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Observations on Spermatogenesis in Polychaete. Species, *Halla parthenopeia* (Family: Oenonidae, Polychaeta)

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Abstract: The polychaetes used as bait for sport and commercial fishing are a natural resource exploited in many beaches in Alexandria and Suez Canal. One of the most popular species captured is *Halla parthenopeia* (family: Oenonidae). Study of its reproductive biology would enable us to understand the period of growth and the spawning period, which are necessary to reach a commercially valuable size in artificial aquaculture in future.Ultra -structure of male germ cells and the character of spermatozoa of *HALLAPARTHENOPEIA* were investigated. Histological studies by Light, TEM and Scanning microscopes indicated that maturation was accomplished in four stages: 1- Immature stage; this was characterized by having spermatogonial cells (8-10 μm) arranged in raceme-like structures and some of them were forming nests of spermatogonia. 2- Mature or recovery stage; which displayed active spermatogenesis and the cells of all stages could be seen (spermatogonia, primary and secondary spermatocytes, spermatids including spermatozoa). Both primary and secondary spermatocytes were always grouped. Moreover, the fine structure of their nuclei were with thick clumped chromatin and a thin layer of cytoplasm. 3- Nearly ripe stage; this was characterized by the active spermiogensis process forming spermatid. (4) Ripe and spawning stage, it began by spermatid development and its metamorphosis into sperm and the appearance of the proacrosomal vesicle, then the formation of implantation fossa, finally the development of flagellum. The spermatozoon of *H. parthenopeia* was morphologically simple and primitive in type. It appeared by Scanning microscope to have round nucleus, cap –like round acrosom, short mid piece with 4 mitochondria and long flagellum with a basic 9 + 2 axonemes. The duration of sperm development took about 4 months. Two peaks of mature sperm appeared in late April and late December while spawning occurred in May and January. Following the classification of Jamieson and Rouse (1) sperms were of ect-aquasperm type.

Key words: Annelida · Polychaeta · Family (Oenonidae) · *Halla parthenopeia* · Spermatogenesis · Ultra-structure

INTRODUCTION

Halla parthenopeia (Dellechiaje, 1828) is one of the most popular polychaete species, it is known as El-Hamra in Arabic. It is free, living marine polychaetes found in unusual group of polychaetes in which many of them are parasitic or have a parasitic phase in their life. The length of most adult Oenonids ranges from 1 to 90 cm long with over 1000 segments [2] but are usually thin. Morphologically, they resemble members of the family Lumbrinereidae with differences in jaw structure and chaetae. It was mentioned that live specimens with orange color and iridescent hue become dark after preservation [3]. This species is wide spread in Suez Canal and Alexandria beaches. It was collected by Selim [4] and Abd-Elnaby [5, 6] in Egyptian Mediterranean Waters from sand bottom, from Lake Timsah [7], Suez Bay [8] and from Suez Canal [9]. It has a commercial economic importance as fishing baits in Alexandria and Suez Canal in Egypt. It is extensively used by thousands of sea anglers fishing from the shore or from small boats.

The importance of polychaetes increased as a resource in relation to the development of world aquaculture. For example in crustacean (Penaeidae) polychaetes has been found to as source of polyunsaturated fatty acids which are essential for egg maturation in cultured crustacean, prawns and fish in aquaculture [10,11]. In 1984, the commercial aquaculture of the polychaete *Nereis virens* was initiated in NE England [12].

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Studies on the reproductive biology are particularly important in species that have a fishery potential. Extensive studies have been carried out for a number of commercially economic polychaete species [13-18].

Polychaetes in Alexandria and Suez Canal were studied from ecological distribution and systematical points of view, but a relatively few investigations have been carried out on the process of gametogenesis and spermatogenesis of polychaetes [6,7,19,20].

