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REPRODUCTIVE CYCLE, GAMETOGENESIS AND EMBRYONIC DEVELOPMENT OF *NITIA TERETIUSCULA* (BIVALVIA: UNIONIDAE), FROM THE RIVER NILE, AT SOHAG GOVERNORATE, EGYPT

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ABSTRACT

The present study dealt with the description of the reproductive and gametogenic cycles and the embryonic development of the freshwater mussel *Nitia teretiuscula* from the River Nile. Macro and micro-anatomy showed that the present species is dioceous and gonadosomatic indices showed that the species is semiannual and had two gonadal growth periods, a short period of gonadal growth from January to March and a long period from April to August. Visceral indices revealed a reciprocal relationship with the gonadosomatic indices of both the two sexes. The recorded sex ratio of females to males was (1.5: 1). The oogenesis displayed five developmental stages; oogonia, previtellogenic oocytes, vitellogenic oocytes, late vitellogenic oocytes and mature ova. Also, spermatogenesis illustrated five developmental stages: spermatogonia, primary spermatocytes, and secondary spermatocytes, spermatids; which clustered to form sperm morula and finally metamorphosed to spermatozoa. Within the gills of females (marsupia), brooding of embryos was occurred till the formation of glochidia larvae. The unfertilized eggs were released from the gonads with distinct germinal vesicle. The fertilized eggs had two polar bodies and a fertilization cone. Successive cleavages were observed. Spiral cleavage, non-ciliated blastula and D-shaped larvae were recorded.

Key Words: *Nitia teretiuscula* - River Nile - Reproductive cycle - Sex ratio - Gametogenesis - Embryonic development.

INTRODUCTION

Family Unionidae is a widely distributed bivalve group of freshwater mussels. Members of these mussels have great importance to human as a source of pearl (Chumnanpuen *et al.*, 2011). They have a great role in the food web and highly influence on the multiple trophic levels (Vaughn *et al.*, 2008). Moreover, they constitute a biofiltration system in removing phosphorus containing materials from agricultural waste water streams (Riley, 2008). Furthermore, they used as a biocontrol system in salmon farming eutrophication (Soto and Mena, 1999) and in fish nutrition as feeding attractants (Myers and Tlusty, 2009). The gonadosomatic indices are good methods in molluscan studies as they give good expression to their gonadal activity and development (Mottet, 1979; Lubet, 1983; Wolff, 1988).

Most species of Unionidae have a complex life cycle that starts with releasing eggs from the ovaries of females to the suprabranchial chamber

(Watters and O'Dee, 1998). Sperms reach the mantle cavity where the fertilization occurs then the females incubate their embryos till the formation of larval stage in their gill chambers (marsupia) (Fritts *et al.,* 2013). Glochidia larvae are released and attach to their hosts which are usually fishes until they metamorphose to the juvenile stage (Chumnanpuen *et al.,* 2011; Fritts *et al.,* 2013). Some studies have been reported concerning gametogenesis in unionids (Grande *et al.,* 2001; Park and Chung, 2004). Embryonic development has been described in few species of fresh water mussels; *Hyridella depressa* and *Anodonta anatina* (Pekkarinen and Englund, 1995).

In spite of the wide distribution of Unionidae in the River Nile, no previous studies have been done on the reproductive and gametogenic cycles and the embryonic development of *Nitia teretiuscula*. Therefore, the present study aims to investigate these items in this species at Sohag Governorate, Egypt.

MATERIAS AND METHODS

Sampling

Specimens of *N. teretiuscula* were collected monthly from early morning to late afternoon from the Western Bank of the River Nile at Bani-Helal Village, EL-Maragha city (26° 43′ 30″N and 31° 35′ 42″ E) about 25 Km north of Sohag Governorate (Fig. 1) from January, 2014 to December, 2015.

The specimens were transported to the laboratory in plastic containers containing water and soil from the site of collection. Specimens were kept in an aquaria (35x60x60 cm) provided with continuous mechanical aeration and water was changed by dechlorinated water; pH (7.8) at room temperature. Each breeding specimen was put in a separate small glass aquarium (15x10x10 cm). For investigation of the gonadosomatic and visceral indices nearly 30 individuals were collected, dissected and the gonads and viscera were separated and dried in foil papers at 60°C for 72 hours, weighing, then the indices were calculated using the equation adapted by Wolff (1988) as follows: gonadosomatic index (GSI) = (gonad dry weight/total body dry weight without the shell) X 100 and visceral index (VSI) = (viscera dry weight/total body dry weight without the shell) X 100. Sex ratio was assured by using smear preparation and the color of the gonads. Numbers of egg masses were observed in the aquaria, collected and counted. Egg masses were examined and different embryonic stages were photographed using Optika Microscope every hour. The adult specimens were identified by Dr. Kevin S. Cummings Illinois Natural History Survey, USA and the mussel project web site http://mussel-project.uwsp.edu/.

Histological studies

For histological studies fresh specimens were used. The male and female gonads were separated and fixed in 5% formalin, dehydrated in graded series of ethanol. Then they embedded in paraffin, sectioned at 5-7 µm and stained with haematoxylin and eosin for microscopical examination.

Scanning electron microscopy

Gills of adult females were separated and the D-shaped larvae were picked from the gills by using a fine hair camel brush. Larvae were fixed in 4% glutraldehyde, washed with cacodylate buffer, post fixed with 1% osmium tetroxide and dehydrated in graded series of ethyl alcohol. The specimens were dried and coated with gold for examination under JSM 5400 LV scanning electron microscope (SEM) at Faculty of science, Assiut University.

RESULTS

Reproductive cycle

The reproductive cycles of males and females of *N. teretiuscula* are presented as GSI in Fig. 2 and Table 1 during the period from January, 2014 to December, 2015**.** Generally, the GSI were slightly higher in the months of 2014 (nearly about 30%) than those of 2015 (nearly about 25%). The male GSI were, to some extent, higher than those of female during the study period, except in some, months (January, 2014 and 2015 and October, 2014 and 2015). The highest values of male GSI were in March and August 2014, (35.5% & 38.5%) and in November and December, 2015 (36.45% and 32.57%), (Fig. 2 and Table 1). The lowest values of the male GSI were in January and October in the two studied years 2014 and 2015 with values of 19.5% and 24.5% and 15.2% and 18.8%, respectively. The male gonadal growth showed two phenomena in both 2014 and 2015. A short period gonadal growth from January to March and a long period from April to August in the two studied years. The drop of the male GSI during the first period was rapid and takes one month only while that of the long period; decreased gradually. Also, the best recorded phenomenon was that: the male gonadal growth in the short period was very rapid and increased from 19.5% to 35.5% in 2014 and from 15.2% to 30% in 2015 with nearly 15% variations. The male GSI indices in the long period (April to August, 2014 and 2015) increased from 24% to 38.5% and from 27.4% to 30% with variations 9.5% and 6.6%, respectively (Fig. 2 and Table 1).

The female GSI presented reciprocal pattern with the male ones during the period January - August, 2014 and nearly parallel pattern after that. The lowest values of female GSI were obtained during August and October, 2014 and reaching 26% and 27%, respectively and July and October, 2015; with a percent of 21.27% and 20.87%, respectively. The highest values were recorded during November and December, 2014; reaching 32% and 31.5%, respectively and February and November, 2015; with a percent of 35.5% and 36%, respectively (Fig. 2 and Table 1). The variations in female GSI between the highest and lowest values were: 2% and 6% in 2014 and 14.23% and 15.3 % in 2015. The shape of GSI presentation displayed two drops in February and August in 2014 and 2015. It is also worth to mention that the peaks of males GSI were one month earlier in 2014 but in 2015 they were synchronous.

Monthly changes in the visceral indices

Monthly changes in the VSI are shown in Fig. 3 and Table 2. The VSI of males and females are parallel to each other with two peaks and two bottoms in each of the two studied years, 2014 and 2015. The highest values of the male VSI were in November and September, 2014 and June and February, 2015. The values were 27.7% & 25.5% and 23% & 21%, respectively. The lowest values of the male VSI were in January and December in 2014 and March and October, 2015 with values 16% & 14.8% and 11.19% & 12.6%, respectively. However, the two lower limits of the female VSI were in February and March 2014 (15% & 15.1%) and in September and October, 2015 (11.54% & 11.8%), respectively. The highest values of female VSI were in November and May, 2014 (27.1% & 24.43%) and June and July 2015 (21.75% & 18.16%), respectively. The most interesting phenomenon was the reciprocal relationship between the GSI and VSI of both sexes.

