# REPRODUCTIVE BIOLOGY OF THE MALE LOBSTER *PANULIRUS HOMARUS*

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A thesis

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*"The more I study nature, the more I stand amazed at the work of the Creator"*

*Louis Pasteur*

***Dedicated with love to my parents***

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# CERTIFICATE

Certified that this thesis entitled " **Reproductive biology of the male lobster** *Panulirus homarus*", is an authentic record of independent bonafide research work carried out by **Ms.S.Lakshmi Pillai**, during the period of study from 2003 to 2006 under my guidance for the degree of Doctor of Philosopy in Zoology and the thesis has not previously formed the basis for award of any degree, diploma, associateship or any other similar title. It is further certified that Ms.S.Lakshmi Pillai, passed the Ph.D preliminary examination held in December 2002, conducted by the University of Calicut.

**Supervisor**

# DECLARATION

I hereby declare that this thesis entitled **"Reproductive biology of the male lobster**, *Panulirus homarus*" is an authentic record of work done by me under the supervision of Dr.M.Nasser, Lecturer, Department of Zoology, University of Calicut in partial fulfillment for Ph.D. degree of the University of Calicut and I also declare that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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# Chapter 1.1

# INTRODUCTION

Spiny lobsters are typically marine and are usually the largest crustacean in their habitat, with a wide distribution. Most numbers and species of spiny lobsters are found in the tropics (Holthuis, 1991). They inhabit rocky or coral areas in shallow waters forming an important ecological entity. The spiny lobsters are so called because of the many spines on their carapace and basal segments of the long second antennae. They are often referred to as rock lobsters. They live relatively in shallow waters, where food is available and where rocks, reefs or coral growths are present for shelter. They carry eggs for a relatively short period and have a long larval life.

In India, lobster fishery is primarily based on eight species of spiny lobsters and four species of slipper lobsters. Of these, *Panulirus homarus*, *P.polyphagus*, *P.ornatus*, *Thenus orientalis* and *Puerulus sewelli* form the main fishery (Radhakrishnan & Vijayakumaran, 1990; Suseelan, 1990; Kagwade, 1991). They are caught by trawls, bottom set gill nets, traps, drag nets and bag nets. *P.homarus* (H.Milne Edwards) (Fig.1) is one of the valuable fishing resources in India having a very good scope for culture and fattening. The genus *Panulirus* is characterized by :

Phylum-Arthropoda
Class-Crustacea
Order-Decapoda
Family-Crustacea
Genus- *Panulirus*
Species-*homarus*

It inhabits a depth of 20-90m and attains a maximum weight of 1.5Kg. They are distributed along Indo-West Pacific region, East Africa to Japan, Caledonia, Australia, South coast and West coast of India, Andaman and Nicobar Islands and Lakshadweep. Though they contribute only about 0.1% to the total marine fish landings along the Indian coast (Radhakrishnan & Manisseri, 2001), they enjoy a lucrative market. The current export value of lobsters is Rs.542.7/Kg amounting to Rs.896.9 million (MPEDA, 2000) which is 1.4% of the total value of the export. They are exported live, as frozen tail and whole cooked frozen.

Spiny lobsters are hardy animals and also easy to handle, which has made them an attractive study material for marine biologists. Since the early 70's an increased urgency has been attached to the study of spiny lobsters owing to their high commercial value. Demand for spiny lobsters has escalated in the past two decades, spurring the need both for better management and for research on which to base that management. Increasing interest in the culture of lobsters has stimulated the need to understand and control reproductive processes. Increased global demand, high production value and recent concern on the sustainability of the wild stock have prompted research into commercial seed production for replenishing of wild stock as well as lobster mariculture (Demestre & Fortuno, 1992).

Furthermore wide tolerance of environmental conditions and higher growth rate make them a suitable candidate species for commercial aquaculture. The progress of lobster mariculture depends on better understanding of their reproductive biology to ensure sufficient supply of high quality larvae.

Despite the importance of *P.homarus* as a candidate species for aquaculture as well as a valuable crustacean fishery resource, knowledge of

Fig.1. *Panulirus homarus*- dorsal view

Fig.2. *Panulirus homarus* -ventral view of male lobster

the basic biology of reproduction of this species is far from well understood. Most of the earlier researchers addressed the physiology of female reproduction rather than that of male reproductive biology, even though reproductive performance of males also play an equally important role in the productivity of captive broodstock. Maturity in males is difficult to define as there is no external criterion (like eggs) for male maturity. There also seems to be a difference between physiological maturity-capability of producing mature spermatozoa and functional maturity-mating capability, since the two seem to occur at quite different sizes.

It is very clearly evident from literature that there are lacunae in the information available on the reproductive biology of the male spiny lobsters (Fig.2). This study focuses on the morphological, histological, ultrastructural and biochemical aspects of the reproductive system of the male spiny lobster, *P.homarus*, which is a commercially important species in our country. The study also provides information on the length at maturity for stock assessment studies.

# Chapter 1.2

# MORPHOLOGY AND HISTOLOGY OF MALE REPRODUCTIVE TRACT

## INTRODUCTION

As already mentioned, spiny lobsters are the largest decapod crustaceans. Inspite of their large size and ubiquitous nature, not much work has been done on their reproductive biology. Inorder to understand postmating selection and how it operates in specific taxa, an understanding of the structure and function of different reproductive organs is imperative (Wortham-Neal, 2002).

In various other decapod crustaceans, detailed investigations on the morphology and histology of the reproductive tract have been accomplished. The first report on the detailed anatomical and histological study of the male reproductive system was that in a crab, *Callinectes sapidus* (Cronin, 1947). The vas deferens of the spider crab, *Libinia emarginata* was studied by Hinsch and Walker (1974). Morphological and functional aspects of the male reproductive tract of the shrimp, *Pleoticus muelleri* was reported (Diaz et al, 2002). Deecaraman and Subramoniam (1980) investigated the male reproductive tract and accessory glands of the stomatopod, *Squilla holoschista*. Similarly in the male Palaemonid shrimp, *Macrobrachium rosenbergii* (Chow et al, 1982) and in the deepwater shrimp, *Aristeus antennatus* (Demestre and Fortuno, 1992), the reproductive tract was studied. Balasubramaniam and Suseelan (2000), reported on the male reproductive system and spermatogenesis in the deepwater crab *Charybdis smithii*.