Information about spawning of this species will contribute to knowledge of their population dynamics and management of the stocks. It was reported that the industry has subsequently sponsored a series of investigations that have stimulated fundamental research into the growth and reproduction of polychaeta. This has resulted in development of techniques for the cryopreservation of the larvae of marine polychaetes, for photoperiodic manipulation of the time of breeding and optimization of the growth process [12]. The ongoing development of commercial culture of polychaeta is likely to give rise to further developments of a fundamental nature, emphasizing the importance of effective links between centers of academic research and commercial exploitation. Therefore, the present study aims to describe the spermatogenesis and morphology of mature sperm of *Halla parthenopeia* to determine spawning time, period of maturation and type of fertilization according to the kind of sperm, which may be help in aquaculture of *H. parthenopeia* depending upon the investigation of its reproductive biology in Egypt.

MATERIALS AND METHODS

Samples of *Halla parthenopeia* were obtained from sediment substrata from El-Ebrahimia beach (Alexandria, Egypt), at monthly intervals from April 2007 to April 2008 by diving, in addition other samples were bought from bait fishermen. Coelomic fluid of a bout 6-10 worms was extracted monthly and microscopically examined to determine sex. In order to evaluate the different spermatogenic 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 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 [21].

For TEM, coelomic contents of mature males 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 tetraoxide 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 100C × TEM.

Sperm cells were also examined by scanning electron microscopy (SEM), once transferred onto cover slips coated by poly-L- Lysine [22], were washed in 0.1M Cacodylate buffer (pH 7.4).

Adhesion of spermatozoa to the poly-L-Lysine coat permitted us to wash and handle cover slips without loss of material. Graded dehydration with ethanol was followed by replacement with hexamethyl disilazane. The cover slips processed in this way were glued with silver paint to SEM steps, coated with gold and observed with Scanning Electron microscope.

RESULTS

There were no morphological differences between males and females in *Halla parthenopeia* and there is no indicator to discriminate mature stage. Therefore, the sex of specimens was determined by puncturing the body wall with a fine needle and examining the extruded coelomic contents. The cloudy white fluid means that is male.

In the most specimens examined, all stages were present; late spermatogonia, spermatocytes, spermatids and mature sperm. The greater number of immature males could be observed in June and February. Immature spermatogonia progressively increased in number and decreased in size until the maximum percentage of spermatid metamorphosed to spermatozoa. The ripening stage started to appear in April and November and spawning took place in May and January. Therefore, the spermatogonial activity was maximum during spring and autumn period in both sexes and spawning peaks were shown in May and January. Spawning period still occurred from May to September and from January to March. After spawning, a small proportion was kept as a reserve material for further gamete production.

Histological and Ultra-structural Examination: The maturation begins by active spermatogenesis. The designation of spermatogenic stages in the following account is based upon the size of cell clusters, size and morphology of nuclei and the appearance of characteristic structures such as centrioles and flagellum.

Spermatogonia Stage: The early stage of spermiogensis are represented by cluster of spermatogonial cells arise from the coelomic epithelium and arranged in raceme- like structures. Some of these spermatgonial cells forming nests of spermatogonia (Fig. 1a and b), then divide mitotically followed by meiotic divisions and terminal differentiation of peripheral cells in the larger cell clusters which were characterized by faintly stained cytoplasm, big eccentric irregular nuclei which were surrounded by thin layer of cytoplasm with an average diameter of 8-10 µm.The nuclear chromatin was two types: chromatin granules and chromatin threads with one nucleoli and the heterochromatin arranged peripherally in discrete clumps. The nuclear membrane is double smooth and had many nuclear pores (Fig. 1c). The cytoplasm was scarce, finely granular and contain granular round mitochondria, they are numerous but small in size. Free ribosomes and Golgi apparatus were also observed in the cytoplasm. Late spermatogonial stages did not differ much from early stage except that the all decrease in diameter and the chromatin material became much more condensed (Fig. 1d).

Spermatocytes Stage: The peripheral cells became detached from the cluster and lost cytoplasmic continuity with adjacent cells. It is likely that these dividing cells represented primary and secondary spermatocytes undergoing meiosis. Both primary and secondary spermatocytes were always grouped into cysts (Fig. 2) and appeared to be connected by intercellular bridges. Cell divisions are frequent within each cyst.