Sex ratio

A total of 609 individuals were examined during the study period from January, 2014 to December, 2015 (Fig. 4 and Table 3). A total of 383 females and 246 of males were clearly identified with 1.5:1 estimated sex ratio. A total of 335 individuals (181 females and 154 males with 1.17:1 sex ratio) were clearly identified during the months of 2014, while in months of 2015 a total of 274 individuals were identified of them one hundred and eighty two females and 92 males with a sex ratio of 1.97:1 (Fig. 4 and Table 3). Statistically, the variation in the density of females and males was biased towards females ($F=8.65.14$, $P=0.005$).

Gametogenesis

The present study showed that the species is dioecious, both male and female gonads are large, creamy in color, located in the anterior-dorsal part of the body cavity and closely associated with the brownish digestive gland, (Fig. 5A). Histologically, both the female and male gonads consist of numerous oval or rounded follicles, in which connective tissue and haemocoel spaces are distributed (Figs. 6A & 7 A).

Gonadal structure showed that, oogenesis can be classified into five stages: oogonia (Fig. 6B&C), previtellogenic oocytes (early primary oocyte), (Fig. 6B), vitellogenic oocytes (late primary oocyte), (Fig. 6C&D), late vitellogenic oocytes (secondary oocytes), (Fig. 6E) and mature oocytes (Fig. 6A&F). Oogonia are spherical in shape and ranged between 3-10.29 µm in diameter (Fig. 6B&C). They developed to give previtellogenic oocytes that ranged between 27 - 60 µm in diameter. At this stage, the nucleus (germinal vesicle) increased in size and the cytoplasm was markedly stained with eosin (Fig. 6B). The vitellogenic oocytes are found at the center of lumina of the follicles and are characterized by presence of nucleus at its center (Fig. 6C&D). Vitellogenic oocytes ranged between 63 to 93.7 µm in diameter. The nucleus of vitellogenic oocytes contains two unequal nucleoli. The late vitellogenic oocytes ranged between 99-113.5 µm

in diameter and are characterized by thick yolk granules and the nucleus shifted to the egg stalk (Fig. 6E). Mature oocytes are round in shape and reached about 121.5-149 µm in diameter. They are characterized by collapsing germinal vesicle and migration of the nucleus to the periphery of the oocyte (Fig. 6A&F). Each oocyte has an egg- stalk with which the wall of the follicle attaches till they are transported to the lumen for spawning.

Spermatogenesis can also be divided into five stages: spermatogonia (Fig. 7B), primary spermatocytes (Fig. 7B), secondary spermatocytes (Fig. 7C), spermatids (Fig. 7D) and spermatozoa (Fig. 7C- E). Spermatogonia are large, oval in shape, growing out of the follicle wall, their diameter ranged between 2-2.3 µm (Fig. 7B). These cells are divided mitotically to produce primary spermatocytes which are spherical in shape, with large homogenous nucleus and measure about 2.6-3.3 µm (Fig. 7B). The nuclear membrane and the nucleolus are not visible within the nuclei. Primary spermatocytes are divided to give secondary spermatocytes which are rounded in shape and measure about 1.06-1.75 µm (Fig. 7C). Secondary spermatocytes are developed to give spermatids. They are polyhedral, intensively stained and having homogenous nuclei (Fig. 7D). Clusters of early spermatids are aggregated to form sperm morula (Fig. 7F). The spermatids metamorphosed to spermatozoa which are strongly basophilic and are smaller than spermatids; its head length measures about 1.65 µm (Fig. 7C&E). Remarkably, mature spermatozoa are observed exiting from the male follicle through the genital duct (Fig. 7A).

Embryonic development

The unfertilized eggs were released from the gonads, they are spherical in shape with distinct germinal vesicle. Eggs adhered to one another and bound together forming egg masses. Each egg mass contains 162 to 300 egg (Fig. 5D) in different stages of development. The fertilized eggs have two obvious polar bodies, a fertilization membrane and fertilization cone (Fig. 8A&B). The first cleavage occurs rapidly and as a result two equal blastomeres are formed (Fig. 8C&D). The following cell division produces four large equal blastomeres (Fig. 8E). After the four cell stage, each blastomere divides asymmetrically giving four small and four large blastomeres that show the start of spiral movement (spiral cleavage) (Fig. 8F). Successive cleavages follow the spiral pattern and result in morula which developed into a non-ciliated blastula (Fig. 8G&J). Gastrula is formed by invagination (Fig. 8K). Many changes took place in the form of the embryos at this time to produce young glochidium (D-shaped larva) (Fig. 9A). The shell plate divides laterally with the formation of a hinge ligament on the dorsal midline which characterizes bivalve shells. From the ventral side, the marginal valves have blunt teeth and attachment threads that are observed coming out between the two valves. The larval valves are perforated with many pores (Fig. 9D) that seen from the outer surface.

Experimentally, it was very difficult to keep egg masses more than few days under observations, although ultimate care was done. So, embryos within the egg

masses were rapidly died. It was vigorously attacked by parasitic cilliophores. So, it was difficult to get precised data in regard to the time of development.

Brooding

Glochidia larvae are brooded in the four demibranches of the female (marsupia). The brooded embryos were observed from the fertilized eggs till mature glochidia. Macroscopic examinations showed that the colour of the female gills was changed from creamy at the start of incubation of embryos to yellow-brown before the exit of glochidia, (Fig. 5B&C). The D-shaped larvae contain two valves attached by straight hinge ligament dorsally (Fig. 9A). Also, attachment threads were coming out between valves that have numerous marginal teeth and countless surface pores (Fig. 9B&D).

DISCUSSION

Most freshwater mussels are dioecious (Aldridge, 1999; Çek and Şereflişan, 2006; Şereflişan, *et al.*, 2009&2013; Hinzmann *et al*., 2013; Hliwa *et al*., 2015). As for example, three species of genus *Unio* (*Unio terminalis delicates*, Unio *pictorum* and *Unio tumidus*) are dioecious. Beside that the *Sinanodonta woodiana* is also dioecious. As for genus *Anodonta*, the three studied species (*Anodonta gabillotia pseudodpsis*, *Anodonta anatina* and *Anodonta cygnea*) are also dioecious. The sexual state of the most common Nile species *Anodonta rubens* and *Unio prasedence* are not clearly evaluated. The present study indicated that one species of genus *Nitia*; *Nitia teretiuscula* in the River Nile is dioecious (see Table 4).

Longevity of the reproductive cycle varies greatly between species of freshwater mussels (Hinzmann *et al*., 2013; Hliwa *et al*., 2015). Some species displayed long reproductive period and single spawning season. *Unio terminalis delicates* (Çek and Şereflişan, 2006) and *Anodonta gabillotia pseudodpsis* have seven months reproductive period from May to November and a single spawning season (Şereflişan, *et al.*, 2009). *Anodonta cygnea* showed 5 months reproductive period from December to April (Aldridge, 1999) while that of *Anodonta anatina* was 4 months from December to March (Hinzmann *et al*., 2013). The short reproductive cycles were shown by three species, *Unio pictorum* and *Unio tumidus* nearly three months from May to July. The third species; *Sinanodonta woodiana* exhibited a long reproductive period nearly 8 months from March to October and a short one 2 months from March to April.

The present studied species *N. teretiuscula* displayed which are two reproductive periods similar to *Sinanodonta woodiana* (Hliwa *et al*., 2015). A long one from April to August (nearly 5 months) and a short one from January to March (nearly 3 months) (see Table 4). The long reproductive period is characterized by gradual gonadal growth and gradual spawning, while the later was rapid and has a short spawning period. Two peaks of gonadosomatic indices showed two seasons of spawning in this species, one in winter and the other in summer. So, this may suggest that water temperature, length of day and night and variation of water level in the Nile is the most important physical factors

affecting gonadal growth and spawning in this species and these suggestions require more intensive studies on the ecology of this species.

Sex ratio in most previously studied freshwater mussels was nearly 1.1: 1 for females: males as in *Unio terminalis delicates*, *Anodonta gabillotia*, *Unio tumidus* and *Sinanodonta* woodiana, (Aldridge, 1999; Çek and Şereflişan, 2006; Şereflişan, *et al.,* 2009; Hliwa *et al*., 2015) and 1: 1.2 in *Potomida littoralis* and *Anodonta* anatina. However, in *Unio pictorum* the sex ratio was 1.3: 1 females: males. The sex ratio of *Anodonta cygnea* varies from the aforementioned species and showed 1: 1.8 females: males ratio. As for the present studied species, the sex ratio was the highest between these species and recorded 1.5: 1 females: males or three females to 2 males and nearly 60% of the population are females and 40% are males (see Table 4). No specimen showed hermaphroditism, but androgamy or gynogamy has not followed in the present study.

