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Gametogenesis and spawning of *Spirobranchus tetraceros* (Polychaeta, Serpulidae) in Abu Kir Bay, Egypt

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Abstract

The serpulid polychaete Spirobranchus tetraceros of Red Sea / Indo-pacific origin, recently has succeeded to establish a foothold in Alexandria Mediterranean waters. Worms were monthly scraped from submerged iron substrates at Abu Kir Bay during the period December 2000 – November 2001. Both light and TEM were used to study gametogenesis and time of spawning of S. tetraceros.

Gametogenesis was asynchronous and oogenesis could be divided into two previtellogenic, two vitellogenic and a spawning stage. Oocyte development took about 8 months, from October to June. Spawning occurred from late May - early June until October. Thus S. tetraceros is a long period spawner. The maximum diameter of ripe oocyte is 78 µm.

The spermatogenic phase could be divided into three stages: spermatogonia, spermatocytes and spermatids (including spermatozoa). The duration of sperm development took about 8 months. Spermatocytes persist from October to March. By March the sperms grew rapidly until they became spermatozoa in May. The sperm could be considered ect-aquasperm with regard to its fertilization biology.

Keywords: *Spirobranchus tetraceros*; Oogenesis; Spermatogenesis; Ultrastructure, Spawning period.

Introduction

Spirobranchus tetraceros (SCHMARDA, 1861) is a serpulid polychaete of a Red Sea / Indo-Pacific origin. It migrated through Suez Canal and now recorded from the coasts of Israel and Lebanon (ZIBROWIUS & BITAR, 1981; BEN-ELIAHU, 1991 & BEN-ELIAHU

& FIEGE, 1996). BEN-ELIAHU & FIEGE (1996) stated that of all polychaete lessepsian migrants, only *S. tetraceros* has reached as far west as Rhodes. Recently *S. tetraceros* succeeded to establish a foothold in Alexandria Mediterranean waters (Egypt) to the extent that it markedly overruns the previously abundant serpulid species, *Hydroides elegans*.

Originally, *S. tetraceros* was recorded in the Eastern Harbour of Alexandria (SELIM 1997). Later fieldwork observations in 1999 – 2001 indicated that this species became the main constituent of the fouling communities along Alexandria shore. Nowadays, it ranked first followed by *H. elegans*.

Despite of the great abundance of the new invader, literature search indicated that gametogenic cycle of *S. tetraceros* remained unstudied (also ten Hove, pers.comm.). This work is the first to deal with that issue.

Gametogenesis of several polychaetes have been studied (FRANZÉN, 1956 DUMONT, 1969, OLIVE & GARWOOD, 1981; CHRISTIE, 1984 & 1986; KOPP, 1985, ECKELBARGER, 1983 & 1988; FRANZÉN & RICE, 1988; JAMIESON & ROUSE, 1989; GIANGRANDE & PETRAROLI, 1994; KUPER & WESTHEIDE, 1997; ROUSE & TZETLIN, 1997; GAMBI *et al.*, 2000 and KUPRIYANOVA *et al.*, 2001). Nevertheless, in Egypt comparatively limited studies have been carried out on gametogenesis of polychaetes. MONA (1992) studied the spawning of *Hydroides dirampha* in Lake Timsah, Suez Canal, Egypt. The only two ultra structural studies in Egypt had been carried out in Lake Timsah on gametogenesis of *Neanthes brandti* (MOSTAFA, 1992) and spermatogenesis of *H. dirampha* (MONA *et al.*, 1994).

The aim of the present work is to study the gametogenic cycle and gamete as ultrastructure of *S. tetraceros*. This study also identifies the spawning period of this species in Abu Kir Bay, Egypt.

Materials and Methods

Samples of *S. tetraceros* were obtained from the submerged iron substrates from Abu Kir Bay (Alexandria, Egypt), at monthly intervals from December 2000 to November 2001. In case of the females, a sample of coelomic fluid from 9 to 14 females (fixed in 4% formaldehyde solution) was extracted monthly

to determine the oocytes diameter. The oocytes were obtained by making a dorsal incision through the body wall and smearing the coelomic contents on to a microscope slide containing water. After oocytes became spherical, the diameters of about 50 oocytes from each female were measured under a microscope by using a calibrated eye piece micrometer. The oocyte diameter was used as an index of the stage of maturation. The oocytes diameters were divided into four groups (according to the egg size and histological studies): < 10 µm, 10 to <20 µm, from 20 to <63 µm and > 63 µm.