Primary spermatocyte divided by meiosis to give secondary spermatocyte. They differed in size and nuclear morphology. The primary spermatocytes measured about 5.5 µm in diameter and the secondary spermatocytes were about 4.7- µm in diameter. And secondary spermatocytes divided also by meiosis to give spermatid (Fig. 3a and b) which metamorphosed into the sperm cell.

The spermatocyte was oval or rounded in shape with a nucleus that had clumps of chromatin distributed centrally (as irregular strands) and at the nuclear periphery.Also cytoplasm was still rich by organelles and densely occupied by ribosomes.

Spermatids Stage: Spermatids were observed during late March and November where, the most spermatids became ripe and developed in spermatozoa by April and December. Spermatids were formed by meiosis from spermatocytes. Early spermatids had spherical nuclei that contained electron-dense and granular chromatin, then during metamorphosis, the nucleus had thick clumped chromatin, which gradually condensed and become homogeneous.

At the beginning of spermatogenesis, the nucleus migrated and became eccentrically placed in an area that corresponded to the anterior region of spermatid (Fig. 3b). At that time a thin layer of cytoplasm separated the nucleus from the cytoplasmic membrane. The basal part formed a depression in nucleus, where the centriolar complex will reside later (Fig. 3c).

Spermatids were with lightly stained heterochromatin and had few inter chromatin spaces with no nucleolus. It is known that during spermatogenesis numerous smaller mitochondria fuse to form the final number. 4 small mitochondria were positioned in shallow nuclear fossae (Fig. 3d and g). The first visible sign of late spermatid, was the appearance of pre-acrosomal vesicles which migrated to the anterior ends of the spermatids and fuse to form the acrosomes (Fig. 3g and Fig. 6a). therefore, late spermatids had rounded nucleus and the pro-acrosomal projection are present (Fig. 3e). The prominent mitochondria and the centriole-flagellum complex occupied the basal region of the cell. The most conspicuous events that distinguished early from late spermatids were the location of the proacrosomal vesicle, the final shape of nucleus, the formation of centriolar fossa at the base of the nucleus and a textural change in the chromatin from granular to fibrillar in late spermatids (Fig. 3f). Also mitochondria were fused and came together forming two pair which migrated to the end of the cell to form the sperm mid-piece (Fig. 3g).

Ripe Stage or Spermatozoa and Fine Structure of Mature Sperm: During this stage a great number of spermatozoa were present beside spermatids and spermatocyte which were observed in the coelomic cavity (Fig. 3h). The spermatids metamorphosed to give rise to spermatozoa. At late spermatid stage the cytoplasm was symmetrically distributed around the nucleus, the pro-acrosomal vesicle is positioned (Fig. 3g) at the anterior end of the sperm while maintaining close contact with the plasmalemma. It appeared by electron microscopy simple in structure. It mainly consisting of 3 regions. The first region was the head which appeared with a rounded cap-Like vesicle with uniform contents. Commonly modification of acrosome takes the form of minor extreme basal invagination of the vesicle. The sub-acrosomal space were filled with granular material (Fig. 3g). In the present study, the nucleus usually shows condensed or compact which darkly stained chromatin, the nucleus was slightly oval and nearly rounded in longitudinal section. The nuclear