The present study assured that oogenesis can be divided into five stages: oogonia, previtellogenic oocytes (early primary oocyte), vitellogenic oocytes (late primary oocyte), late vitellogenic oocyte (secondary oocytes) and mature oocytes which are similar to some other species (Aldridge, 1999; Çek and Şereflişan, 2006; Hinzmann *et al*., 2013; Şereflişan *et al.,* 2009&2013; Hliwa *et al*., 2015). Except some notes in their sizes, the mature oocyte was smaller than that recorded in *Unio terminalis delicates* (Çek and Şereflişan, 2006) and larger than that found in in *Anodonta anatina* and *Sinanodonta woodiana* (Hinzmann *et al*., 2013; Hliwa *et al*., 2015). Also, the vitellogenic oocytes had only two nucleoli while the other unionid species had many nucleoli as in *Unio terminalis delicates* (Çek and şereflişan, 2006), *Anodonta gabillotia* (Şereflişan *et al.,* 2009), *Anodonta anatina* (Hinzmann *et al.,* 2013) and *Sinanodonta woodiana* (Hliwa *et al.,* 2015). It seems likely to say that there is a positive relationship between the number of nuclei and the number of eggs produced in these species.

The morphology of spermatozoa of *N. teretiuscula* was similar to most freshwater unionid species. It characterized by a bullet-shaped head, uniflagellated tail as those recorded by Rocha and Azevedo (1990). The head length of *N. teretiuscula* spermatozoa was approximately 2.65 µm while it was 2.8 µm in *Truncilla trincata* (Waller and Lasee, 1997); 4.0 µm for *Anodonta grandis* (Lynn, 1994); 4.2 µm for *Prisodon alatus* (Matos *et al.,* 1998) and 4.3 µm for *Sinanodonta woodiana* (Hliwa *et al.*, 2015).

Spermatogenesis of *N. teretiuscula* revealed the presence of sperm morula, the role of this structure is still unknown. Some authors suggested that sperm morulae were an aggregation of spermatids that converted into mature sperms during certain environmental conditions (Matos *et al.,* 1998; Çek and Şereflişan, 2006, 2011; Şereflişan *et al.,* 2009&2013). The present study suggests that the morulae of spermatids may be an aggregation around food material during spermatogenesis since there are no Sertoli cells. According to some authors the presence of sperm morula is an evidence for abnormal spermatogenic pathway under certain condition (Hliwa *et al.*, 2015). However, typical spermatogenic

pathway occurred in the normal conditions (Hliwa *et al.*, 2015). Noteworthy, the present species follows the abnormal mode of spermatogenesis and Sertoli cells were not observed and this was agreed with that described in the Chinese pond mussel *Sinanodonta woodiana* by Hliwa *et al.* (2015). The two mentioned spermatogenic pathways were recorded in unionid *venustaconcha ellipsiformis* (Shepardson *et al.,* 2012).

Generally, Unionaceans are classified according to the position and organization of the brood pouches (Wu *et al.,* 1999). In the most primitive type in which incubation occurred in all the four demibranchs with lamellae separated by connective tissue. However, in the second type, brooding was restricted to the inner or outer demibranchs only or to particular portions within the demibranchs (Wu *et al.,* 1999).

Examination of gills of the present species illustrated that the embryos are incubated in all the four demibranchs leaving only a narrow strips in the anterior and posterior regions. This is in consistent with some other fresh water mussels where the embryos are found only in the inner demibranchs as in *Hyridella depressa* (Jupiter and Byrne, 1997), *Anodontites trapesialis* and *Monocondylaea minuana* (Silva-Souza *et al*., 2011). Whereas, in *Mycetopoda legumen* the marsupia occupied only the anterior half of the inner or outer demibranch (Mansur and Veiteinheimer- Mendes, 1979). The early embryonic development of the present species is quite similar to that described for some species belonging to genus *Unio* (Lillie, 1895).

D-shaped larvae of the present studied species *N. teretiuscula* are characterized by the presence of attachment threads. This finding was in accordance with some unionids like *Margaritifera margaritifera* (Wood, 1974); *Anodonta cataracta* (Rand and Willis, 1982); *Utterbackia imbecillis* (Schwartz and Dimock, 2001) and in the pearl mussel, *Hyriopsis (Hyriopsis) bialatus* (Chumnanpuen *et al*., 2011). In contrast, some other unioniods lack such threads e.g. *Anodonta implicate* (Rand and Willis, 1982). The investigated D- shaped larvae of the present species showed distinct pores on their outer surface of the shell and this agreed with that described in *Anodonta arcaeformis* (Wu *et al.,* 1999). These pores are suggested to be sites of nutrition or gaseous exchange as have been observed in *Pyganodon cataracta and Utterbackia imbecillis* (Rand and Wiles, 1982; Schwartz and Dimock, 2001). Within Unionidae, there were another five different described shapes of larvae; the wide triangular shape with pits on their surface which was recorded in *Unio douglasiae, Lanceolaria cylindrica* (Wu *et al*., 1999). The elongated triangular larvae are present in Anodontinae excluding the free living *Anodonta arcaeformis where it is semicircular in shape and has* pores on its surface (Wu *et al*., 1999). The semi elliptical larvae with pores and shallow pits on their surface as in *Anodonta woodiana, Anodonta pacifica, Anodonta angula, Hyriopsis cumingii* and *Lamprotula caveata* (Pekkarinen and Englund, 1995; Wu *et al*., 1999). The fifth

shape was the gourd-shape with shallow pits which are present on the larval surface of *Acuticosta aurorae* and *Acuticosta chinesis* (Wu *et al*., 1999).

Beside the pores on the outer surface of the larval shell in the present species, numerous teeth were present on the outer margins of the two valves. Also, the larval thread is not a single thread but a compile of threads observed coming out from the two valves which may suggest that, the larvae may be attached by them to any other body to achieve their dispersal.

As for as the present authors aware, the present investigation is considered as the first study concerning the reproductive cycle, gametogenesis and embryonic development of the fresh water mussel *Nitia teretuiscula* from the River Nile. Further studies are recommended to investigate the role of sperm morula.

REFERENCES

Aldridge, D. C. (1999). The morphology, growth and reproduction of Unionidae (Bivalvia) in a fenland waterway. J. Moll. Stud., **65(1):** 47-60.

Çek, Ş. and Şereflişan, H. (2006). Certain reproductive characteristics of the freshwater mussel *Unio terminalis delicates* (Lea, 1863) in Gölbaşı Lake, Turkey. Aquacult. Res., **37(13):** 1305-1315.

Çek, Ş. and Şereflişan, H. (2011). The gametogenic cycle of *Leguminaia whaetleyi* (Lea, 1862) in lake Gölbaşı, Turkey (Bivalvia: Unionidae). J. Exp. Zool. Ecol Genet. Physiol., **315(1):** 30-40.

Chumnanpuen, P.; Kovitvadhi, U., Chatchavalvanich, K.; Thongpan, A. and Kovitvadhi, S. (2011). Morphological development of glochidia in artificial media through early juvenile of freshwater pearl mussel, *Hyriopsis (Hyriopsis) bialatus Simpson*, 1900. Invertebr. Repr. Dev., **55(1):**40-52.

Fritts, M. W.; Fritts, A. K.; Carleton, S. A. and Bringolf, R. B. (2013). Shifts in stable-isotope signatures confirm parasitic relationship of freshwater mussel glochidia attached to host fish. J. Moll. Stud., **79(2):** 163-167.

Grande, C.; Araujo, R. and Ramos, M. A. (2001). The gonads of *Margaritifera auricularia* (Spengler, 1793) and *M. margaritifera* (Linnaeus, 1758) (Bivalvia: Unionoidea). J. Moll. Stud., **67(1):** 27-36.

Hinzmann, M.; Lopes-Lima, M.; Teixeira, A.; Varandas, S.; Sousa, R.; Lopes A.; Froufe, E. and Machado, J. (2013). Reproductive cycle and strategy of *Anodonta anatina* (L., 1758): Notes on hermaphroditism. J. Exp. Zool. A. Ecol Genet. Physiol., **319(7):** 378-390.

Hliwa, P.; Zdanowski, B.; Dietrich, G. J.; Andronowska, A.; Król, J. and Ciereszko, A. (2015). Temporal changes in gametogenesis of the invasive Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) from the Konin lakes system (Central Poland). Folia. Biologica., **63(3):** 175-185.

Jupiter, S. D. and Byrne, M. (1997). Light and scanning electron microscopy of the embryos and glochidia larvae of the Australian freshwater bivalve *Hyridella depressa* (Hyriidae). Invertebr. Reprod. Dev**., (32):**177-186.

Kat, P. W. (1984). Parasitism and the Unionacea (Bivalvia). Biol. Rev., **59(2):** 189-207.

Lillie, F. R. (1895). The embryology of the Unionidae. A study in cell-lineage. J. Morphology, **(10):**1-100.

Lubet, P. (1983). Experimental studies on the action of temperature on the reproductive activity of the mussel (*Mytilus edulis*). Malac. Soc. Lond., **(12)A:** 100-705.