The reproductive system and its histological nature have been studied only in a few species of spiny lobsters. Since most of the studies on the reproductive tract has centered around vitellogenesis and related aspects, knowledge about male maturation are scarce. The reproductive anatomy of several species of the family Palinuridae has been described. Ortmann (1896) described that of *Panulirus vulgaris* and Mathews (1951) made a detailed study of the male reproductive organs of *P.pencillatus*. Von Bonde (1936) studied the reproduction and embryology of *Jasus lalandii*. The external and internal reproductive organs and the arrangement of the genital apertures have been described in *P.japonicus* (Okamura, 1956) and *J. lalandii* (Paterson, 1968). Reproduction in *J.lalandii* was studied by Fielder (1964). The origin and chemical nature of the spermatophoric mass of

*P.homarus* was investigated by Radha and Subramoniam (1985). The vas deferens of the Spanish lobster (Hinsch & Mcknight, 1988) and spermatid pathway with associated reproductive structure of the squat lobster was also reported (Burton, 1995).

## MATERIAL AND METHODS

Male lobsters, *P.homarus* were collected from Vizhinjam, South of Trivandrum for the studies. They were fished at a depth of 80 m using trammel net and gill net. The animals were transported from the landing centre to the laboratory in jerry cans containing seawater of salinity 35 ppt. Aeration was provided throughout transportation. In the laboratory the animals were maintained in one tonne rectangular FRP tanks and two tonne circular FRP tanks. Seawater salinity in the holding tanks ranged from 33-35 ppt and temperature 26.5°C-27°C. The lobsters were fed mussel/clam meat on alternate days. The animals were grouped as per size into separate tanks. Seawater was changed once in three days so as to keep the dissolved oxygen content above 50% saturation level. Changing of water also helped in the removal of faeces and nitrogenous wastes.

The carapace length (CL) of the animals was measured with a Vernier caliper (CL is the distance between the dorsal midline from the transverse ridge between the supra-orbital horns to the posterior extremity of the cephalothorax) (Berry, 1971). The lobsters were weighed in a monopan balance and excised, by cutting open the carapace. Few drops of 10% formalin were applied after opening the carapace, so as to obtain the reproductive tract intact. The whole reproductive tract was photographed.

Histological sections were prepared by fixing the tissues, which were removed from the lobsters after excising them. The paired testis, proximal vas deferens and distal vas deferens were fixed in Davidson's fixative. Initially, Bouin's fixative was tried but this made the tissues brittle. Hence tissues were exposed to Davidson's fixative for 24hrs and then transferred to 50% Isopropyl alcohol, for storage till further processing (Bell & Lightner, 1988).

### Composition of Davidson's Fixative

95% Ethyl alcohol - 30ml

Formalin - 20ml

Glacial acetic acid - 30ml

| | | |
|---|---|---|
| Glycerol | - | 10 parts |

**Processing**

The tissues were subject to alcohol processing as follows-:

70% Isopropyl alcohol- Two changes, each 30 minutes

80% Isopropyl alcohol- Two changes, each 30 minutes

100% Isopropyl alcohol- Two changes, each 30 minutes

Xylene- Two changes, 30 minutes each

Chloroform- Two changes, 30 minutes each

Molten paraffin- Two separate changes, 30 minutes each

**Embedding**

The tissues were introduced into molten paraffin wax (m.p.56°C) and allowed to solidify. Extreme care was taken to get the correct orientation of the tissue and to avoid air bubbles. The paraffin blocks were cut into 7μm thick ribbons in a hand microtome. Before sectioning the tissue embedded paraffin blocks, were trimmed to suitable size.

## Staining

Modified Mayer's Haemotoxylin and Eosin stain (H &E)

Hemo De- 5 minutes, two changes

100% Ethyl alcohol- 10 dips, two changes

85% Ethyl alcohol- 10 dips, two changes

80% Ethyl alcohol- 10 dips, two changes

50% Ethyl alcohol- 10 dips, two changes

Distilled water- 6 rinses (change water for each rinse)

Haemotoxylin- 4-6 minutes

Running tap water- 4-6 minutes

Eosin- two changes

95% Ethyl alcohol- 10 dips, two changes

100% Ethyl alcohol- 10 dips, two changes

Hemo De- 10 dips, four changes

Histological sections were photographed using a Nikon Fd x 35 microphotography system. The study was conducted during 2003-2006.

## RESULT

The sexes of *P.homarus* are easily distinguishable externally. The male reproductive tract is located in the cephalothoracic region dorsal to the alimentary canal consisting of paired testis and vas deferens (Fig.3). The testis extends from the level of eyes to the insertion of the abdomen. The anterior extension of the testis is partly concealed by the dorsally situated heart. The testis is 'H' shape, semi transparent closely approximated. The mesentery covering the testis extends along its entire length. Each vas deferens originates from the mid region of the testis and extends to the gonopore located at the base of the fifth walking leg. In the immature lobsters, the testis is extremely delicate and transparent structure which could be located and removed only with great difficulty. As the animal mature, the testis change little in appearance except to grow in size and become somewhat opaque.

The reproductive system could be discerned from 35 mm carapace length onwards. In animals with 35-55 mm carapace length, the testicular lobe if left unwound measure 20-22 mm in length and in animals of 55 mm carapace length could measure 35-50 mm.