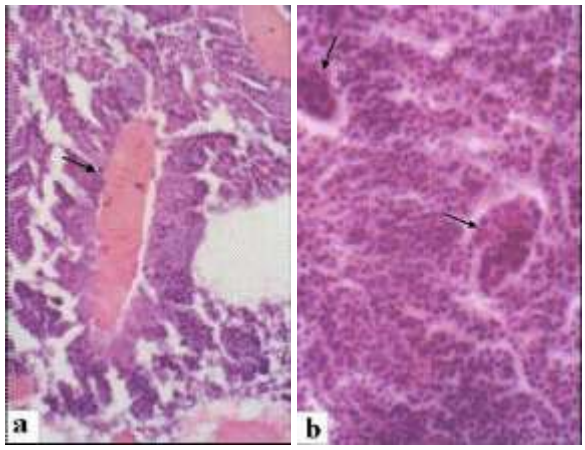

Fig. 1: a. Photomicrograph of TS in the abdomen of *Halla Parthenopeia* at immature stage showing small and large spermatogonia in a racm- like shape (arrow). Staining with Hematoxylin and Eosin.X 400. b: Photomicrograph of TS in the abdomin at immature stage showing nests of spermatogonia (arrow). Staining with Hematoxylin and Eosin. X 400

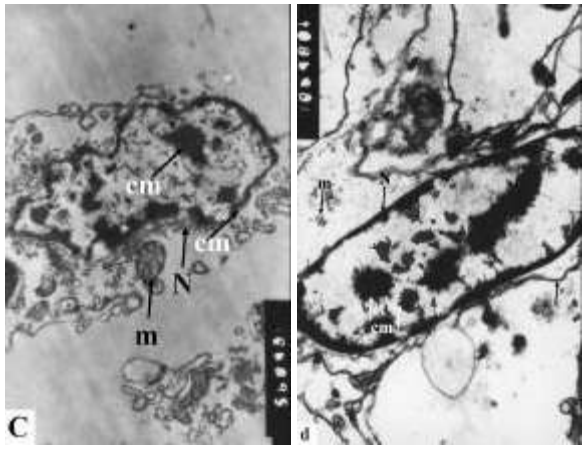

Fig. 1: c. Electronmicrograph (TS) in early spermatogonia showing chromatin material (cm), nucleus (N), mitochondria (m). X10000. d : Electronmicrograph (TS) in late spermatogonia of immature worm, showing dense chromatin material (cm), plasma membrane (pm), nucleus (N). X 2000.

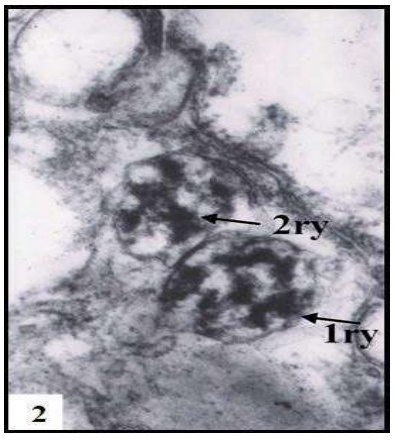

Fig. 2: Electronmicrograph (TS) in (1ry) primary and (2ry) secondary spermatocytes.X 25000