In order to evaluate the different gametogenic stages and the time of spawning; serially histological sectioning was examined by both light and transmission electron microscopes (TEM) as follows.

For light microscopy, worms were fixed in Bouin's solution for 24 hours and then were washed in 70% ethanol for two days prior dehydration. Then worms were processed by routine methods of dehydration, cleaned in xylene and embedded in paraffin wax. Sections (5 µm) were cut and stained with haematoxylin and eosin (LUNA, 1968).

For TEM, mature males and females were fixed by immersion in 2.5% glutaraldehyde buffered to pH 7.3 Millonig's fluid for 2 hours, then post-fixed with buffered osmium tetroxide at 4 °C. After fixation, the material was rapidly dehydrated in graded series of ethanol and embedded in Epon. Semithin sections were stained with toluidene blue. Ultrathin sections were stained with lead citrate and Uranyl and examined with Jeol 100CX TEM.

Results

Sex differentiation

In specimens investigated sexes of *S. tetraceros* were separate. Colour of the worm usually was used as an indicator to discriminate mature stage; the abdomen of female was distinguished by an orange-red colour, while

male abdomens creamy in colour as in many serpulid polychaetes.

Development of ova:

Oogenesis had been divided into five stages: the first two previtellogenic followed by two vitellogenic stages and the final spawning stage. Previtellogenic oocytes were suspended in the coelom and underwent vitellogenesis while floating in the coelomic fluid.

Stage I: Immature stage:

This stage included oocytes less than 10 μm diameter. It was detected from October-February. Generally, the occurrence of this stage was rare (from 0.2 to 15.1%) throughout the period of investigation (Fig 1). Oocytes in this stage (Pl. 1) were sub-spherical, polygonal or hexagonal in shapes depending upon the stresses imposed on them by the expanding neighbouring oocytes. The nucleus was large (about 3 – 5 μm diameter), with peripheral nucleolus (about 2 μm diameter). Each oocyte

was surrounded by a thin layer of cytoplasm covered with several layers of squamous epithelial cells. Oocytes were attached to each other by connective tissue.

Stage II: Premature stage:

Oocytes reached its maximum diameter of 20 μm . This stage was observed throughout the year. Its occurrence was frequent from October-February (from 63.8 to 33.2%) but was rare (3.8 – 1%) during other months (Fig. 1). The oocytes were still covered by several layers of squamous epithelial cells as in the previous stage. Oocytes of early stage II (Pl. 1) surrounded by condensed cytoplasm, with a maximum diameter of about 12 μm . The nuclei and nucleoli had spherical shapes (about 9 μm & 3 μm respectively). The oocytes of late stage II (pl. 1) increased in size and the cytoplasm became more condensed. The nucleus and the nucleolus diameters were about 12 μm and 3 μm respectively. The nucleolus was still peripheral. Golgi complexes, endoplasmic

