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Reproduction and gonad development of gastropod *Thais carinifera* in Lake Timsah, Suez Canal, Egypt

Nancy A. Radwan[1], Samya H. Mohammad[2], Saad Z. Mohamed [3] and Ahmed E. Yaseen[4]

1-Department of Zoology, Faculty of Education, El Arish, Suez Canal University, Egypt

2- Department of Zoology, Faculty of Science, Port Said, Suez Canal University, Egypt

3- Department of marine Biology, Faculty of Sciences, Ismailia, Suez Canal University, Egypt

4- Department of Zoology, Faculty of Science, Suez, Suez Canal University, Egypt

ABSTRACT

Gonad development, reproductive cycle and sex ratio of *Thais carinifera* were studied through a histological investigation of its gonads. Samples of *Thais carinifera* were monthly collected during the period from February 2007 to May 2008, from Lake Timsah, Suez Canal, Egypt. Staging criteria for the gonad of male and female snails were described. The ovary and testis in both sexes were composed of a number of oogenic follicles and several spermatogenic follicles respectively. Reproductive cycle of *Thais carinifera* could be categorized into five stages: early active, late active, ripe, fully ripe and partially spawning phase (in males), and, early active, late active, ripe, spawned and spent phase (in females). Sex ratio departed from 1:1 and the males dominated the population.

Keywords: Gastropoda, *Thais carinifera*, reproductive cycle, imposex

INTRODUCTION

An organism's relationship to its environment can be indicated by its life history: the average growth rate, size at maturity, frequency of reproduction, and life expectancy of its population members (Spight *et al*., 1974). Life history patterns vary enormously from species to species and from population to another within the same species (Spight, 1972; Tinkle & Ballinger, 1972). Spight *et al*. (1974) concluded that these variations follow rules and when these rules are known, the life history parameters of most populations can be predicated and controlled. There are many methods used to determine the course of the annual reproductive cycle of marine invertebrates. These include the relative size of gonads, the appearance of ripe gametes in gonads, spawning, numbers of larvae and other body components have been used by different investigators to define the reproductive season (Giese, 1959). Reproduction of prosobranch gastropods

is more varied than any other group of molluscs. This is related to their wide range of structure and habitat (Fretter, 1984). Webber (1977) summarized the reproduction in three groups of prosobranch gastropods, where the gonad is widely situated on the surface of the digestive gland, located in the posterior spiral part of the shell.

Large number of vessels passing across the Suez Canal makes the waterway vulnerable to pollution. Since 1960, Tributyltin (TBT) and other organotin based compounds have successfully been used as effective biocides in antifouling marine paints for more than 2 decades (Hanafy, 1996). It was discovered that the organotin compounds have toxicological effects on prosobranch molluscs. Among these effects, imposex, the phenomenon whereby male sex characters are superimposed on females – has proved to be the most sensitive parameter to assess TBT pollution (Gibbs *et al*. 1987, Alzieu *et al*. 1991). Induction of imposex in gastropod snails by organotin has been reported world wide.

The present study aimed to identify maturity stages, spawning time in relation to the imposex phenomena that have been reported by Hanafy (1996) in the Suez Canal.

MATERIALS and **METHODS**

A total of 275 snails of different sizes (3.5-9.5 cm in shell height) were used for histological study. The snails were monthly collected from February 2007 to May 2008. They were kept alive during transportation to the laboratory. A vernier caliper to the nearest 0.1 mm was used to measure the height & width of the snails. After that, the snails were weighed to the nearest gram using an electric balance with accuracy of 0.01g. Then the shells were carefully broken using a hammer, and the soft parts were carefully removed from the shells using a small forceps. A sharp scalpel was used to dissect the gonad out of the body, since it was widely situated on the surface of the digestive gland and located in the posterior part in the shell. All individuals were preserved in Bouin's solution for 24 hours, dehydrated in a graded series of ethyl alcohol (70-100% with an hour for each change), cleared in terpineol for 2-5 days and embedded in paraffin wax blocks (three changes, each for half an hour). Serial sections were cut at 6μm, and stained with Hematoxylin and Eosin (H & E) and mounted by Canada balsam. The histological preparations were examined under a light microscope at different magnifications from 10-100x. Photomicrographs were taken with a compact digital camera (Canon PC1200) fixed with axioster plus corl Zeiss microscope.