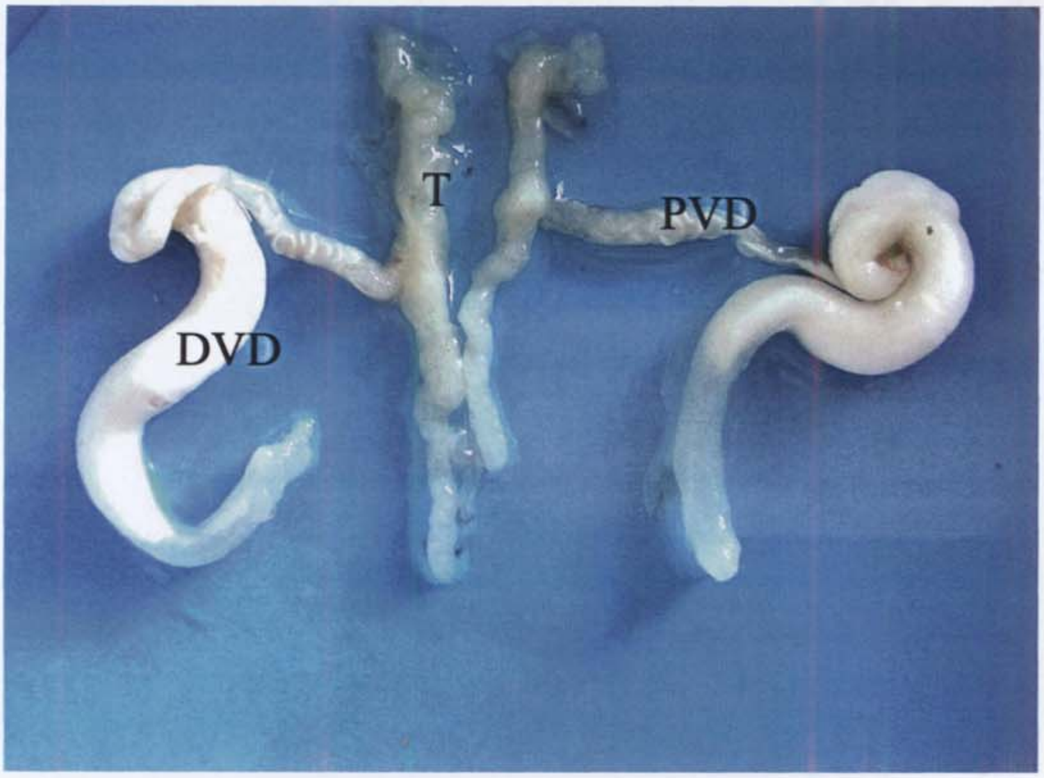


Fig.3. Reproductive tract of male *P.homarus* showing Testis (T), Proximal Vas Deferens (PVD) and Distal Vas Deferens (DVD)

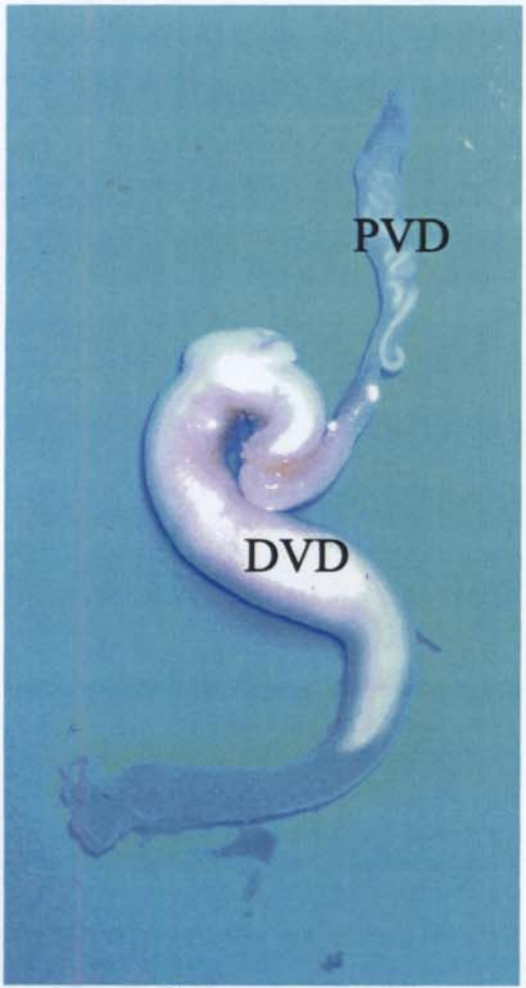


Fig.3a. A portion of the reproductive tract of male *P.homarus* showing Proximal (PVD) and Distal (DVD) Vas Deferens

The vas deferens can be divided into two distinct portions- the highly coiled proximal vas deferens and the distal vas deferens (Fig.3a) - this is dull white and the portion leading to the gonopore is transparent. The distal portion of the vas deferens is encircled by muscles. The vas deferens could not be measured due to the highly coiled nature of the proximal vas deferens. The androgenic gland is located just before the distal vas deferens opens out through the gonopore. Each vas deferens extends from the mid region of the testis to the gonopore located at the base of the 5th walking leg.

## Histology of the reproductive tract

### Testis

The testis is multilobed or with many follicles separated by seminiferous duct (Fig.4 & 4a). Each lobule is independent of the other and connects to the proximal vas deferens through the ducts. The paired testis lying along the alimentary canal, in immature lobsters has the spermatogonial cells filling the entire follicles (Fig.5). These cells measure 6.9-13.3 μm in diameter (Table.1). The lightly basophilic cells in the testis with large round nuclei are the spermatogonial cells and those which are elongate with dark chromatophilic nuclei are the nurse cells or nutritive cells

**Table.1 Characters of spermatogenic cell types of the reproductive tract of *P.homarus*.**

| **Germ cell type** | **Size range (µm)** | **Cytological characteristics** |
|---|---|---|
| Spermatogonium | 6.6-13.8 | Largest among the germ cells, spherical in shape with larger nucleus. |
| Primary spermatocyte | 5.4 -5.9 | Produced by multiplication of spermatogonia, smaller in size. |
| Secondary spermatocyte | 2.8-3 | Produced as a result of reduction division of primary spermatocyte, less cytoplasmic content. |
| Spermatid | 2.2-2.4 | Produced by second meiotic division of secondary spermatocyte, smaller in size, appear as dotlike structures. |
| Spermatozoa | | Produced by metamorphic changes in spermatids by orientation and reorganization of nucleoplasm and cytoplasm, rounded and present in clusters. |

(Fig.4a). Lumen was not observed in the testis of both juvenile and adult lobsters. The development of male gametes from spermatogonia through primary and secondary spermatocytes to spermatids takes place in the acini or follicles. The spermatids measure 2.2-2.4 µm in diameter (Table.1). The testis is covered by a thin outer epithelial layer and inner connective tissue layer. No muscle layer was apparent. In mature testis, spermatogonia are present towards the periphery and the primary and secondary spermatocytes towards the centre (Fig.6). The primary spermatocytes measure 5.4-5.9 µm and the secondary spermatocytes, 2.8-3 µm in diameter (Table.1).