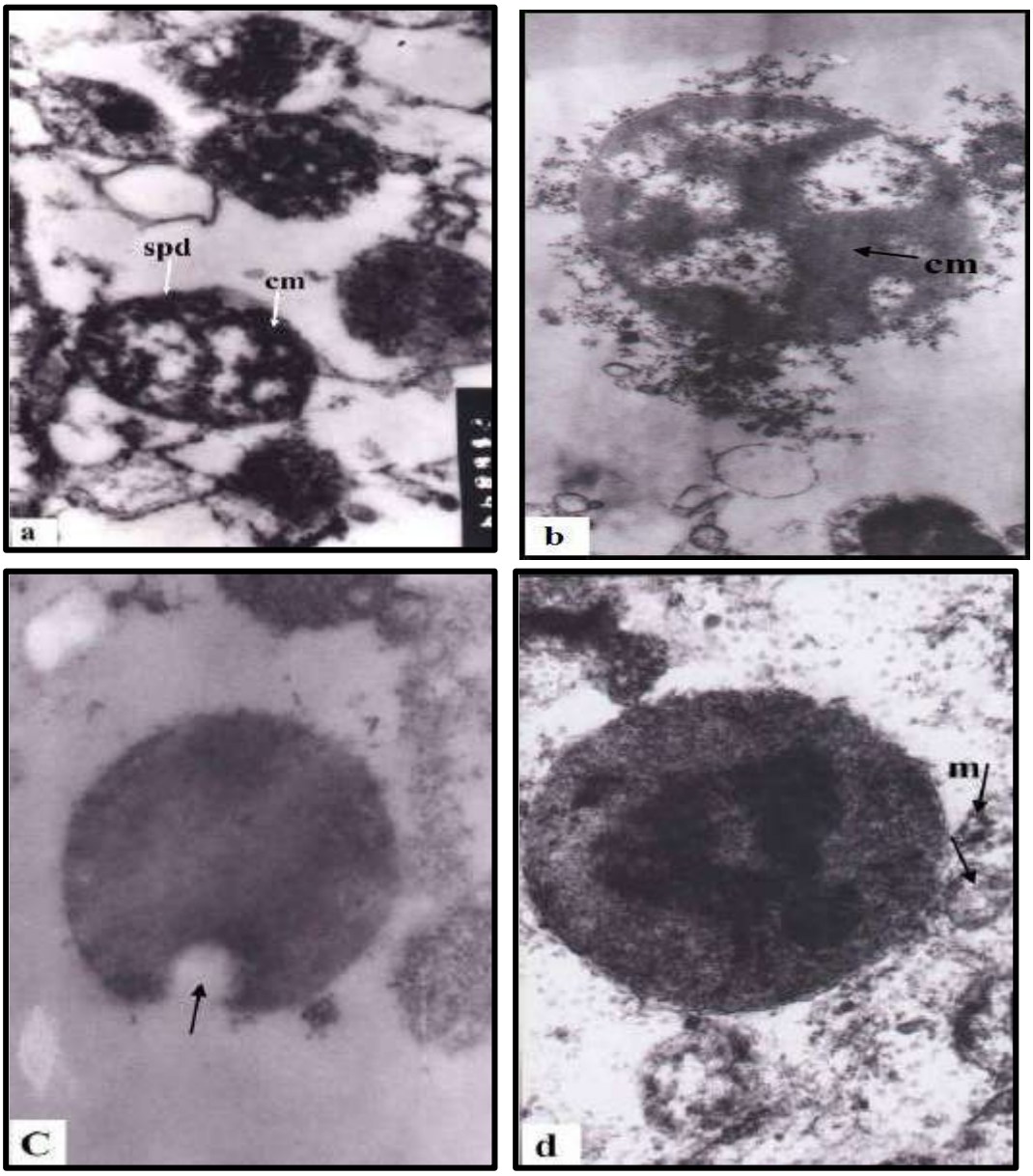

Fig. 3: a. Electronmicrograph (TS) in spermatocytes showing chromatin material (cm) and spermatid (spd). X 25000. b: Magnification of early spermatid showing irregular strands of chromatin material (cm).X 15000. c: Electronmicrograph (LS) in late spermatid showing the basal part of spermatid form a depression (arrow).X 40000. d: Magnificatiom of early spermatid showing a number of mitochondria (arrow). X 40000