Determination of the gonadal phases was based on the histological preparations, external appearance of the gonads and the percentage occurrence of mature follicles, as more than two phases occurred simultaneously within the gonad especially in male snails. Monthly percentages of maturation phases were calculated and were graphed for males and females separately.

RESULTS

Position and external feature of the gonad

Thais carinifera possessing a large sex gland, where the snail sex can be identified. The gonad was lightly located over the digestive gland. The gonad sizes varied according to its different phases. The external color of ovary was white or creamy while that of the testis was orange or yellow so the color of the gonad was used to differentiate the snails sexes. From both sexes, ripe eggs and sperms could be readily obtained by gentle scratching over their gonads.

Gametogenic phases

Histological sections of gonads of both male and female gastropod were used to follow and describe their reproductive cycle. Gametogenic phases of both sexes could be divided into five phases according to the gonad shape, size and color, percentage of testicular and ovarian follicle fullness, follicle shape and the abundance of the connective tissue. The amount of the connective tissue between the follicles play an important role in the classification of the gametogenic phases as it declined with gonad's ripening in both sexes.

1-Male gonadal phases

The testis is composed of several spermatogenetic follicles. Spermatogenetic phases of *Thais carinifera* are present in figure (1). The stages of male reproductive cycle can be illustrated as follows:

A- Early active stage

The gonad has numerous distinct follicles; most of them are filled with spermatogonia and few spermatocytes. The connective tissue inbetween the follicles appears by enormous amount (Fig. 1A).

B- Late active stage

The gonad follicles increased in number with decreasing the amount of connective between them. At this stage, spermatogonia and spermatocytes are found in addition to few spermatides that appeared with dark color near the center (Fig. 1B).

C- Ripe phase

The gonad becomes larger, while the follicles increase in size and become elongated. In addition to the previous germ cells. Sperms appeared and occupied the lumen of the follicles (Fig. 1C).

D- Fully ripe phase

The testes have numerous elongated lobulated follicles with large lumen. Countless sperms filled the majority of the lumen with tails oriented towards the lumen. The outer layers and the follicular wall of the testis decline in thickness (Fig. 1D).

E- Partially spawned phase

At this stage, the gonad follicles become very large and more lobulated. Males have partially empty follicles due to discharge of some sperms. Spermatogenetic cells in various developmental stages were also visible in the follicles (Fig. 1E).

2-Female gonadal phases

Due to the presence of imposex phenomena observed frequently in this gastropod, the degenerated ova were found in the lumen of the follicles at all stages. So, determination of maturity stages was based on the follicular wall and the appearance of various germ cells. Reproductive cycle of female *Thais carinifera* is illustrated in figure (2).

A-Early active stage

The gonad is white in color and small in size, containing large amount of connective tissue and a number of oogenetic follicles inbetween them.

Each follicle contains oogonia and a few early oocytes that bud from the follicular wall (Fig. 2A).

B- Late active stage

The follicles become larger with little amount of connective tissue between follicles. The follicles contain oogonia, early oocytes and few growing oocytes. Degenerated ova were also found in the follicles lumen (Fig. 2B).

C- Ripe phase

The gonad becomes larger than the previous phases with larger follicles. Ripe ova appeared in the lumen (Fig. 2C).

D- Spawning phase

The follicle lumen was completely filled with ripe ova, where most of them were degenerated. At this time, the follicles wall become very thin and some vacuoles appeared due to partial spawning (Fig. 2D).

E- Spent phase

In spite of the large size of the ovary at this phase, its rigidity is less than the former phase. At this phase, ripe oocytes in the oogenic follicles were discharged into the surrounding environment and a few degenerate ova remained in the follicles. The lumen of the follicle becomes considerably empty. The wall of some follicles is teared off in the late stage (Figs. E,F).

Reproductive cycle of *Thais carinifera*

1- Males

The early active stage of males was recorded in February 2007, March and February 2008. The percentage of the population comprising late active phases of males was low in March (13%) and then recorded the highest peak in April (71%). Ripe males were recorded in most months except in June, July & November, 2007, and January and May, 2008. Fully ripe males were found in all months except in July, 2007. Partially spawning males were found in May to August and in October to December, 2007 and January – May, 2008 period. All

male populations have partially spawned in July 2007, (Fig.3A).