### Proximal vas deferens

The proximal vas deferens is thinner and highly coiled than the distal vas deferens and its lumen serves as a collecting area for the clusters of germ cells entering from the collecting tubule of the testis. In the juvenile lobsters, the outer walls of the proximal vas deferens are not well differentiated. The beginnings of the formation of the spermatophoric mass are visible (Fig.7). Spermatophores housing the spermatocytes are found towards the periphery and spermatozoa in the centre (Fig.8). The spermatophores are embedded in a gelatinous matrix which is brightly stained and basophilic in nature. This

Fig.4. Transverse section of the testis of *P.homarus* showing spermatogonia (SG) and seminiferous duct (SD)

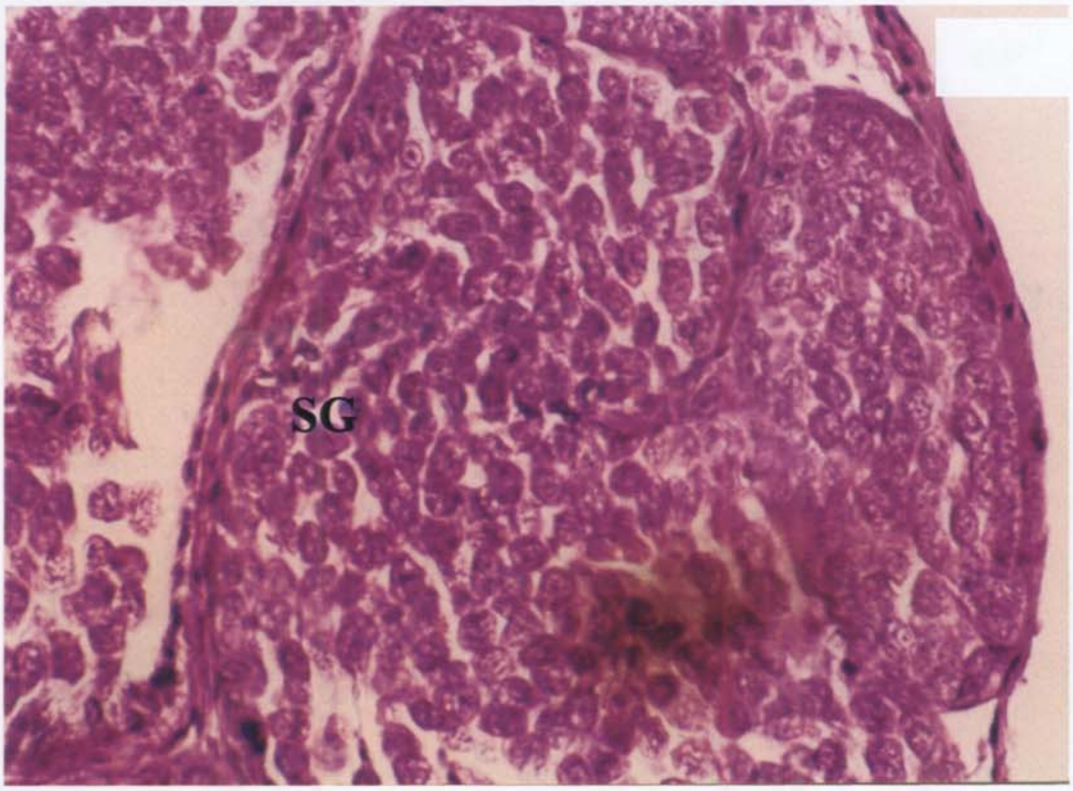


Fig.4a. Closer view of spermatogonial cells (SG) within testis

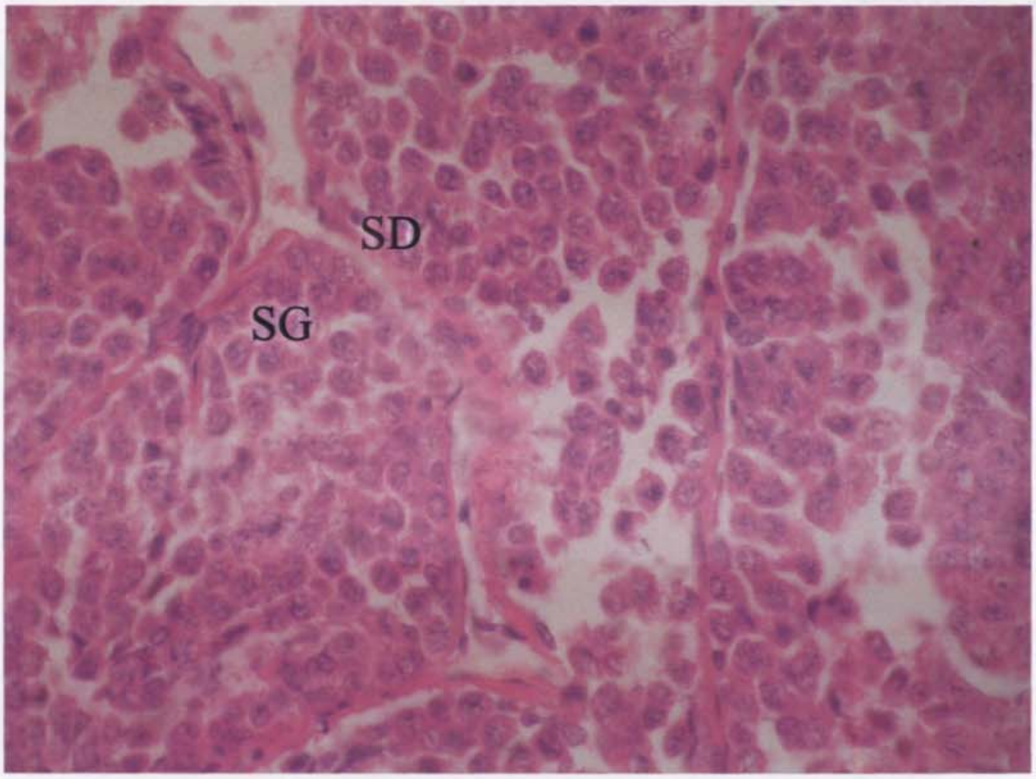


Fig.5. Transverse section of the seminiferous duct (SD) and spermatogonia (SG) in immature testis