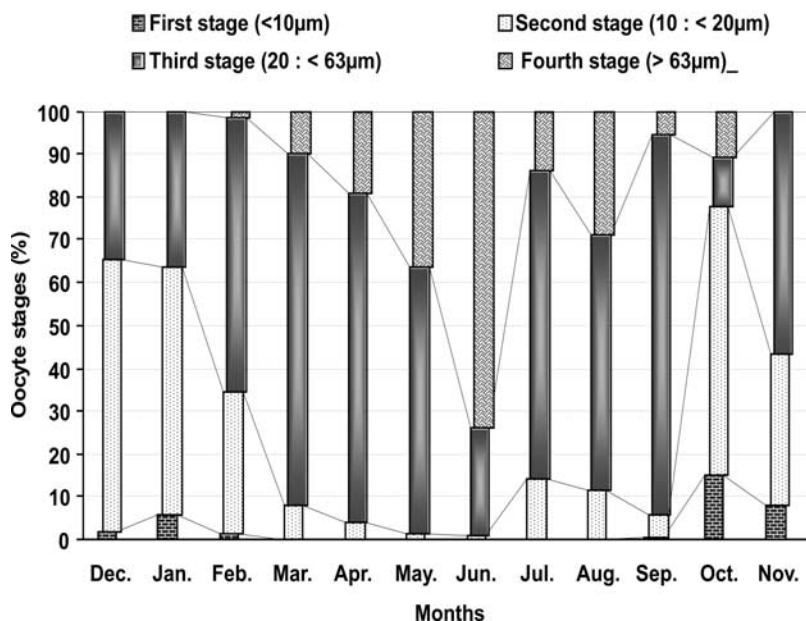


Fig. 1: Monthly variation of the oocyte stages (%) throughout a year.

reticulum and mitochondria began to appear in late stage II (Pl. 2).

Stage III: Mature stage:

In this stage (Pl. 3A&B) diameters of the majority of coelomic oocytes ranged from $> 20 \mu\text{m}$ to $< 63 \mu\text{m}$. This was the most common stage throughout the year. Fig. 1 showed that the highest occurrence of this stage was in September (88.9 %) while the least was in October (11.8%). In this stage oocytes were characterized by the onset of yolk deposition i.e. they entered the vitellogenic stage. Initially, lipid droplets appeared peripherally in the cytoplasm. Both cytoplasm and nucleus started to be vacuolated and less condensed (Pl. 3A). Lipid droplets diameter ranged from 2 to $4 \mu\text{m}$. The nucleolus was still peripheral ($5 \mu\text{m}$ in diameter). Later, granule elements increased in size and number with small developing yolk spheres. In addition, the cytoplasm and nucleus became less condensed and more vacuolated (Pl. 3B). While the oocytes increased in size (about $63 \mu\text{m}$ in diameter), the nuclei started to decrease in size and reached $30 \mu\text{m}$ in diameter. Eventually, the nucleoli disappeared and the distal layer of the oocytes became thin.

Stage IV: Fully mature ova stage:

This stage appeared from February to October (Fig.1). In June the diameters of most coelomic oocytes were $> 70 \mu\text{m}$, most of them reached their maximum size, $78 \mu\text{m}$ (74%). During this stage (Pl 4&5) the oocytes were free floating in the coelomic fluid. They were orange-red in colour with great numbers of spherical yolk granules (Pl. 4&6A) that varied in size ($1\text{--}3 \mu\text{m}$ in diameter), in addition to great numbers of lipid droplets ($2\text{--}5 \mu\text{m}$ in diameter), mitochondria and endoplasmic reticulum connected with cytoplasm (Fig. 6A). Nucleus size decreased until it reached its smallest size in late stage IV ($20 \mu\text{m}$ diameter). In early stage IV (Pl. 5A), the oocyte began to develop a thin vitelline envelope ($2 \mu\text{m}$ thick) instead of epithelium cells. In the late stage IV (Pl. 5B&6B) a double vitelline envelope developed

further and thickened ($4 \mu\text{m}$ thick). Oocytes in the earliest stages of vitellogenesis lacked microvilli that appeared in vitellogenesis (Pl. 6 A&B).

Stage V: Spawning stage:

The greatest percentage of fully mature oocytes stages was observed in June (74%). The first indication of spawning onset was a clear decline in the number of oocytes in the samples collected from July (14%) to October (10.6%) and disappearance of oocytes from samples in November (Fig.1). During spawning, oocytes appeared more rounded, seemed to be free, ready for shedding and often showed signs of degeneration. Females were not completely devoid of gametes after spawning, but spawning extended from July to October. While spent females disappeared, a very large numbers of immature worms started to appear during October. The development of oocytes from their first appearance in the coelom to mature ova took about 8 months, from October to June (Fig. 1).

Spermatogenesis:

Spermatogenesis took place in three stages, which could be detected in coelomic cavity at the same time except fully mature active spermatozoa. They were; spermatogonia, spermatocytes and spermatids (including spermatozoa) (Pl. 7).

Stage I: Spermatogonia:

The immature males appeared in early June. Sections of peripheral abdominal regions contained compact mass of dense connective tissue. Embedded in connective tissue small packets of spermatogonia. Spermatogonia were first detected in July and their occurrence was scarce and irregular throughout the year.