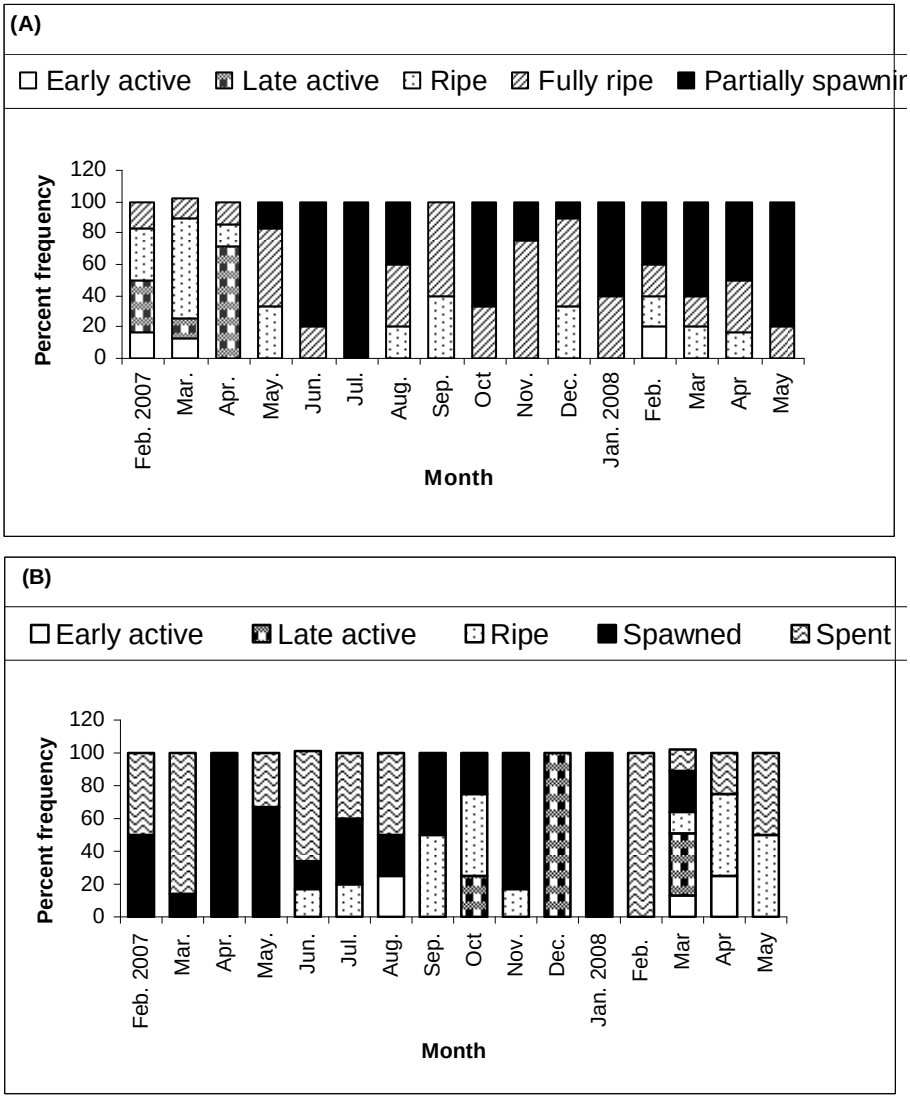

Figure (3). Monthly variations in gonad developmental stages of males (A) and females (B) of *Thais carinifera*.

2- Females

Early active stage was detected in August, 2007 and March & April, 2008. All females sampled in December, 2007 were found in late active stage. This stage was also detected in October, 2007 with low percentage (25%). Ripe phase was recorded in June, July, and September – November 2007 period and

in March - May 2008 period. Spent females had two peaks in winter. The first was in March 2007 (86%) and the other in February 2008 (100%). This stage was not recorded during autumn and early of winter (Fig. 3B).

However, no synchronization was recorded between male and female snails during early and active phases. A simple synchronization between both sexes was recorded during September 2007 in the ripe phase, and increased with increasing gonad ripening in the following phases (fully ripe, spawning and partially spawning) (Fig. 4).