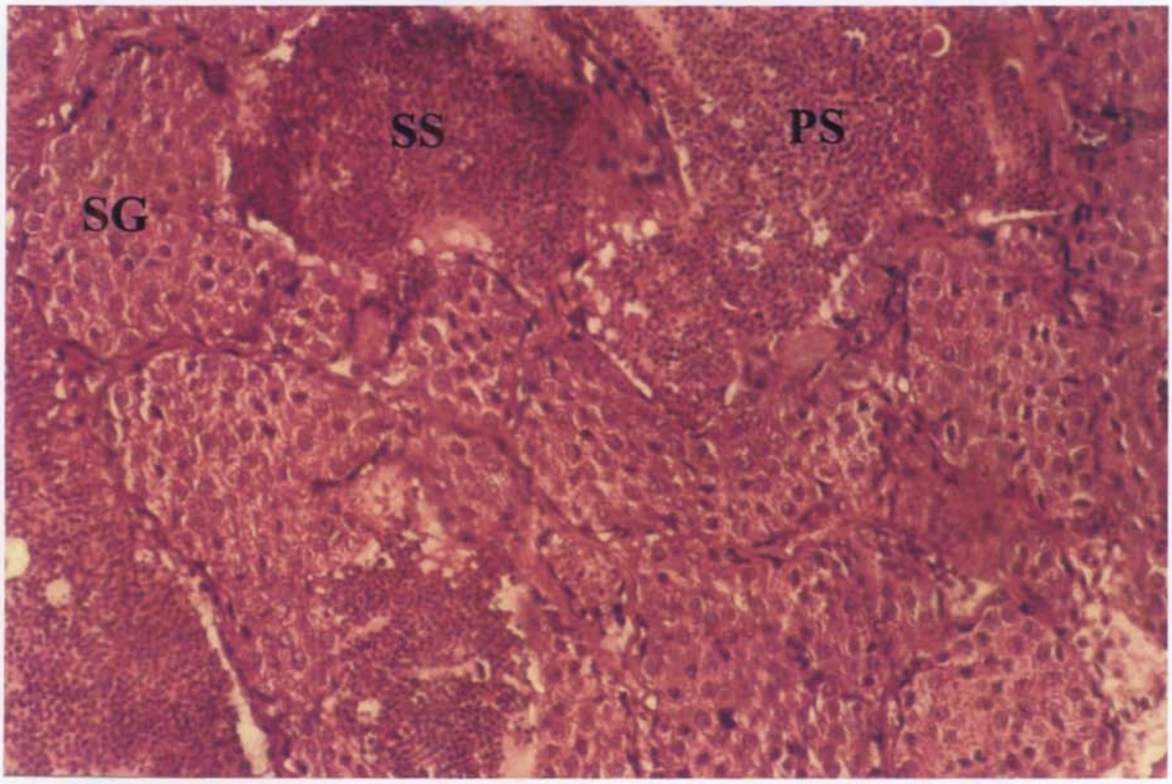


Fig.6. Transverse section of mature testis showing spermatogonia (SG),primary spermatocytes (PS) and secondary spermatocytes (SS)

matrix is formed by the epithelial wall of the proximal vas deferens. In the mature lobsters, the outer covering of the proximal vas deferens are well defined with an outermost connective tissue layer followed by epithelial layer (Fig.8). The spermatozoa filled spermatophore is arranged as discrete masses, filling almost the entire matrix of the proximal vas deferens (Fig.7). The matrix is eosonophilic in nature. The proximal vas deferens conveys the sperms from the seminiferous ducts of the testis to the distal vas deferens and it is also responsible for secreting the acellular layers around the sperms to form the spermatophoric mass. The proximal vas deferens is highly secretory as evidenced by the very thick inner epithelial layer and a very thin outer connective tissue layer (Fig.9).

## Distal vas deferens

The distal vas deferens is almost transparent in the juvenile lobsters. They contain the primary and secondary spermatocytes, which are embedded in a gelatinous matrix. In the mature lobsters the muscular layer is well defined (Fig.14). This is followed by an innermost layer of glandular epithelium, consisting of columnar cells. The epithelial layer invaginates to form the typhlosole (Fig.10). Later towards the posterior end it becomes

Fig.7.PVD of *P.homarus* showing the beginning of the formation of the spermatophoric mass (SM) in the gelatinous matrix (GM).

Fig.8. A closer view of the transverse section of the PVD with spermatozoa (SZ), the acellular layer (AL) and gelatinous matrix (GM)

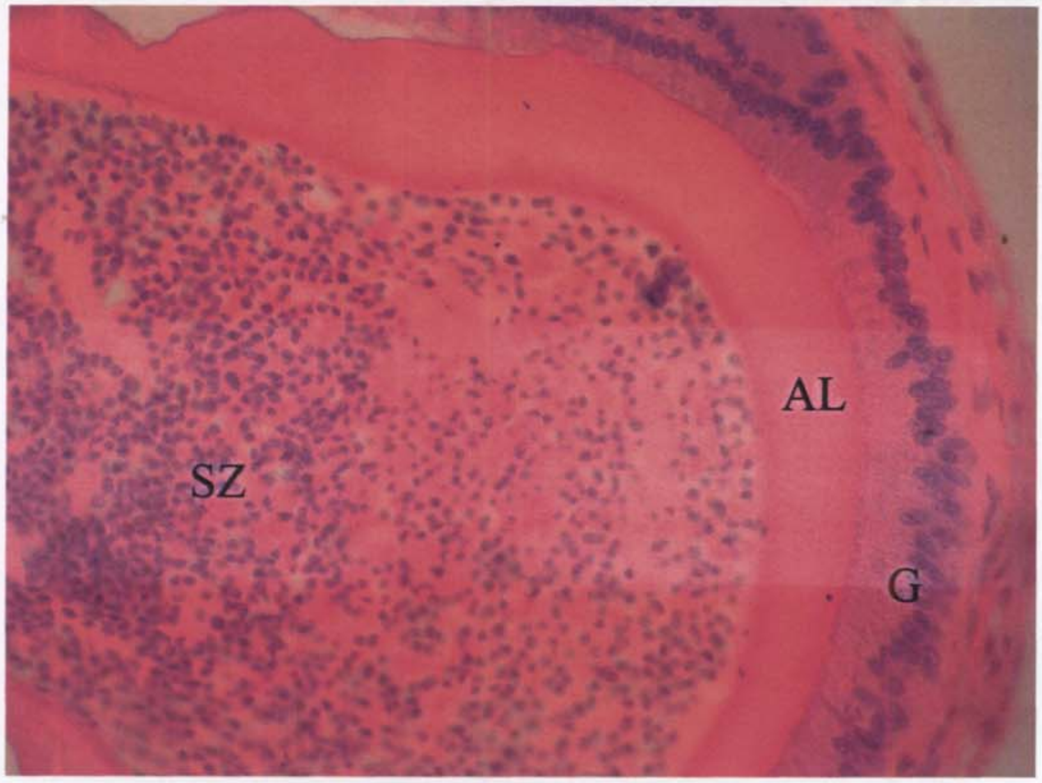


Fig.9. A portion of the spermatophoric mass forming within the PVD with spermatozoa (SZ), acellular layer (AL) and glandular epithelium (G)