TEM showed that spermatogonial cells (Pl. 7&8) were large, $5 \mu\text{m}$ in diameter. A spermatogonium had a large sub spherical nucleus that occupied most of the cell and was enveloped by cytoplasm. The nucleus contained clumps of condensed chromatin.

The nuclear membrane was double, smooth and had many nuclear pores. The cytoplasm was full of fine granules of free ribosomes, containing few mitochondria and diffuse endoplasmic reticulum (Pl. 6).

Stage II: Spermatocytes:

Spermatocytes were formed from October to March. Spermatocytes were smaller in size (4 µm in diameter) than spermatogonia (Pl. 7&9). A spermatocyte had a large nucleus occupying most of the cell. The nucleus contained clumps of condensed chromatin. The cytoplasm was still rich in free ribosomes and contained few mitochondria and diffuse endoplasmic reticulum.

Stage III: Spermatids (including spermatozoa):

Spermatids were observed from March to May. During May all spermatids became ripe and developed to spermatozoa. They appeared during May, became free in the coelomic fluid and were expelled during spawning period that lasted from late May - early June to October. The development of gametes in females and males occurred at the same time. Spermatid cells (Pl. 7) were slightly smaller (3 µm in diameter) than spermatogonia and spermatocytes, spherical in shape and stained very densely with osmic acid. Spermatid had little cytoplasm and mitochondria. The nucleus contained more condensed chromatin. Finally, the spermatid nucleus became gradually smaller, more or less spherical and condensed. The mitochondria were fused together forming one pair. They migrated to the basal part of the nucleus to form the sperm mid-piece.

TEM showed that a ripe spermatozoan (Pl. 10A&B) consisted of a head, a middle piece and a flagellum. The head had more or less rounded nucleus (barrel-shaped). Anteriorly, the nucleus is flattened where it is in contact with the acrosome. The acrosome formed a cap over the tip of the nucleus. The anterior tip of the nucleus formed a ledge upon which the acrosome rested. A smooth nuclear

membrane extended into the convex space below the acrosome. The acrosomal contents were homogeneous. The acrosome was bounded by an acrosomal membrane and overlain by the closely apposed plasma membrane. The middle piece was short and contained five spherical mitochondria (Pl. 11A) surrounding the centriolar complex at the base of the nucleus. The centriolar complex was composed of the proximal centriole located at the base of the nucleus and the distal centriole located between the mitochondria and continued with the sperm flagellum. The proximal centriole contained nine microtubules and was positioned perpendicular to the long axis of the sperm in a shallow centriolar fossa. The distal centriole also contained nine microtubules as well as nine lateral branching extensions forming an anchoring structure of the base of the flagellum. Therefore, it showed the 9 + 2 axonemal pattern (Pl. 11B).

Discussion

Now *S. tetraceros* is considered as the principal fouling organism in Alexandria water instead of *Hydroides elegans*. BEN-ELIAHU (1991) stated that this species is a migrant that succeeded to traverse to the Eastern Mediterranean via ship hulls as foulers or as free planktonic larvae.

The pattern of oogenesis in polychaete is generally divided into two main types, extra ovarian and intraovarian (ECKELBARGER, 1983&1988). Results of this study revealed that oogenesis clearly falls into the extraovarian category, where previtellogenic oocytes encountered in the coelom and completed vitellogenesis floating freely in the coelomic cavity. Oocytes of stage I occurred scarcely in the coelomic fluid around the year. This may be attributed to the rapid oocytes growth of this stage. This observation was noticed in *Nereis virens* (BRAFIELD & CHAPMAN, 1967). On the contrary, oocytes of stage III was the most common during the investigated