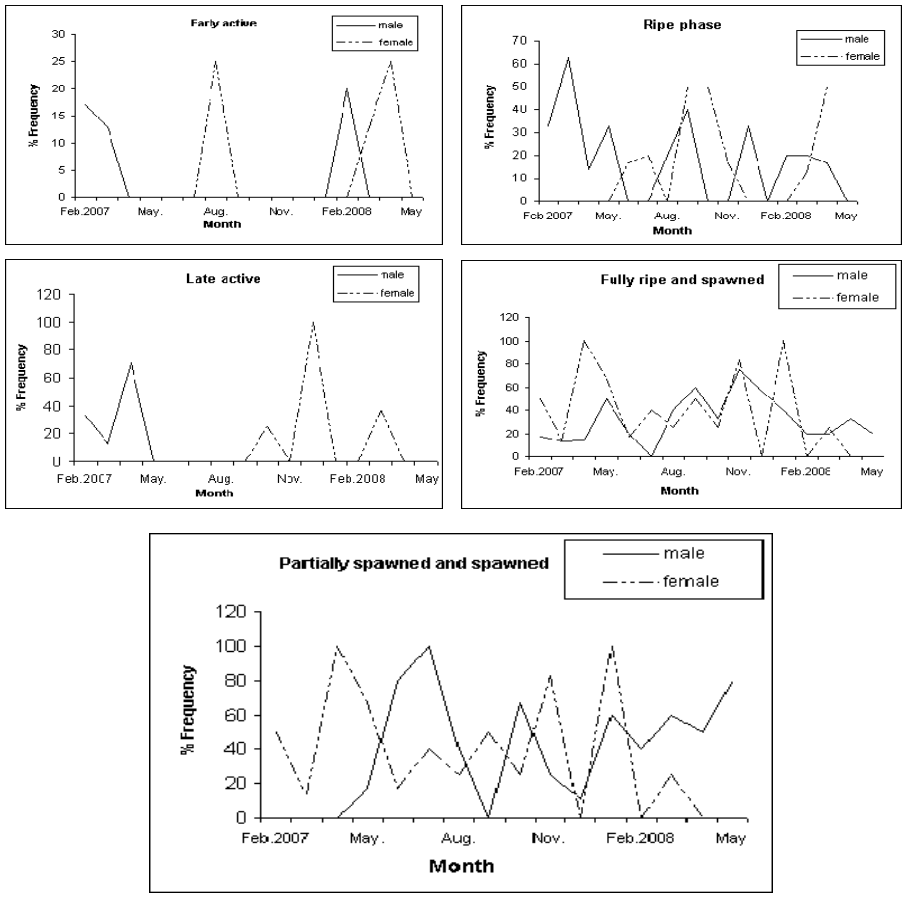

Figure (4). Synchronization in the percentage frequency of male and female follicles in *Thais carinifera* for different maturity stages.

Sex ratio

Figure (5) shows a dominance of the size class 5.6-6 cm. The percentage of the males reached 56.23% and the females formed the rest of the total individuals. Males with size class 5.1-5.5 and 5.6-6 cm contributed 14.96% and

20.50% of the population respectively (Table, 1). On the other hand, the percentages of females were 13.57% and 11.63% for class intervals 5.6-6 and 6.1- 6.5 respectively. No females were detected in the smallest size classes 3.5- 4 cm and the largest sizes 8.5-9 cm in shell length, whilst males were only recorded in these class intervals.