Lynn, J. W. (1994). The ultrastructure of the sperm and motile spermatozeugmata released from the freshwater mussel *Anodonta grandis* (Mollusca, Bivalvia, Unionidae). Can. J. Zool., **72(8):** 1452-1461.

Mansur, M. C. and Veitenheimer-Mendes, I. L. (1979). Redesciçao de *Mycetopoda legumen* (Martens, 1888) (Bivalvia: Mycetopodidae). Rev. Bras. Biol., **(39):** 695–702.

Matos, E.; Corral, L. and Azevedo, C. (1998). Fine structure of spermiogenesis with special reference to the spermatid morula of the freshwater mussel *Prisodon alatus* (Bivalvia, Unionoidea). J. Morpholo., **238(1):** 63-70.

Mottet, M. G. (1979) A review of the fishery biology of scallops. Technical Report, Department of the fisheries. Washington, USA, PP. 39-97.

Myers, A. and Tlusty, M. F. (2009). A long-term assessment of the physiological effects of herring (*Clupea harengus*) as a dietary component of the American lobster (Homarus americanus). New Zeal. J. Mar. Fresh., **43(1):** 173-183.

Park, G. M. and Chung A. E. (2004). Histological studies on hermaphroditism, ametogenesis and cyclic changes in the structures of Marsupial gills of the introduced Asiatic clam, *Corbicula fluminea*, and the Korean clam, *Corbicula leana*. J. Shellfish Res., **3:**179-184.

Pekkarinen, M. and Englund, V. P. M. (1995). Description of unionacean glochidia in Finland, with a table aiding in their identification. Arch. Hydrobiol., **134(4):** 515-531.

Rand, T. G. and Wiles, M. (1982). Species differentiation of the glochidia of *Anodonta cataracta* Say, 1817 and *Anodonta implicata* Say, 1829 (Mollusca: Unionidae) by scanning electron microscopy. Can. J. Zool., **60(7):** 1722-1727.

Riley, L. W. (2008). Freshwater Clams as a Treatment Mechanism for Phosphorus in Agricultural Wastewater. PhD Thesis, University of Florida, USA.

Rocha, E. and Azevedo, C. (1990). Ultrastructural study of the spermatogenesis of *Anodonta cygnea* L. (Bivalvia, Unionidae).Int. J**.** Inver. Rep. Dev., **18(3):** 169-176.

Schwartz, M. L. and Dimock, R. V. (2001). Ultrastructural evidence for nutritional exchange between brooding unionid mussels and their glochidia larvae. Invertebr. Biol., **120(3):** 227-236.

Şereflişan, H.; Çek, Ş. and Şereflişan, M. (2013). The reproductive cycle of *Potomida littoralis* (Cuvier, 1798) (Bivalvia: Unionidae) in Lake Gölbaşi, Turkey. Pak. J. Zool., **45(5):** 1311-1319.

Şereflişan, H.; Menderes, S. and Soylu, S. (2009). Description of glochidia of three species of freshwater mussels (Unionidae) from southeastern Turkey. Malacologia, **51(1):** 165-172.

Shepardson, S. P.; Heard, W. H.; Breton, S. and Hoeh, W. R. (2012). Light and transmission electron microscopy of two spermatogenic pathways and unimorphic spermatozoa in *Venustaconcha ellipsiformis* (Conrad, 1836) (Bivalvia: Unionoida). Malacologia, **55(2):** 263-284.

Silva-Souza, A. T.; Guardia-Felipi, P. and Arrebola, N. R. (2011). Embryonic development of *Anodontites trapesialis* (Lamarck, 1819) (Bivalvia: Mycetopodidae). Braz. J. Biol., **71(1):** 139-144.

Soto, D. and Mena, G. (1999). Filter feeding by the freshwater mussel, *Diplodon chilensis*, as a biocontrol of salmon farming eutrophication. Aquaculture, **171(1):** 65-81.

Vaughn, C. C.; Nichols, S. J. and Spooner, D. E. (2008). Community and food web ecology of freshwater mussels. J. North. Am. Benthol. Soc., **27(2):** 409-423.

Waller, D. L. and Lasee, B. A. (1997). External morphology of spermatozoa and spermatozeugmata of the freshwater mussel *Truncilla truncata* (Mollusca: Bivalvia: Unionidae). Am. Midl. Nat., **138(1):** 220-223.

Watters, G. T. and O'Dee S. H. (1998). Glochidial release as a function of water temperature: beyond bradyticty and tachyticty. In: Proceedings of the Concervation, Captive Care and Propagation of Freshwater Mussels Symposium, pp. 135-140. Ohio, USA.

Wolff, M. (1988). Spawning and recruitment in the Peruvian scallop *Argopecten purpuratus*. Mar. Ecol. Prog. Ser., **(42):** 213-217.

Wood, E. M. (1974). Development and morphology of the glochidium larva of *Anodonta cygnea* (Mollusca: Bivalvia). J. Zool., **173(1):** 1-13.

Wu, X.-P.; Liang, Y.-L.; Wang, H.-Z. and Ouyang, S. (1999). Morphological characters of glochidia of Unionidae and the taxonomic significance. Acta Hydrobiologica Sinica, **23:** 139-147.

EXPLANATION OF FIGURES

Fig. (1): A map showing the location of studied site.

Fig. (2): Monthly changes in the gonadosomatic indices of male and female *Nitia teretiuscula* during the period from January, 2014 to December, 2015. Vertical bars show standard error.

Fig. (3): Monthly changes in the visceral indices of male and female *Nitia teretiuscula* during the period from January, 2014 to December, 2015. Vertical bars show standard error.

Fig. (4): Monthly changes in the sex ratio of males and females *Nitia teretiuscula* during the period from January, 2014 to December, 2015.

Fig. (5): Light microscopic photographs of dissected specimens of *Nitia teretiuscula* showing: (A) The gonad, the foot, the mantle and the shell. (B) Two preserved creamy gills with early embryonic developmental stages. (C) A yellow-brown gills with mature larvae. (D) Part of an egg mass containing embryos in different developmental stages. G: gonad, Gi: Gills, F: Foot, M: mantle, Sh: shell. Scale par 1.49 mm.

Fig. (6): Light microscopic photographs of female gonads of *Nitia teretiuscula* showing: (A) Overall view of ovarian follicles and mature oocyte. (B) Oogonia and previtellogenic oocytes. (C, D) Vittelogenic oocytes. (E) Late vittelogenic oocytes with egg stalk. (F) Mature oocytes. Ff: female follicle, Oo: oogonia, N: nucleus, Nu: nucleolus. Pvo: previtellogenic oocytes, Vo: vittelogenic oocytes, Lvo: late vittelogenic oocytes, Es: egg stalk and Mo: mature oocytes. Scale par: A = 50 µm, B-F = 10 µm. (Haematoxylin and Eosin stain).

Fig. (7): Light microscopic photographs of male gonads showing: (A) Overall view of testis follicle, genital duct and spermatozoa. (B) Enlarged part of testis showing spermatogonia, primary spermatocyte. (C) Secondary spermatocyte and spermatozoa. (D) Spermatids and spermatozoa. (E) spermatozoa. (F) Sperm morula. Tf: testis follicle, Gd: genital duct, Sz: spermatozoa, Sg: spermatogonia, Ps: primary spermatocyte, Ss: secondary spermatocyte, Sd: spermatids, Sm: morula. Scale par: A =10 µm; B-F = 5 µm (Haematoxylin and Eosin stain).

Fig. (8): Light microscopic photographs of the different embryonic developmental stages of *Nitia teretiuscula* showing: (A) Fertilized egg with fertilization cone. (B) Fertilized egg with polar body. (C, D) Two embryos in the beginning of first cleavage and the two cell stage (two similar blastomeres). (E) Embryo having four similar blastomeres. (F) Eight-blastomere stage with four large and four small blastomeres and beginning of spiral cleavage. (G)16-blastomere stage, embryo with clear spiral cleavage. (H) 32-blastomere stage. (I) Early blastula stage. (J) Late blastula stage. (K) D-shaped larva during gastrulation. Fc: fertilization cone, Pb: polar body. Scale par: A-J = 100 µm; K = 68 µm.

Fig. (9): Scanning electron micrographs of the D-shaped larvae of *Nitia teretiuscula* showing: (A) Straight hinge ligament. (B) The Two valves and larval threads. (C) The two valves, larval threads, shell dentation and growth lines. (D) Outer surface of the shell having numerous pores Hl: hinge ligament, V: valves, T: shell dentation, Lt: larval threads, GI: growth lines, P: numerous pores.

Table (1): Monthly changes (Mean ± SE) in gonadosomatic index (GSI) of *Nitia teretiuscula* during the period from January 2014 to December 2015.