period (Fig. 1), seemingly as a result of long developmental period that is necessary for oocytes of this stage. This stage is similar to stage II in males, in the long period that necessary to develop fully. This long period helps the sperms and eggs attain their full maturation simultaneously. Oocytes of stage IV were present in more or less moderate numbers. This may explain the continuous liberation of the ripe oocytes from the animal into the sea. This stage is equivalent to stage III in males in rapid and continuous liberation of spermatozoa from the animal into the sea as ripe oocyte in females. It was apparent that there was an inverse relation between both third and fourth stages in females, i.e. when oocytes of stage III increased, oocytes of stage IV decreased and vice versa. This may be interpreted as steady and continuous development of stage III into IV. The occurrence of high proportions of ripe oocytes in early June, followed by a clear decline in early July (Fig. 1), coincides with the first appearance of new generation of new immature worms among samples of early June. This suggests that the spawning began in late May-early June. The duration of coelomic phase of gametogenesis in female takes about 8 months (October - June). In both males and females, all stages could be recognized in the coelomic fluid with a detectable difference through the year. Hence, there is a degree of asynchrony during spermatogenesis and oogenesis. Therefore, males and females do not completely devoid of gametes after spawning. They do not completely shed all coelomic contents. So, spawning of *S. tetraceros* is prolonged and extended through several months (July – October). This type of breeding is referred to as semi-continuous iteroparous breeding, where gametes are produced in small batches at intervals during a prolonged breeding season (ECKELBARGER, 1983). This observation was recorded in serpulid species such as *Spirobranchus giganteus* (ALLEN, 1957), *S. polycerus* (LACALLI, 1976), *S.*

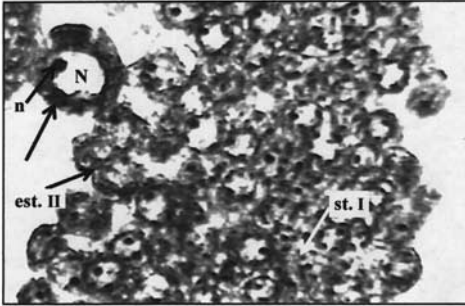
corniculatus (SMITH, 1984) and *Hydroides ezoensis* (MIURA & KAJIHARA, 1984).

The serpulid mature egg size ranges from 45 to 200µm (KUPRIYANOVA *et al.*, 2001). Present study results reveal that ripe ova of *S. tetraceros* measures 78µm in diameter. These small sized ova give rise to feeding planktonic larvae (KUPRIYANOVA *et al.*, 2001). Gaikwad, 1988 (cited from KUPRIYANOVA *et al.*, 2001) found that mature egg size of artificially fertilized *S. tetraceros* reached 60 µm, which is smaller than that recorded in natural populations of the present investigation. The egg sizes in *S. polycerus* measure 65 µm (MARSDEN, 1992). On the other hand, egg sizes of both *S. corniculatus* (SMITH, 1984) and *S. giganteus* (ALLEN, 1957), slightly differ from that recorded in the present study (80 & 83 µm respectively). In the present study, the vitelline envelope reached 4µm thick, while in serpulids it usually is approximately 2 µm (KUPRIYANOVA, *et al.*, 2001). In some polychaetes the envelope is more thickened as in *Trichobranchus glacialis*, 10 µm thick (CHRISTIE, 1986).

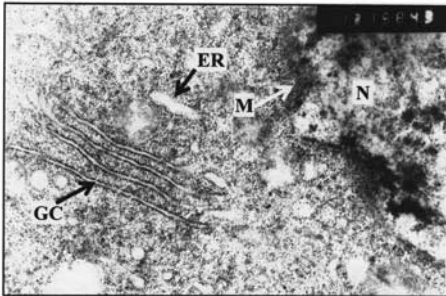
Morphological studies suggest that Golgi complex and endoplasmic-reticulum participate in the synthesis of yolk materials in the majority of polychaetes, while microvilli have nutritive function (ECKELBARGER, 1988). Therefore, the appearance of Golgi complex and endoplasmic-reticulum during late previtellogenic phase in the present study may confirm their rule in yolk synthesis. On the other hand, the occurrence of microvilli during the late vitellogenic stage in *S. tetraceros* may interpret their rule in active transport of nutrients from the coelom to the oocytes during oogenesis.