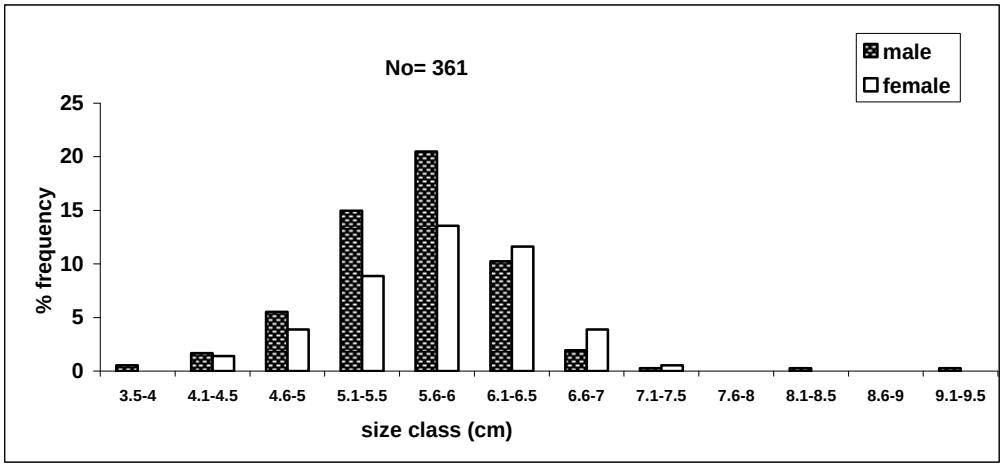

Figure (5). Size frequency distribution of male and female *Thais carinifera*.

Table (1). Numbers of males and females and percentages recorded in each size interval of *Thais carinifera* collected from Lake Timsah.

Size class	**Lake Timsah**			
(Cm)	Males	Percentages	Females	Percentages
3.5-4	2	0.55%	0	0
4.1-4.5	6	1.66%	5	1.39%
4.6-5	20	5.54%	14	3.88%
5.1-5.5	54	14.96%	32	8.86%
5.6-6	74	20.50%	49	13.57%
6.1-6.5	37	10.25%	42	11.63%
6.6-7	7	1.94%	14	3.88%
7.1-7.5	1	0.28%	2	0.55%
7.6-8	0	0.00%	0	0.00%
8.1-8.5	1	0.28%	0	0.00%
8.6-9	0	0.00%	0	0.00%
9.1-9.5	1	0.28%	0	0.00%

DISCUSSION

Documentation of the reproductive cycle in a fishery is one logical step to determine when recruitment might occur (Malachowski, 1988). Most marine invertebrates have their own unique breeding patterns. Boolootian *et al.* (1962)

stated that their breeding habits could be divided into three types: year round breeds, summer breeds, and winter breeds.

Histological examination of the present gastropod gonad provided an accurate description of its structure reproductive cycle. It could be divided into five phases, early active, late active, ripe, fully ripe and partially spawning phases in males and early active, late active, ripe, spawning and spent phases in females.

The snail *Thais carinifera* is characterized by its continuous spawning, as pronounced in most months without a defined pattern. The continuous reproduction had a direct relationship with the food abundance (Sutherland, 1970). Mohammad (2002) reported the availability of *Thais carinifera* food (bivalves) in Lake Timsah throughout the year, though they can feed on barnacles.

In the present study the highest percent of spawning activity was coincident with the periods of spring and summer in males and the periods of winter, spring and autumn in females. A similar finding was reported by Cronin (2000) who stated that the peaks in maturity and spawning of *Melarhaphe neritoides* were coincident with the period of winter storms. Several authors demonstrated that gonadal development, gametogenesis, reproductive cycle and spawning in molluscs are correlated with other exogenous environmental factors such as specific gravity of seawater (Taki, 1949), photoperiodism (Simpson, 1982), abundance of food for adults and their planktotrophic larvae (Jaramillo and Navarro, 1995), an endogenous factor, that is, the endocrine system (Euler and Heller, 1963).

The length of the reproductive cycle may depend upon geographical location (Cronin, 2000). Little is known about the mechanism that regulates the yearly reproductive cycles in Prosobranchia, but it has been suggested in early studies that these cycles are influenced by temperature. Temperature plays an important role in spawning (Fretter and Graham, 1962). Differences are often attributed to local climatic conditions (Lee, 2001), water temperature (Kim *et al*., 1999 and Lee, 1999).

The histological investigations of the present study revealed the presence of degenerated ova by large amount in all phases in females. This may be attributed to the imposex phenomena. It thus appears that the majority of neogastropods are susceptible to imposex (Bright & Ellis, 1990). However, very clear evidence has been provided for induction of imposex by tributyltin (TBT) in many neogastropod populations of surrounding areas of boating activity that have been shown to have the highest degrees of imposex, such as *Nucella lapillus* (Anon, 1986; Bryan *et al*., 1986, 1988 and Spence, 1989), *Urosalpinx cinera* (Gibbs *et al*., 1991), *Thais haemastoma* (Spence *et al*., 1990), *Thais carinifera* (Hanafy, 1996), *Thais orbita*, (Stewart *et al*., 1992, Foale, 1993). Suez Canal is extremely exposed to such contamination due to the intensive shipping activity (EEAA, 2006).