Months	**Female**	**Male**
	gonadosomatic index **%**	gonadosomatic index **%**
Jan., 2014	30.0±1.42	19.5±6.35
Feb.	28.5±2.11	34.0±2.47
Mar.	29.5±1.41	35.5±3.53
Apr.	30.5±2.45	29.0±3.09
May	30.5±1.02	32.5±1.63
Jun.	30.0±1.06	33.5±4.33
Jul.	28.0±1.88	34.5±1.58
Aug.	26.0±2.56	38.5±2.75
Sep.	31.0±2.84	33.0±1.64
Oct.	27.0±2.21	24.5±2.18
Nov.	32.0±0.69	31.5±0.48
Dec.	31.5±1.25	34.0±2.07
Jan., 2015	26.0±1.76	15.23±4.33
Feb.	35.5± 1.5	27.61±2.01
Mar.	26.26±1.83	24.81±2.26
Apr.	26.26±1.56	27.42±1.89
May	30.18±1.42	27.0±3.00
Jun.	23.51±1.49	23.07±4.04
Jul.	21.27±1.69	24.6±7.07
Aug.	24.66±2.21	30.0±8.37
Sep.	24.0±5.59	24.3±4.00
Oct.	20.87±2.71	18.81±2.26
Nov.	36.0±1.59	36.45±1.17
Dec.	31.81±1.62	32.57±1.52

Table (2): Monthly changes (Mean ± SE) in visceral index (VSI) of *Nitia teretiuscula* during the period from January 2014 to December 2015.

	Female	**Male**
Months	**Visceral index**	**Visceral index**
Jan., 2014	15.0±0.36	16.0±1.32
Feb.	15.0±1.89	16.6±0.98
Mar.	15.1±1.67	17.2±1.43
Apr.	17.0±2.37	19.0±2.08
May	24.43±1.07	23.2±0.95
Jun.	22.0±0.89	21.8±1.56
Jul.	19.0±1.73	16.1±1.35
Aug.	15.84±0.82	16.6±4.04
Sept.	19.88±1.43	25.5±2.07
Oct	23.6±2.67	20.0±1.61
Nov.	27.1±0.67	27.7±0.75
Dec.	15.99±1.01	14.8±1.06
Jan., 2015	18.11±1.63	17.38±1.27
Feb.	18.0±0.82	21.0±1.03
Mar.	14.19±2.02	11.19±0.56
Apr.	14.82±1.01	16.18±0.91
May	14.5±1.53	15.87±1.28
Jun.	21.75±3.88	23.0±1.45
Jul.	18.16±1.16	18.74±1.09
Aug.	15.0±0.36	13.87±0.26
Sep.	11.54±1.43	13.07±0.36
Oct.	11.79±2.37	12.6±1.77
Nov.	14.3±1.12	16.46±1.06
Dec.	13.67±1.84	13.36±1.07

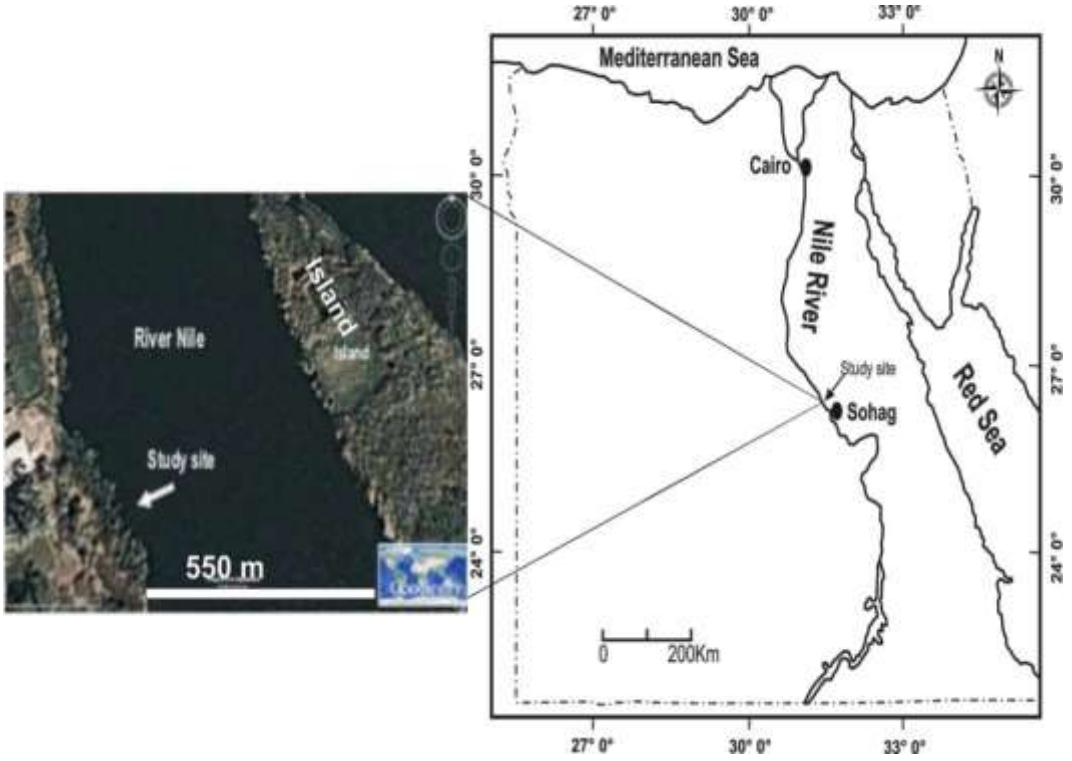
Table (3): Monthly changes in the number of females and males of *Nitia teretiuscula* and the calculated sex ratio during the period from January 2014 to December 2015.

	Female	**Male**	**Total**	**Sex ratios (Female: Male)**
Jan., 2014	6	3	9	2 : 1
Feb.	10	18	28	1:1.8
Mar.	19	11	30	1.7:1
Apr.	15	15	30	1 : 1
May	24	6	30	4 : 1
Jun.	18	12	30	1.5:1
Jul.	18	12	30	1.5:1
Aug.	11	19	30	1:1.7
Sep.	10	18	28	1:1.8
Oct.	13	17	30	1:1.3
Nov.	14	16	30	1:1.1
Dec.	23	7	30	3.3:1
Total 2014	181	154	335	1.17:1
Jan., 2015	18	12	30	1.5:1
Feb.	9	5	14	1.8:1
Mar.	19	7	26	2.7:1
Apr.	20	10	30	2 : 1
May	24	4	28	6 : 1
Jun.	22	3	25	7.3:1
Jul.	17	2	19	8.5:1
Aug.	13	3	16	4.3:1
Sep.	5	4	9	1.3:1
Oct.	11	7	18	1.6:1
Nov.	8	22	30	1:2.8
Dec.	16	13	29	1.2:1
Total 2015	182	92	274	1.97:1
Total	363	246	609	1.5:1

Table (4): Differences in sex, reproductive cycle and sex ratio in some freshwater mussel species and the present species.

Species	Sex	Reproductive cycle	Sex ratio	References
Unio terminalis delicates	Dioecious	Long reproductive cycle (long gametogenesis and spawning periods) from May to November approximately 7 months	1.1:1	Çek and Şereflişan, 2006
Anodonta gabillotia pseudodpsis	Mostly dioecious	Long reproductive cycle (long gametogenesis and spawning periods) from May to November approximately 7 months	1.1:1	Şereflişan *et al*., 2009
Potomida littoralis	Dioecious	Single large spawning event in June	1:1.2	Şereflişan *et al*., 2013
Anodonta anatina	Dioecious	Long reproductive cycle (long gametogenesis and spawning periods) from December to March approximately 4 months	1.2:1	Aldridge, 1999 & Hinzmann *et al*., 2013.
Anodonta cygnea	Dioecious	Long reproductive cycle (long gametogenesis and spawning periods) from December to April	1:1.8	Aldridge, 1999.
Unio pictorum	Dioecious	Short reproductive period from May to July	1.3:1	Aldridge, 1999.
Unio tumidus	Dioecious	Short reproductive period from May to July	1.1:1	Aldridge, 1999.
Sinanodonta woodiana	Dioecious	Spawning through March to October and higher reproductive activity in females from March to April	1.1:1	Hliwa *et al*., 2015.
Nitia teretiuscula (present species)	Dioecious	Semiannual and had two gonadal growth periods, a short period of gonadal growth from January to March and a long period from April to August	1.5:1	Present study