Differences in spermatozoa morphology reflect different modes of fertilization. Spermatozoa are grouped into externally fertilizing sperms (ect-aquasperm) and sperms which released into water at some stage and stored by the female prior to fertilization (ent-aquasperm) (JAMIESON & ROUSE, 1989). In the present work, mature sperm of *S. tetraceros*

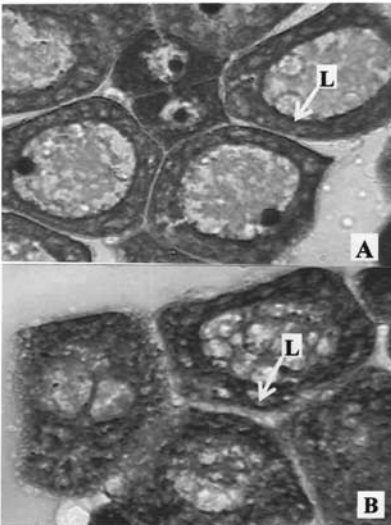
Plates



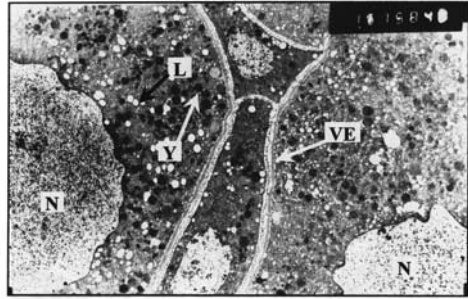
PL. 1: Photomicrograph cross section (cs) showing groups of stage I (st. I), early (est. II) and late (arrow), stage II oocytes with large nucleus (N) and peripheral nucleolus (n). X600.



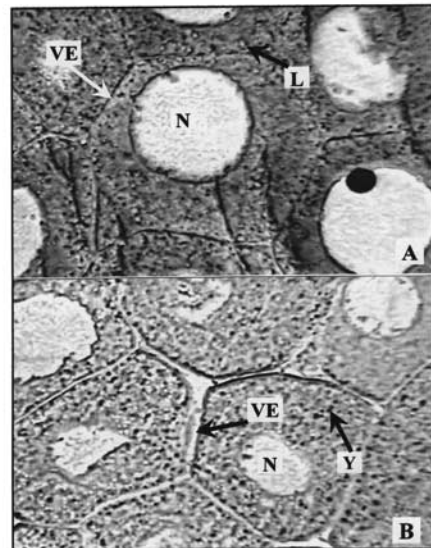
PL. 2: TEM section illustrating a portion of cytoplasm of late stage II oocytes, showing a portion of the nucleus (N), mitochondria (M), Golgi complex (GC) and endoplasmic-reticulum (ER). X 13000.



PL. 3: Photomicrograph of cs showing early (A) and late (B) stage III oocytes. A: showing lipid droplets (L), the nucleolus still present. B: Note the increase of lipid droplets in size and number, the nucleus more vacuolated and the nucleolus disappeared. X600.

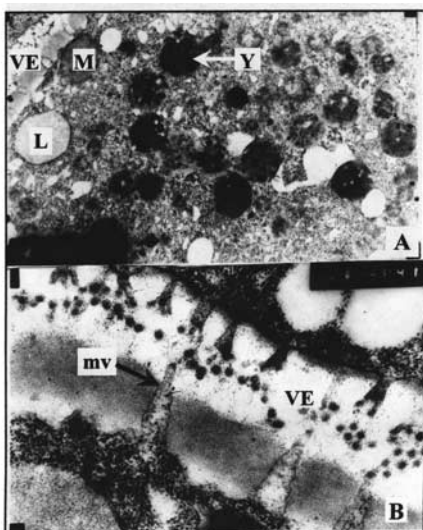


PL. 4: TEM section showing stage IV oocytes with two portions of nuclei (N), yolk spheres (Y), lipid droplets (L) and vitelline envelope (VE). See the difference in size of both yolk spheres and lipid droplets. X1500.

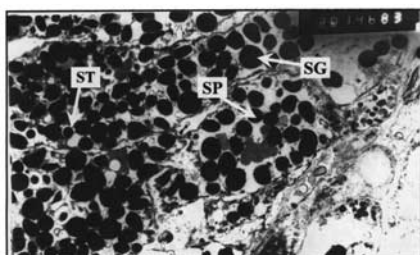


PL. 5: Photomicrograph of semithin cs showing stage IV oocytes. A: Early stage IV; nucleus (N), yolk spheres (Y), lipid droplets with minute sizes (L) and sharp vitelline envelope (VE) appeared. B: Late stage IV; note the increase of yolk sphere numbers and thick vitelline envelope, oocytes with small nucleus. Stained with Toluidine blue. X600.