Fig.10. Transverse section of the DVD showing beginning of the formation of the typhlosole by the invagination of the muscular epithelium (M)

single leaf like structure (Fig.13). The epithelial cells which cover the typhlosole projections are longer than those which line the lumen. A shaft of connective tissue forms the core of each projection. From one to three longitudinal muscle fibers may be seen in the base of each projection. Spermatophoric mass containing the spermatozoa are neatly arranged towards the periphery, below the typhlosole (Fig.11). This is due to the extensive dorsal production of the matrix by the typhlosole. It is formed in an eosonophilic matrix and spermatophore in a basophilic matrix. The matrix surrounding the spermatphore is much more compact than that found in the lumen. The secretory material originating from the ventral epithelial cells is granular. The typhlosole and adjoining dorsal epithelial cells produce a rather less viscous fluid that is deposited in a lamellar fashion. The posterior part of the distal vas deferens stores the mature spermatozoa prior to ejaculation (Fig.14). In the terminal portion of the distal vas deferens the muscular layer thickens partly by increasing the number of outer circular fibres and also by increasing two masses of inner longitudinal fibres (Fig.14 &15). The circular muscle layer here may function as a sphincter to constrict the continuous spermatophore into a precise length during ejaculation. Vas deferen were not observed to contain spermatophores distally to this region.

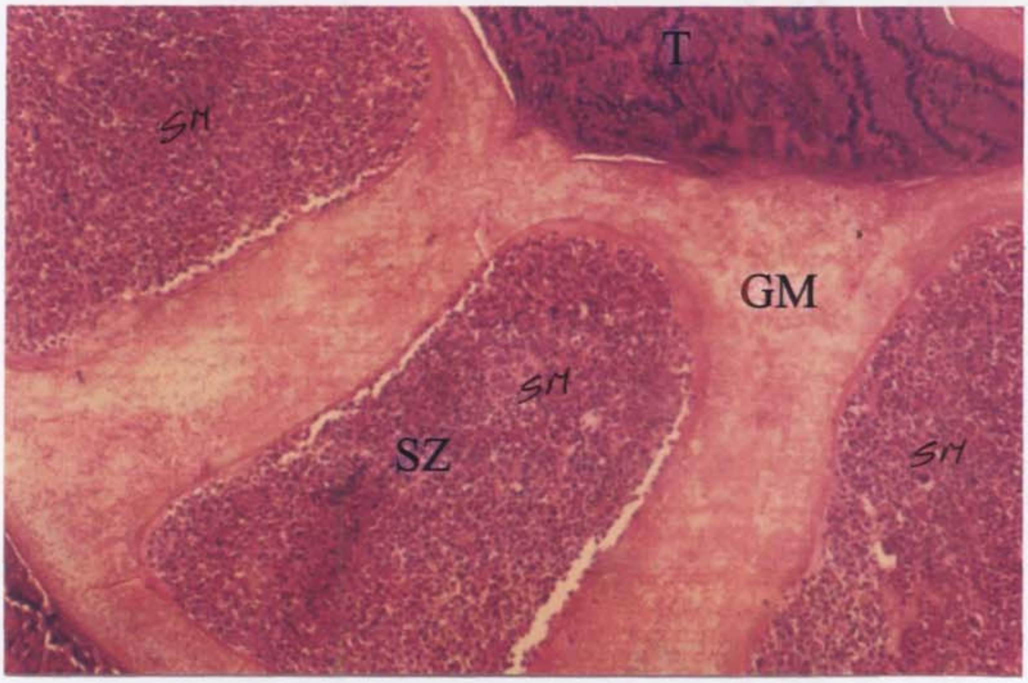


Fig.11. Transverse section of the DVD showing arrangement of spermatophoric mass (SM), spermatozoa (SZ), gelatinous matrix (GM) and a portion of typhlosole (T)

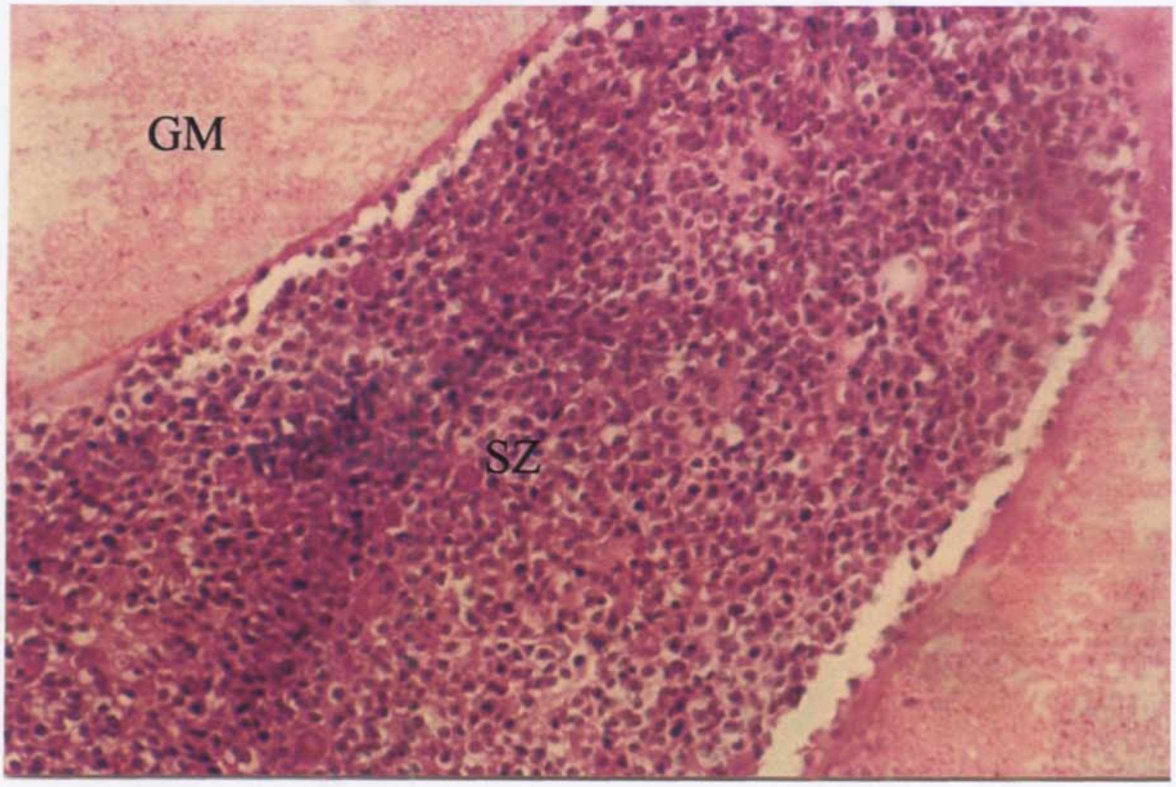


Fig.12. A spermatophoric mass containing the Spermatozoa (SZ) surrounded by the Gelatinous Matrix (GM)