Fig. (1)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33° 0°
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Cairo
Nile River
Red Sea
Study site
Sohag
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27° 0°
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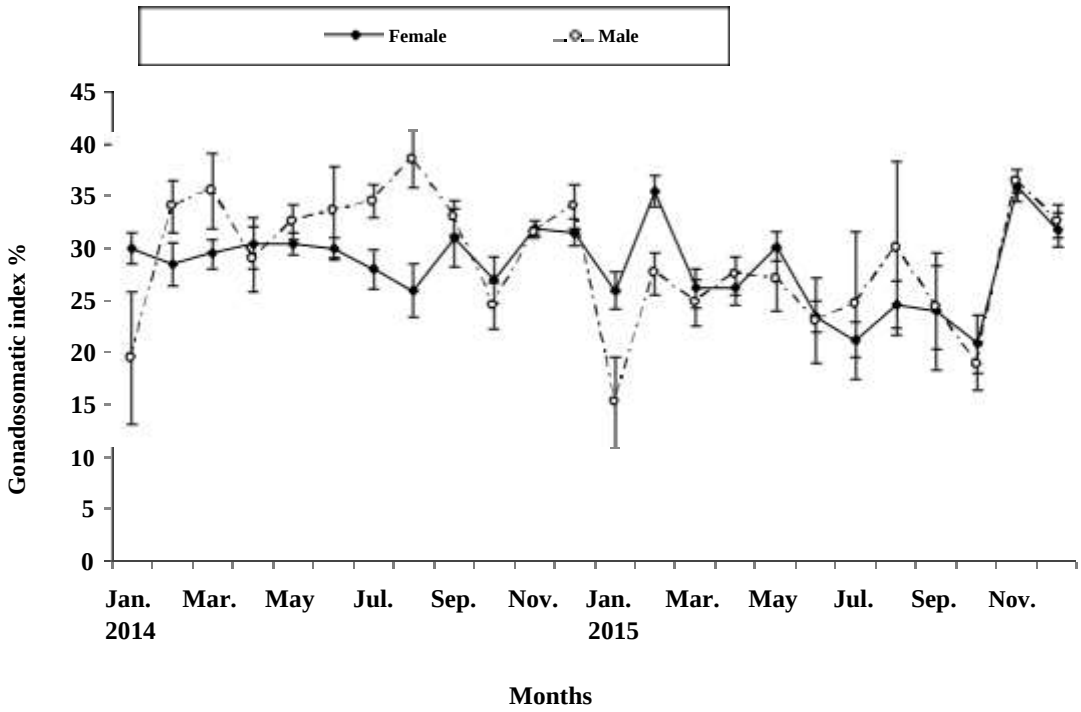
0
200Km
River Nile
Island
Study site
550 m

Fig. (2)


Female
Male
Gonadosomatic index %
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40
35
30
25
20
15
10
5
0
Jan. 2014
Mar.
May
Jul.
Sep.
Nov.
Jan. 2015
Mar.
May
Jul.
Sep.
Nov.
Months

Fig. (3)

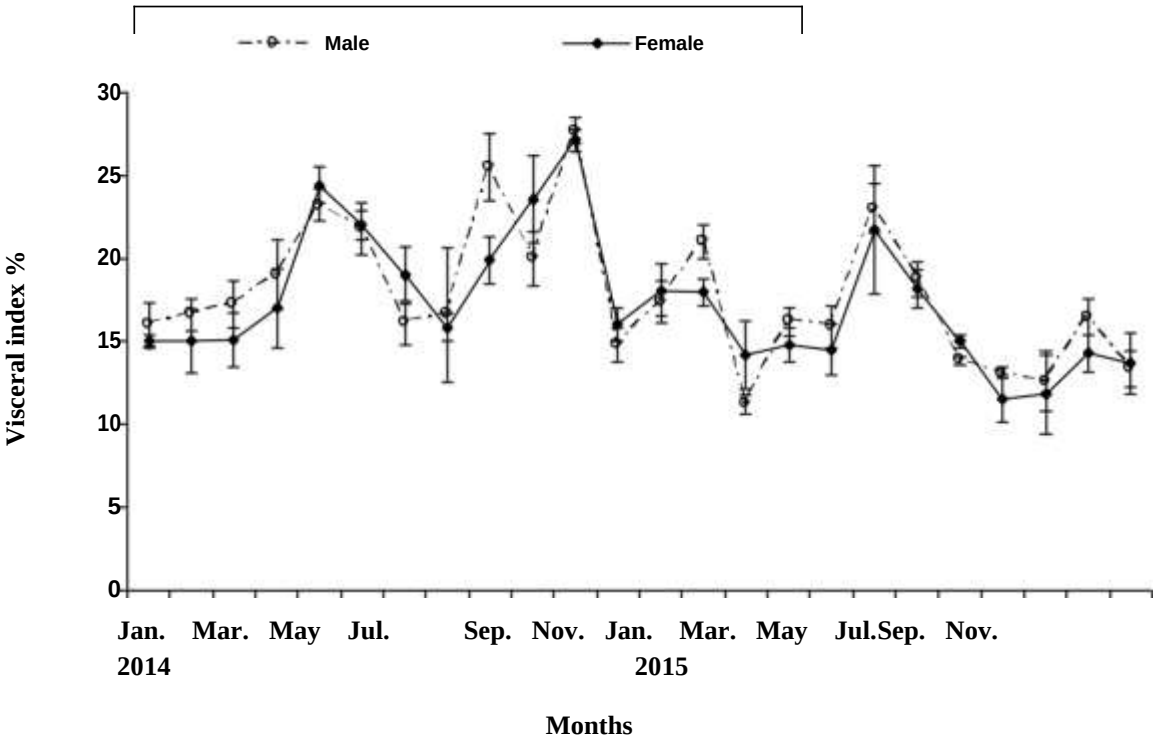

Male
Female
Visceral index %
30
25
20
15
10
5
0
Jan. Mar. May Jul. Sep. Nov. Jan. Mar. May Jul. Sep. Nov.
2014
2015
Months

Fig. (4)

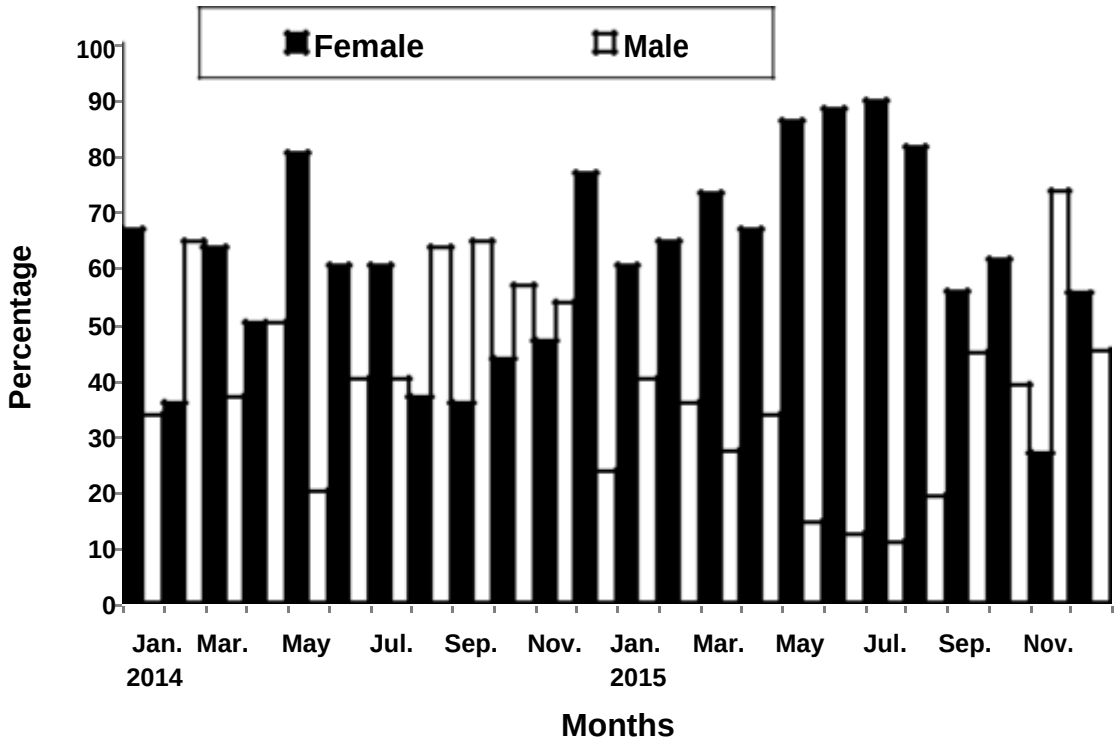
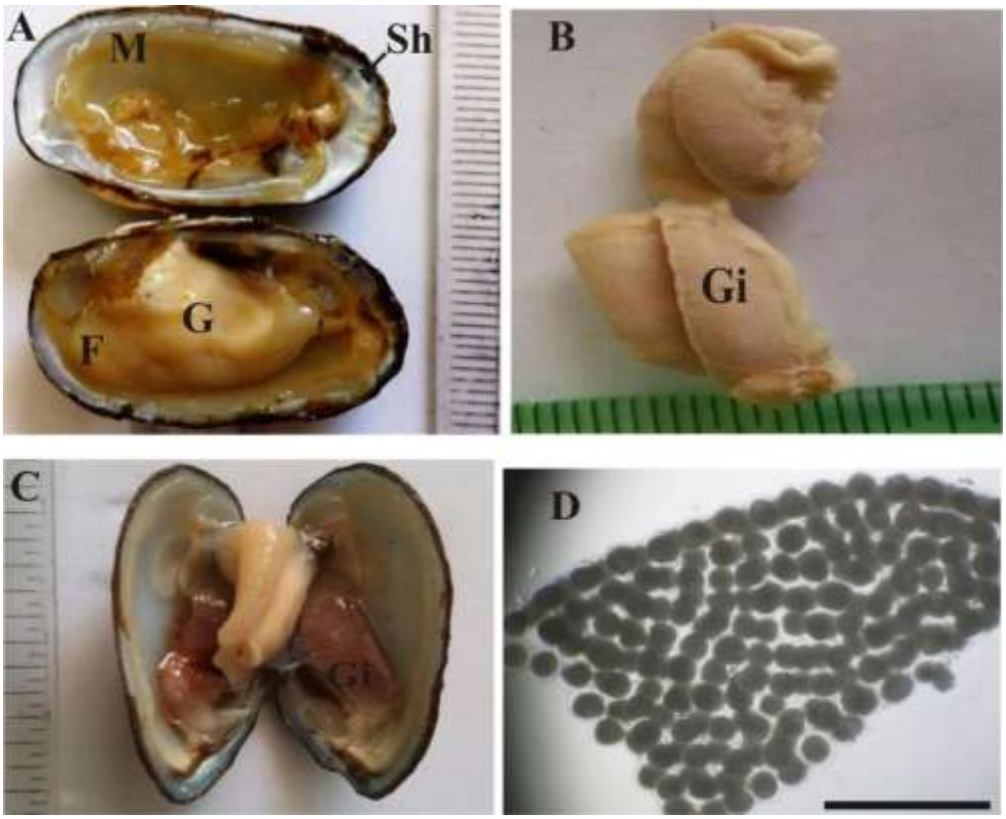