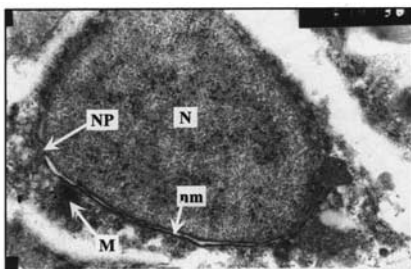
has nearly spherical head and in the middle piece there are five spherical mitochondria surround the distal centriole below the nucleus, similar to those of ect-aquasperm. This type of sperm is a common feature in broadcast spawners such as *Pomatoleious kraussi*, *Spirobranchus corniculatus* and *Serpula* sp. (JAMIESON & ROUSE, 1989). There are many broadcast serpulid spawners having



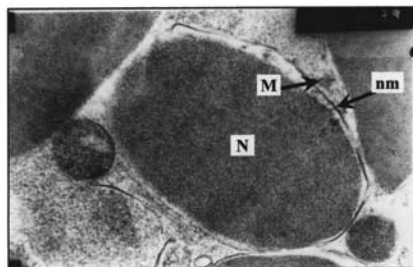
PL. 6: TEM section of stage IV oocytes. A: Section showing a portion of cytoplasm and vitelline envelope (VE). See the variation in size of both lipid droplets (L) and yolk spheres (Y). Note large mitochondria (M). X 7500. B: Magnification of a portion of vitelline envelope with microvilli (mv). X 25000.



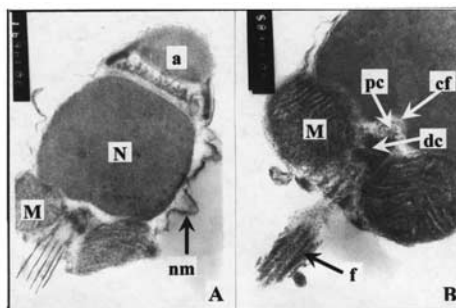
PL. 7: TEM section of coelomic fluid in male showing the various developmental stages: spermatogonia (SG), spermatocytes (SP) and spermatids (ST). X 3000.



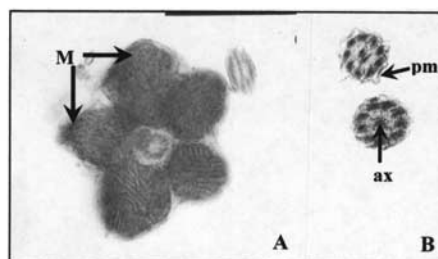
PL. 8: TEM section showing spermatogonium with large nucleus (N), nuclear membrane (nm) with nuclear pores (NP) and mitochondria (M). Note cytoplasm full of free ribosomes. X 15000.



PL. 9: TEM section showing spermatocyte with large nucleus (N), nuclear membrane (nm) and mitochondria (M). X 40000.



PL. 10: Sections of mature spermatozoa illustrating its details. A: Section through mature spermatozoa showing acrosome (a), nucleus (N), nuclear membrane (nm), mitochondria (M), centriolar complex and axoneme. X 30000. B: Magnification of the mid piece showing anchoring apparatus for the axoneme with two perpendicular centrioles; proximal centriole (pc), distal centriole (dc), centriolar fossa (cf), spherical mitochondria and flagellum (f). X 40000.



PL. 11: TEM section; A: Middle piece region showing five mitochondria (M) surrounding the axoneme (ax). X 20000. B: Section through axoneme with 9+2 axonemal pattern and plasma membrane (pm). X. 40000.

sperm morphology similar to that described in the present study; with a midpiece containing spherical mitochondria and a flagellum, such as *Hydroides dianthus*, *H. norvegicus*, *Pomatoleios kraussi* *Spirobranchus corniculatus* (KUPRIYANOVA *et al.*, 2001).

While other broadcast spawners, sperms have different shapes, such as *Hydroides elegans* that characterized by spherical to conical head (CLAPAREDE, 1870, cited from KUPRIYANOVA *et al.*, 2001) and *H. dirampha* with elongated head (MONA *et al.*, 1994).

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