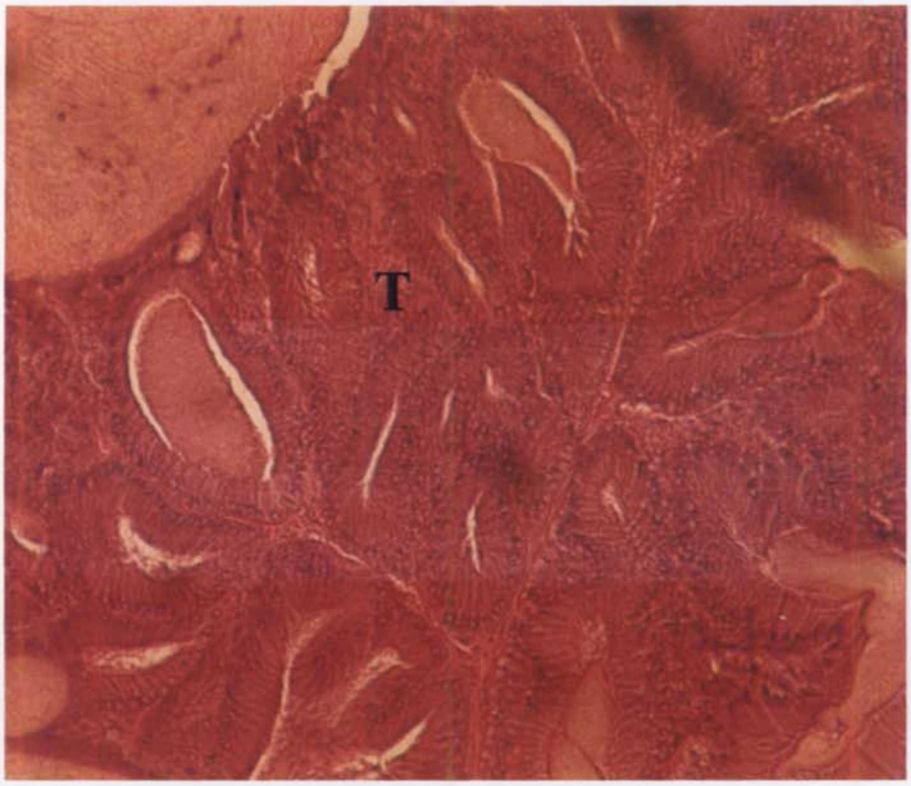


Fig.13. Transverse section of the typhlosole (T)

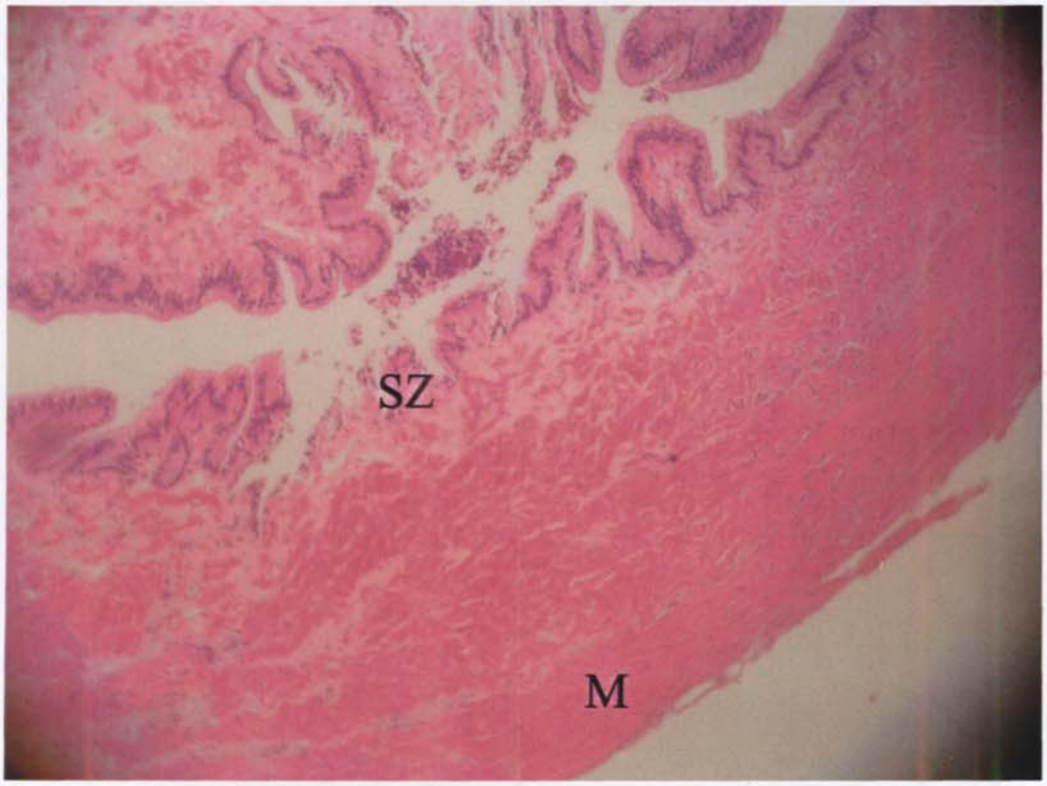


Fig.14. The posterior portion of the DVD showing the muscular epithelium (M), with mature spermatozoa (SZ)