Hanafy (1996) stated that all female specimens of *Thais carinifera* collected from the Suez Canal exhibited the late stages of imposex (large penis, blockage of genital pore, sterility), and 60% of the Mediterranean female specimens manifested early stages of imposex. So, he attributed the decline in the Suez Canal's populations of *Thais carinifera* "and the absence of juveniles to the recruitment failure resulting from TBT pollution and the female sterility. The present study agreed with him in the first interpretation, about the continuous spawning of females *Thais carinifera* along the study period, while the imposex affected the amount of spawned ova leading to the decline in the population.

The sex ratio of *Thais carinifera* was 1.2 male: 1 female in the present study. Males dominated females in most months and size groups, except at the size intervals of, 6.6-7 & 7.1-7.5, where the reverse was obtained. The departure of sex ratio from equality (4.97:1 & 3.82: 1) was previously mentioned (Hanafy, 1996) on the same species at two sites in Suez Canal. In addition he also recorded a tendency of higher proportion of females than males in the largest size of the Mediterranean Sea population, and in the smallest size in Suez Canal one. In many prosobranchs, predominance of females in large size groups is well known (Abbott, 1960; Paine, 1969; Feare, 1970; Fotheringham, 1971 and Hanafy, 1993). It may be proposed that females could withstand environmental hazards than males in a restricted size. Accordingly, the non-representation of female individuals in the smallest and largest sizes may be attributed to the sensitivity of females to water pollution than males in the smallest size. Females suffer from spawning in the largest size that exhausted them and led to their mortality.

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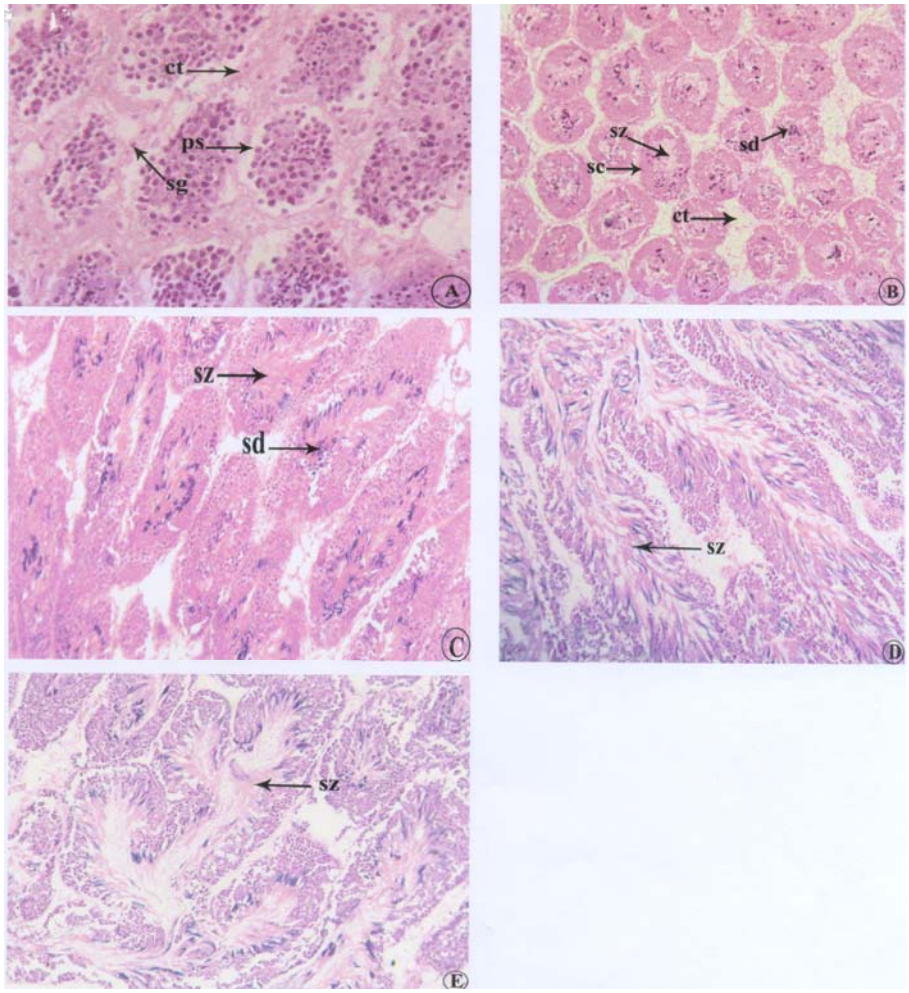

