			1	1	1	2			1	1
Mar.		3	2		1	1	1	1			1	
Apr.		1	4					3			1	1
May		2	3						1	2	1	1
Jun.		1	3	1					2	3		
Jul.				5					2	3		
Aug.				5						5		
Sep.				4	1					4	1	
Oct.				2	3					1	4	
Nov.				1	4					2	3	
Dec.					5						5	
Total No. of samples examined	62						58					

b-

Month	males						females					
	Early developing	Developing	Mature	Spawning	Spent	resting	Early developing	Developing	Mature	Spawning	Spent	resting
Jan.	3	2					3	1				1
Feb.	2	3					2	2				1
Mar.	1	1	3				1	1	3			
Apr.		1	3	1					4	1		
May				5					2	3		
Jun.				5						4	1	
Jul.				4	1					3	2	
Aug.				1	4					1	4	
Sep.				1	4						5	
Oct.					5						5	
Nov.	1				2	2	2				1	2
Dec.	1	1			1	2	1	2			1	1
Total No. of samples examined	60						64					

3.1.6. Stage 6: Resting stage:

Figure (1 – F) illustrates, sex can not be identified and gonad in males is actionless after spawning; only connective tissues are observed in this degenerative state. Some time known as stage (0) of indeterminate or inactive gonad where the follicles are collapsed and reduced in volume.

Figure (2 – F) in females also sex can not be recognized and gonad is inactive after spawning; with connective tissues in this degenerative state. It is noted here also that there are no yolk nuclei visible in the cytoplasm of the oocytes as ova.

3.2. Maturity and spawning condition:

Table (1 a&b) and Figure (3 a&b) summarizes the frequency distribution of the monthly gonad histological preparations during the period 2006 – 2007 for *P. margaritifera* collected from two localities,

Northern part, North of Hurghada area and Southern part around Shalatein area. It can be seen that early gametogenesis, as detected by the early developing and developing stages, took place during January, February, March and April for the populations collected from the southern areas. While these stages extends during May and June especially for males in the population collected from the Northern areas as in Table (1a). During May and June, in the two areas, gonads of both sexes showed a rapid transition to ripe and spawning stages and then maintained a nearly 80 – 100% in spawning stage through June and July for southern oyster population while it extends to August, September and October for the Northern oyster population in both of males and females. This is followed by an abrupt decline in the following months with a corresponding increase in the spent stage in both sexes of about 80 – 100% during August, September and October of southern population and it remains to November and December for the northern population, suggesting the commencement to the summer peak spawning. The majority of gonads after summer spawning were collapsed and they entered a resting phase during winter when early gametogensis took place in all areas.

The histological observations on gonadal development in *P. margaritifera* females as shown in figure (3 a&b) indicates that this species had one spawning period a year in summer season in both of studied areas. The spawning continued till the early autumn months, however by December all the collected individuals were spent and some of them were at the resting stage with synchronization between both males and females. Spawning of southern area population south of Shalateinstarts as early as April Figure (3b) at which the water temperature is normally higher by about five degrees in south part than that of north part.

4. Discussion

Reproductive patterns and processes are quite varied in marine invertebrates as reported by Giese and Pearse (1979). Some species of potential culture as commercially shell fish are known to have annual reproductive cycles, but some bivalves have two spawning peaks per years, such as hard clams *Mercenaria* spp., Hesselman *et al* (1989). Similar characteristics have also been seen in pearl oysters, Hwang (2007).

A ripe oyster is identifiable superficially by the size of the gonad and macroscopically by examing live gamete smears and microscopically by examing histological preparations of gonads. In this study, the gonadal development of *P. margaritifera* was found to be reliable and simplified into 6 stages of reproduction according to Hwang (2007) scheme.

The histological observations on gonadal development indicated that *P. margaritifera* exhibited annual cyclical patterns and has one spawning period in the year from April to August in the southern population and from May to November in the northern population in the Egyptian Red Sea water.

According to the above mentioned results it is cleared that the population of *P. margaritifera* in the north of Hurghada area starts spawning during the summer season and continues till November with synchronization between both males and females.

Meanwhile for the southern population, spawning starts as early as April with an increase in spawning individuals till July as the water temperature is higher by 5 °C than the northern area at that time while in August spawning showed a sharp decrease. By the end of the year most of the two populations were in the spent condition with few individuals exhibiting signs of new development. This comes in agreement with the findings of Hwang (2007) on *P. margaritifera* in Taiwan waters which confirmed that temperature may play an important role in oyster maturity and spawning.

The same results as the present study were also achieved by Crossland (1957) through his study on *P. margaritifera* in the Sudanese Dongonab Bay, the Red Sea, who suggested that spawning took place between July to October with gonadal development from March to June. Nicholls (1931), Galtsaff (1933), Crossland (1957), Tranter (1958, d) and Bullivant (1962) considered that the temperature (26 – 28 °C) was the spawning inducer in *P. margaritifera*.; considering the biogeographical distribution of the species of Pinctada being between 30 ° north and south of the equator (Galtsoff, 1933; Tranter, 1958 d). According to Khamdan (1993) it seems that the temperature is more likely to be the controlling factor for spawning at least for the summer peak.