Female
Male
Percentage
100
90
80
70
60
50
40
30
20
10
0
Jan. Mar. May Jul. Sep. Nov. Jan. Mar. May Jul. Sep. Nov.
2014
2015
Months

Fig. (5)



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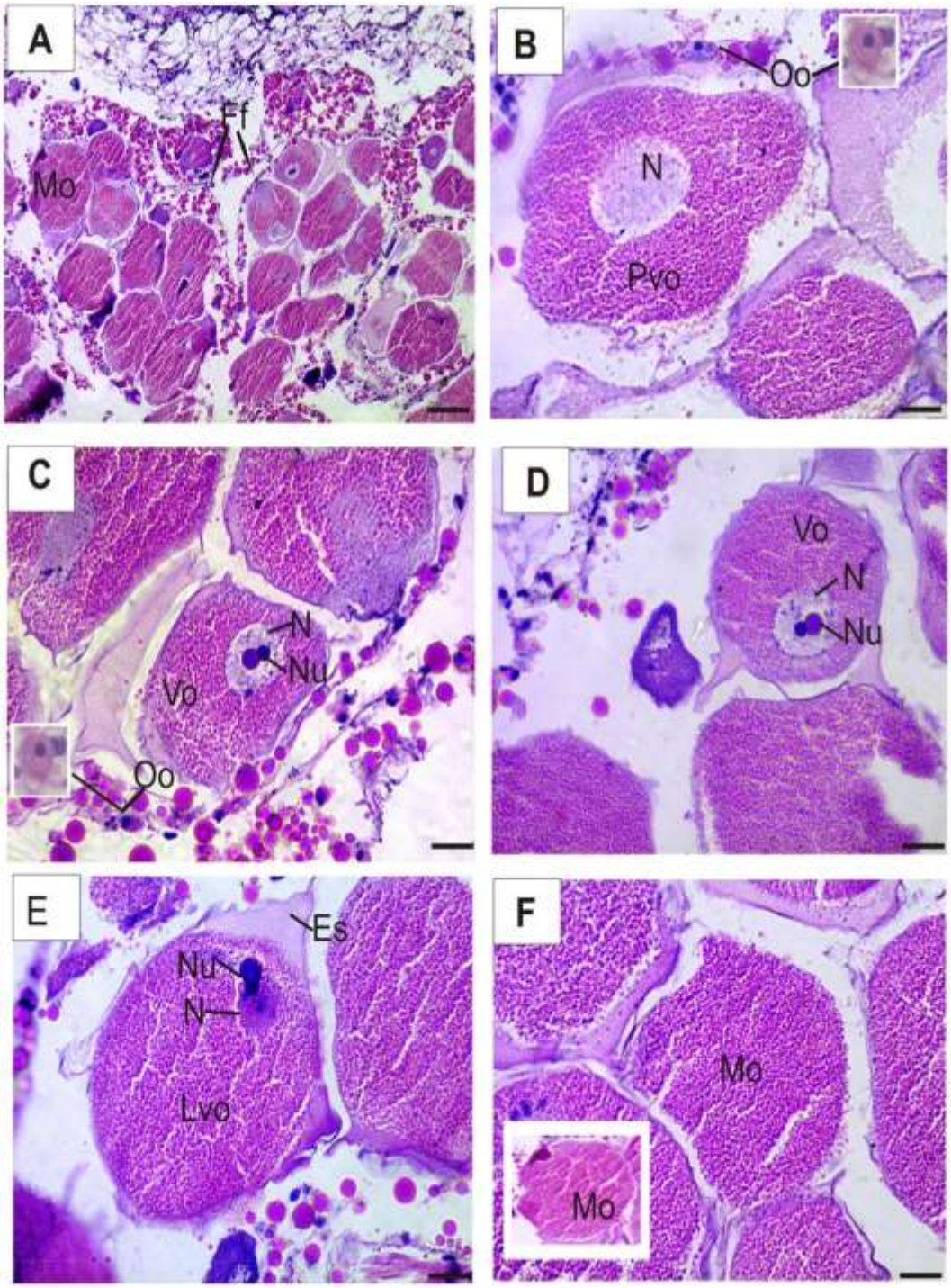

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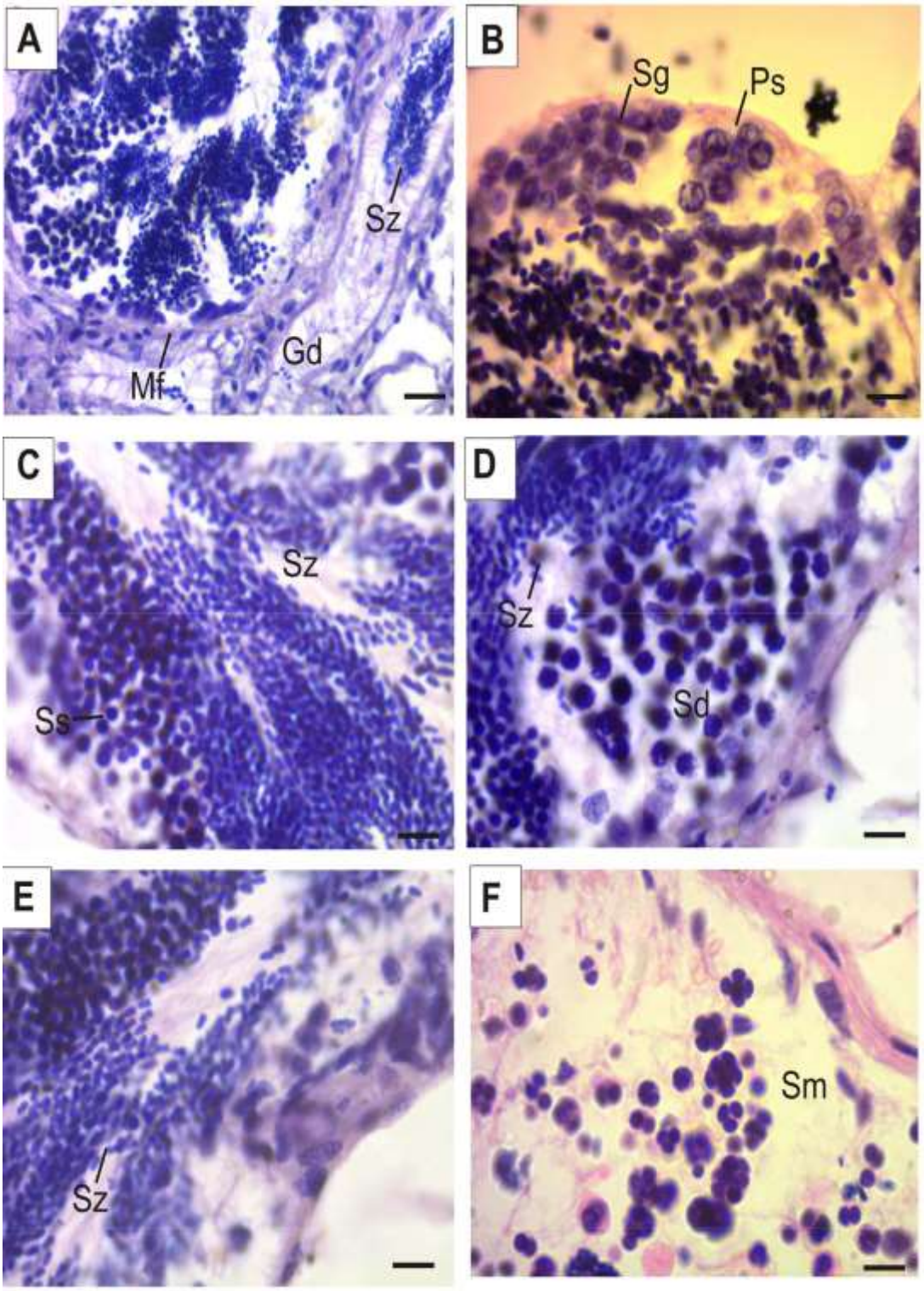