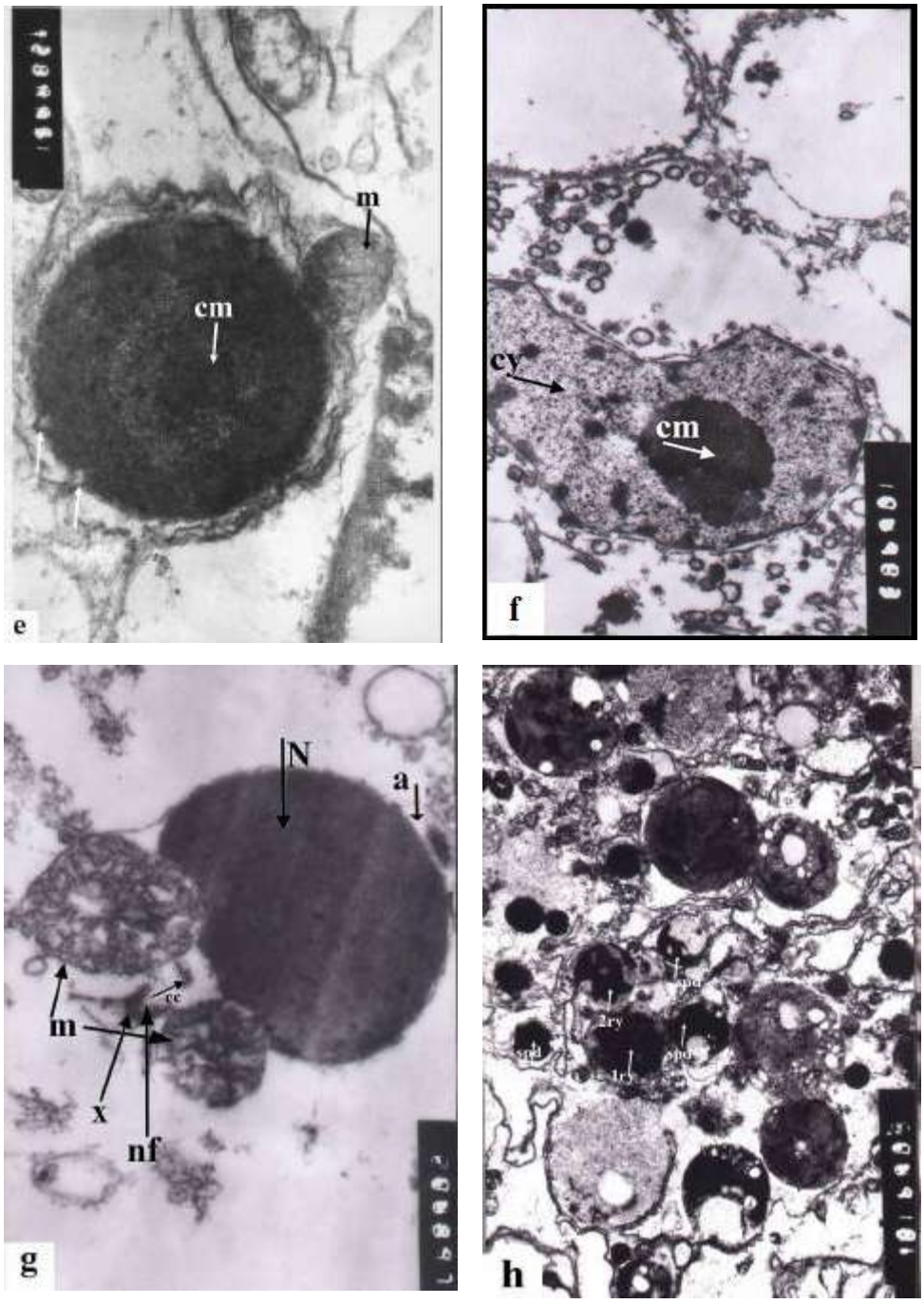

Fig. 3: e: Electronmicrograph (TS) in late spermatid showing chromatin material (cm), mitochondria (m), two projections showing the beging of acrosome (arrow). X 30000. f: Electronmicrograph (TS) in tate spermatid showing dark stain nucleus, cytoplasm (cy), plasma membrane (pm).X 10000. g: Electronmicrograph (LS) in spermatozoa showing the rounded shape head, with rounded nucleus (N), cap-like acrosom (a), mitochondria (m), the nuclear fossa (nf) and centriolar coplex (cc) and part of axoneme (x). X 20000. h: Electronmicrograph (TS) showing (1ry) and (2ry) spermatocyte, spermatid (spd) and spermatozoa (spa).X 5000