Also in the present study the latitudinal variations between Hurghada area (lat. 27°) and Shalatein area (lat. 22°) may produce such variations in reproductive strategy of *P. margaritifera*. This was also suggested by O'Connor (2002) and supported the previous observations of latitudinal changes in the reproductive behaviour of pearl oyster in which populations at higher latitudes have truncated breading seasons that tend to occur in the warmer months. Also, Rosa *et al* (1990) considered variations in reproductive cycle to be due to the temperature range in different locations as influenced by latitudinal position.

References

Abdel Razek F.A.; Mohamed M. Abou Zaid and El Sayed A.E. Hamed: 2010, Abundance and ecological observations of the Black-lip pearl Oyester, *Pinctada margaritifera* (L) (Bivalvia: Pteriidae), in Red Sea Egyptian Waters (in press).

Abou Zied, M.M.A.: 1991, Biological studies on some bivalves from the Suez Canal. *Ph.D.* thesis, faculty of Science, Al-Azhar University.

Bullivant, J.S**.** (1962): Direct observation of spawning in the blacklip pearl shell oyster (*Pinctada margaritifera*) and the thorny oyster *(Spondylus* sp.). Nature 193(4816): 700-701.

Crossland, C.: 1957, The cultivation of the mother of pearl oyster in the Red Sea. *Australian Journal of Marine and Freshwater Research*, 8: 111-130.

El-Eisawy, A.M.: 1974, Spawning and larval development of the Red Sea oyster, *Crassostrea forskali* chemnita. *Bulletin Institute of Oceanography & fisheries,* A.R.E., 4: 204-219.

El-Sayed, A.A.S. Hamed: 2009, Biological and ecological studies on some pyster species from the coast of the Egyptian Red Sea. M.Sc. Theis, Faculty of Science, Al-Azhar University.

Gabal, M.N.A.: 1982, Feeding and food of pearl oyster of the Red Sea; *Pinctada margaritifera* and *Pinctada vulgaris*. M.Sc. Thesis, Faculty of Science, Ain Shams University.

Galtsoff, P.S.: 1933, Pearl and Hermes Reef, Hawaii hydrographical and biological observations. *Bernice P. Bishop Museum Bulletin* no. 107:49pp.

GEF.: 1998, Development of coastal and marine resources of the Red Sea, World Bank, GEF, Project, Report No. 5. The Marine Protected Areas. 322pp.

Giese, A.C. and Pears, J.S.: 1979, Reproduction of marine invertebrate, Vol. V, Academic press, new York.

Hesselman, D.M., Barber, B.J. and Blake, N.J.: 1989, The reproductive cycle of adult hard clams, Mercenaria spp. In the Indian River Lagoon, Florida. *Journal of Shellfish Reearch.* 8: 43– 49.

Hwang, J.J.: 2007, Reproductive cycle of the pearl oysters, Pinctada fucata (Gould) and Pinctada margaritifera (Linn) (Bivalve:Pteriidae) in Southwestern Taiwan waters. *Journal of Marine Science and Technology*, Vol. 15 (2): 67-75.

Khamdan, S.A.A.: 1993, The reproduction, larval rearing and production of triploid embryos and spot of the pearl oyster, *pinctada radiate*. In: The proceeding of second Arab conference on perspective of modern biotechnology, 24-28 April 1993. Amman Jordan. Biodynamic international, San Diego, California USA., pp. 190-207.

Mohammed, T.A. and Yassien, M.H.: 2008, Bivalve assemblages on living coral species in the northern Red Sea, Egypt. *Journal of Shellfish Research*. 27 (5): 1217-1223.

Nicholls, A.G.: 1931, On the breeding and growth rate of the black lip pearl oyster (*Pinctada margaritifera*). *Rep. Gt. Barrier Reef Gmm.,* 2: 26-31.

O'Connor, W.A.: 2002, Latitudinal variation in reproductive behavior in the pearl oyster, Pinctada albina sugillata Aquaculture 209: 333–345.

Rose, R.A., Dybdahl, R. and Harden, S.: 1990, Reproductive cycle of the Western Australian silver lip pearl oyster, *Pinctada maxima* (Jameson) (Mollusca: Pteriidae). d. *Journal of Shellfish Reearch,* 9(2): 261-272

Tranter, D.J.: 1958a, Reproduction in Australian pearl oysters (Lamellibranchia). I. *Pinctada albina* (Lamarck): primary gonad development. *Australian Journal of Marine and Freshwater Research* 9(1): 135-143.

Tranter, D.J.: 1958b, Repmduction in Australian pearl oysters (Lamellibranchia). 11. *Pinctada albina* (Lamarck): gametogenesis. *Australian Journal of Marine and Freshwater Research,* 9(1): 144-1 58.

Tranter, D.J.: 1958c, Reproduction in Australian pearl oysters (lamellibranchia) III. Pinctada albina (Lamarck): breeding season and sexuality. *Australian Journal of Marine and Freshwater Research. 9, 191– 216 Freshwat. Rea.* 9(4): 509-523.

Tranter, D.J.: 1958d, Reproduction in Australian pearl oysters (Lamellibranchia). IV. *Pinctada margaritifera (W. Aust. J. Mar.*

Wada, K.T.: 1984, Breeding study of the pearl oyster, *Pinctada fucata. Bull. Natl. Res. Inst. Aquaculture. (Japan)/Yoshokukenho.* 6: 79-157

Yassien, M.H.: 1998, Biological and ecological studies on the pearl oyster, Pinctada radiata (Mollusca, Lamellibranchia) from the Red Sea, with special reference to its tolerance to water pollution. PhD Thesis, Faculty of Science, Ain Shams University, 191 p.