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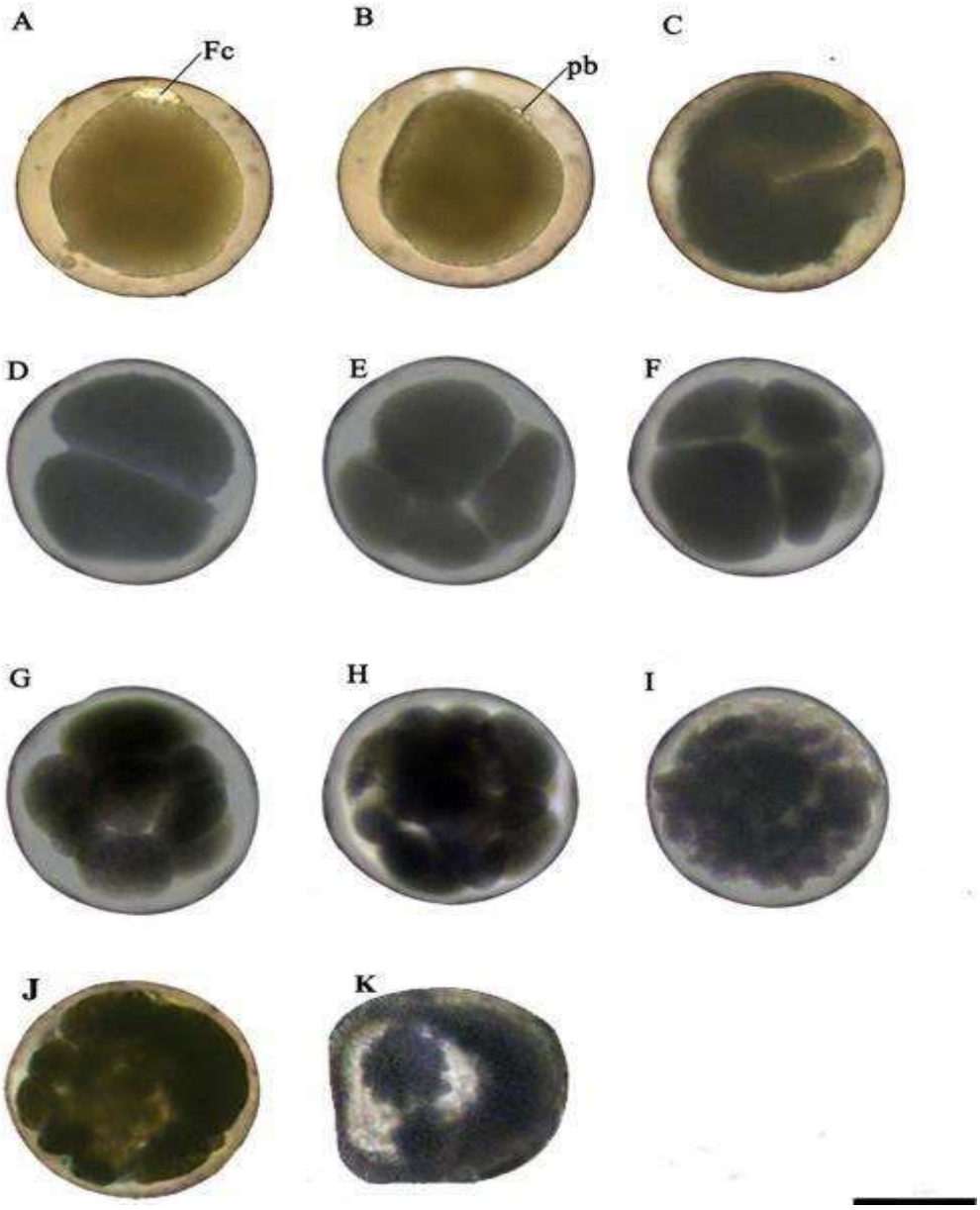

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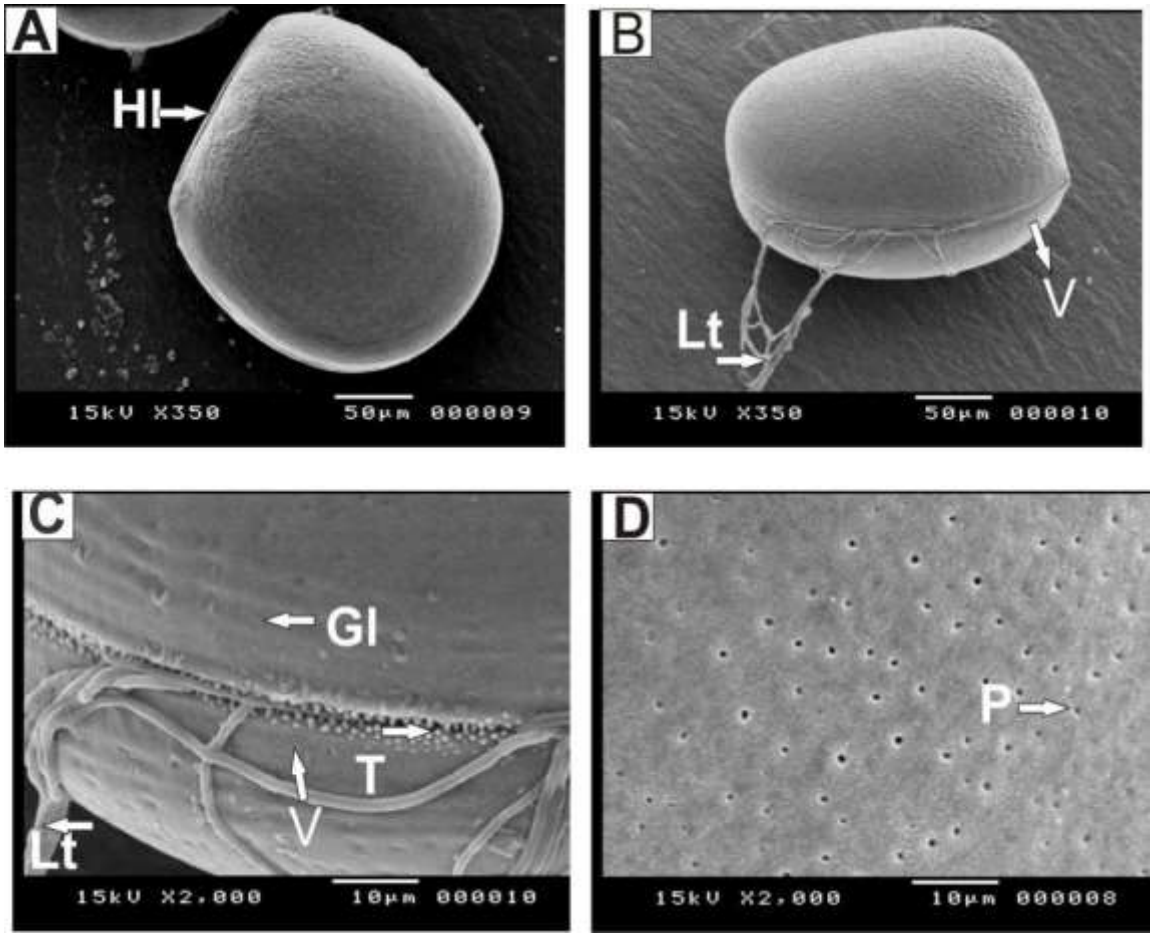
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B
Lt
V
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C
Gl
T
V
Lt
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10µm 000010
D
P
15kV X2,000
10µm 000008