Figure (1). Photomicrogarphs of gonadal phases of male *Thais carinifera.* A, Transever sectiolJ of the spermatogenic follicles in the Early active stage. B, Late active stage. C, Ripe phase D, Fully ripe phase. E, Partially spawned phase. Sg, spermatogonia; Ps, prima'l spermatocyte; Sc, secondary spermatocyte; Sd , spermatid; Sz , spermatozoa; Ctconnective tissue. (200X)

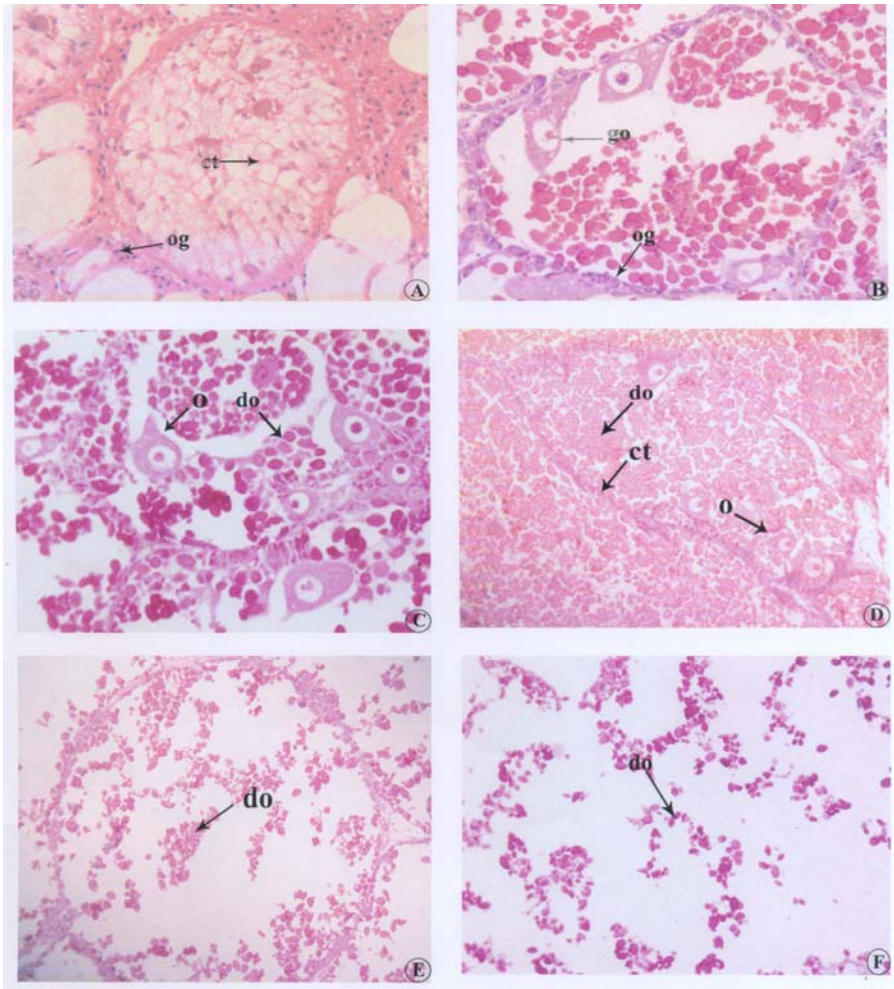

Figure (2). Photomicrogarphs of gonadal phases of female *Thais carinifera.* A, Transever section of the oogenic follicles in the Early active stage. B, Late active stage. C, Ripe phase. D, spawning phase. E, Partially spent phase. F, Spent phase. og , Oogonia ; go, growing oocyte; o , ripe ova; do , degenerated ova; Ct , connective tissue. (200 X)