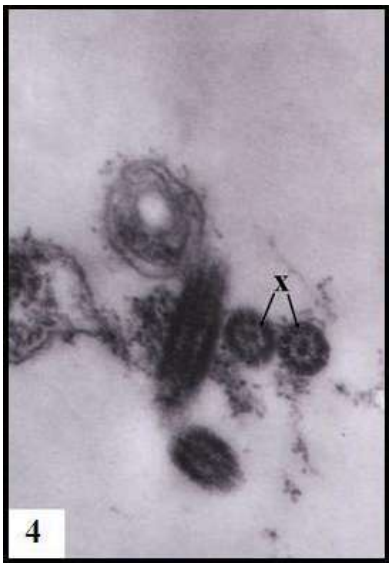

Fig. 4: Electronmicrograph (TS) in tail region of spermatozoa showing axoneme microtubules (x). X 40000

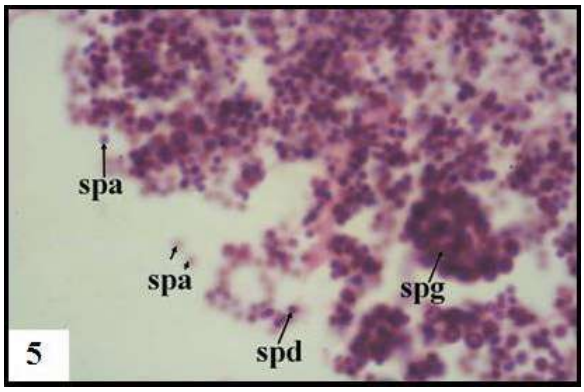

Fig. 5: Photomicrograph of TS in spwning stage showing spermatozoa (spa) and new generation of spermatogonia in nests (arrow). X 400

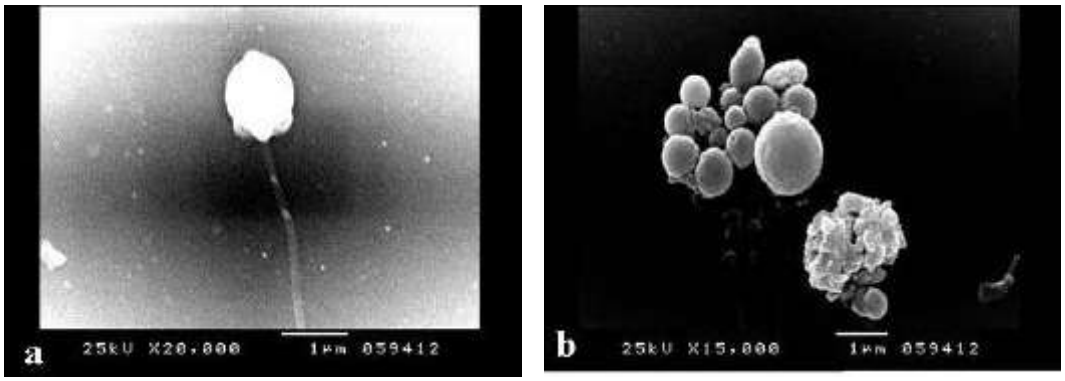

Fig. 6: a: Scan photo showing complete sperm with round cap acrosome, b: Scan photo showing nests of spermatid. rounded nucleus, four mitochondria

membranes ran close together along the anterior side of the nucleus. (Fig. 3g and Fig. 6).

As for the second region of the sperm. It was the mid piece which short with 4 spherical mitochondria and prominent cristae. Two large mitochondria could be seen arranged in the mid piece. The posterior end of the nucleus surrounded 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 mitochondrial which continued with the sperm flagellum (Fig. 3g).

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 or rays extensions forming an anchoring structure of the base of the flagellum. Therefore, it showed the 9 + 2 axonemal pattern (Fig. 4). In addition the flagellum was very long and thin (Fig. 6a and b).

The last stage of spermatogenesis corresponds to mature reproductive stage where spermatids and spermatozoa constitute the majority of the abdomen (Fig. 5).

The disappearance of mature sperm from samples at the beginning of May and January suggests strongly that spawning occurs at this time.

DISCUSSION

Studies of the reproductive biology of polychaetes are very important to know the period of maturation, spawning time, type of fertilization which depend on the type of sperm and its characters [1]. All these aspects are necessary for small attempt in laboratory or on a large scale production that will reduce the anthropogenic disturbance to biological communities in Alexandria.

Polychaetes are readily available, easy to sample and available commercially. Besides marine polychaetes have the highest level of protein and vitamin C. The biochemical composition of *Halla parthenopeia* was also assessed to evaluate its nutritive value since it is known that polychaetes can provide a nutritionally correct balanced food for cultured fish and prawn [9]. In addition, the high economic value of these worms and the size of their catch due to its good price, have led to their intense exploitation as commercial bait for many economically important fishes in Alexandria and Suez Canal. Which can impact the population structure in the short term and may also affect fishing ground on long term causing severe disturbance in the area. Therefore, aquaculture could preserve wild population by supplying the markets with cultured stock of *H. parthenopeia*. However, there no work which has been done to collect any information that are necessary to control the catch or for the species's future culture in artificial systems. This study was an attempt to fill this gap.

In the present study, there were no morphological difference between males and females in *H. parthenopeia* and there was no indicator to discriminate mature stage. It was mentioned that, maturation is accompanied by significant morphological changes (mainly on increase in pigmentation) in the abdomen [23]. However, all samples were orange color which means; that they were adult.

Study of spermatogenesis of *H. parthenopeia* showed that spawning occurs in May and January continuously during an extended reproductive cycle. Therefore, spawning usually restricted to a definite breeding season, which may be very short through few day or which may be through several months. In such cases there is a period prior to the breeding season where the gametes are ripening and a period after breeding season when the worms do not contain coelomic gametes [24]. Such a pattern which agrees with the reproductive behavior recognized in *H. parthenopeia* in the present study. Monthly examination of maturity stages revealed that *H. parthenopeia* had a long spawning period extended through several months (4 months). This type of breeding is referred to as semi-continuous breeding, with gametes produced in small batches at intervals during a prolonged breeding season. Moreover, they do not completely shed all coelomic contents. This type of behavior was similarly shown in *Owenia fusiformis* as recorded by Gentil *et al*. [25]. Additionally, [26] reported that polychaetes are referred to as iteroparous, polytelic, or multiannual with respect to the frequency of reproduction, all these are used to describe species that spawn several times in lifetime. Also they mentioned that the breeding periods for a species may vary geographically.

The present study showed that, *H. parthenopeia* produced new generation twice a year which means extensive number of worms. When it compared with any monotelic polychaete species. Which means that this species can be exploited commercially on large scale.

Results of the study by Osman [9] revealed that oogenesis of *H. parthenopeia* falls into the extraovarian category, where oocytes encountered in the coelom and completed their development then floating freely in the coelomic cavity. In the present study, no copulatory organs or indications of any sort of sperm storage have

been found in male of *H. parthenopeia*, so that external fertilization is the most probable strategy as occurs in many species of polychaetes [27]. Moreover, The ultra-structure of the spermatozoa showed almost the same characteristics found in species clearly having this fertilization type [1, 28, 29].

The distinctive changes that were observed in *H. parthenopeia* spermatogonia to develop into spermatocytes were as follows: decreased size, increased nuclear cytoplasmic ratio and a reduced number of mitochondria. These characteristics of germ cells were similar to finding in many polychaete species [1].

It is well known that, the morphology of spermatozoa also became of interest from a taxonomic point of view in recent year. In particular the head morphology, the shape of acrosome which differ considerably among different polychaetes. It reflects different mode of fertilization or sperm transfer [28].

In the present study it was found that, mature sperm of *H. parthenopeia* possess small rounded acrosome and small spherical nucleus followed by four cristae- rich mitochondria and long tail, similar to those of ectaquasperm for broadcast spawning, corresponding to the so called "primitive type" [28,30,31].

In conclusion, aquaculture of *H. parthenopeia* can be attempt depending upon the investigated reproductive biolog in this study.

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