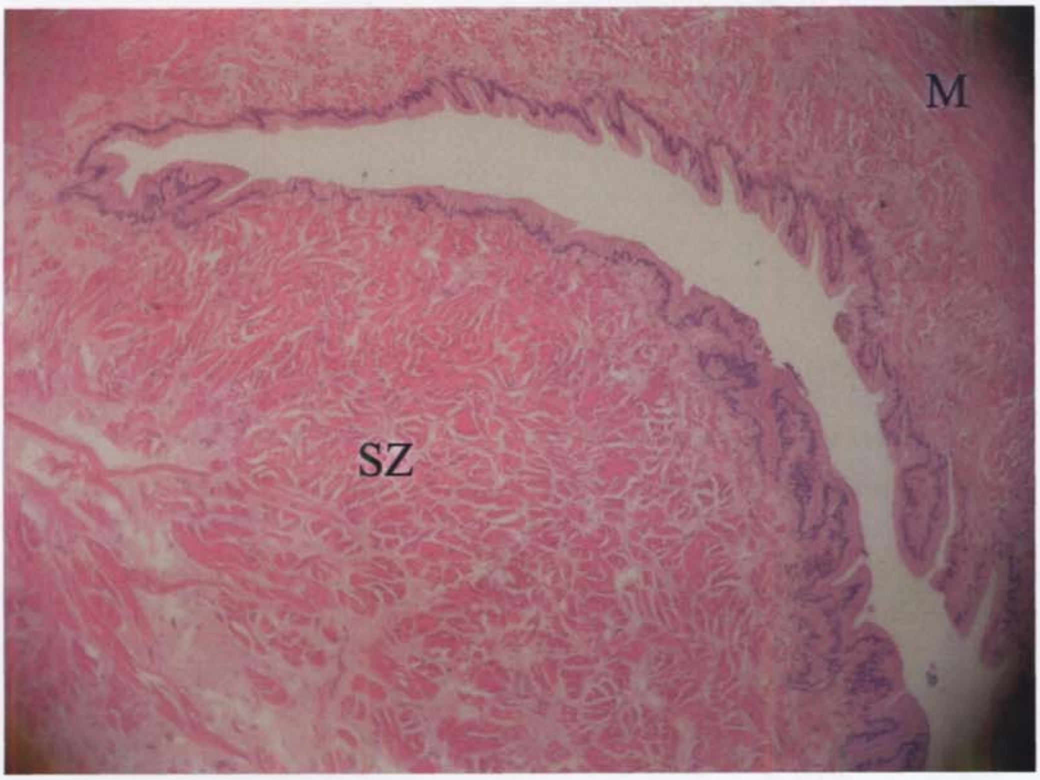


Fig.15. A closer view of the distal portion of DVD showing the mature spermatozoa (SZ) and muscular wall (M)

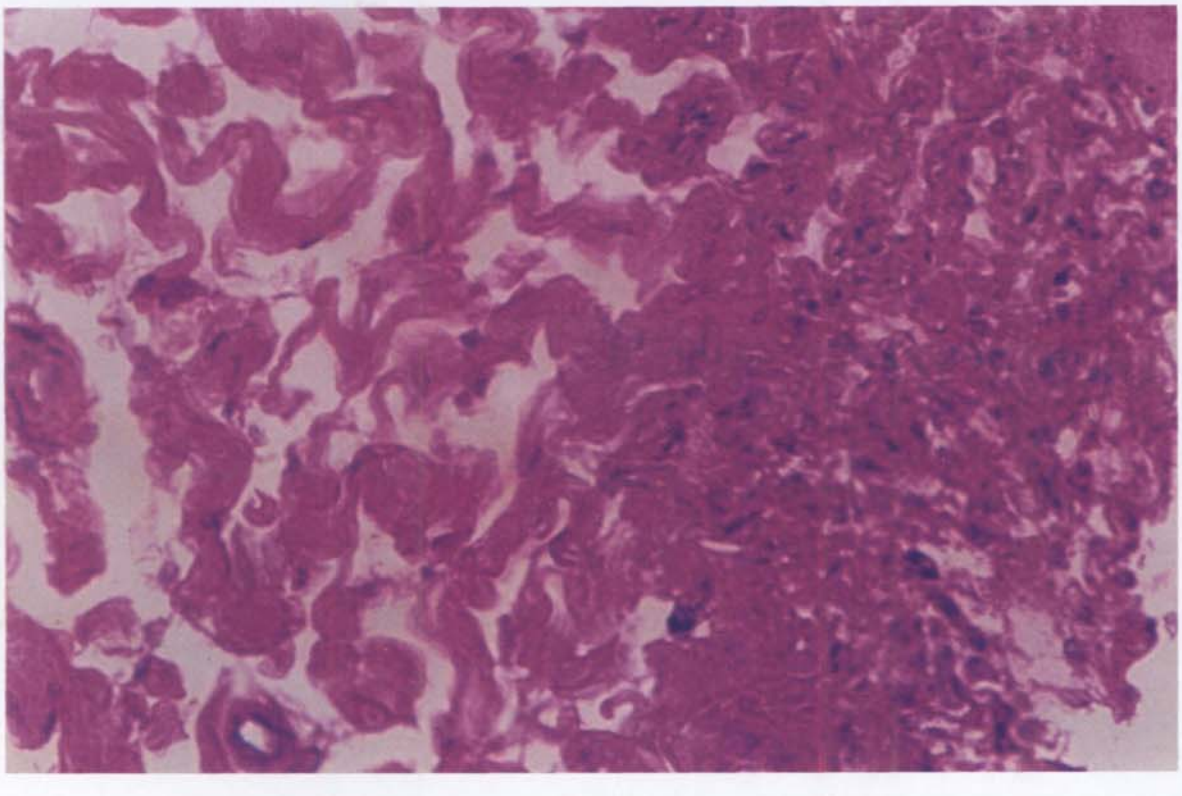


Fig.18. Transverse section of androgenic gland.

## Androgenic gland

This gland is located between the muscles of the coxopodite of the last walking leg and is attached to the subterminal region of the vas deferens (Fig.18). It appears as a transparent gel like cord. Some gaps were observed and the rest of the gland was attached to the distal vas deferens. The gland consists of strands of cells folded back on itself. It is 2-3 cm in length. The cells were found to be oval in shape with large nuclei and distinct celluar boundaries and vacuoles. The nuclei are towards the periphery. Some portions of the gland are degenerate.

## Spermatogenesis

Spermatogensesis is the development of germinal cells from spermatogonia through primary and secondary spermatocytes to spermatids. In spermatogenesis, spermatogonia can either divide mitotically to produce more spermatogonia or undergo cytodifferentiation to become primary spermatocytes. Secondary spermatogonia undergo meiotic prophase to become primary spermatocytes. Secondary spermatocytes form at the end of the first meiotic division. They are smaller than primary spermatocytes. Spermatids are formed from the second meiotic division.

In *P.homarus*, spermatogenesis is synchronous, that is only one or two developmental stages occur concurrently in any segment of the seminiferous tubules. The epithelium of the follicles thickens when it is filled with spermatids and spermatozoa. They move into the collecting duct and are conveyed to the proximal vas deferens. The development of male gametes from spermatogonia through primary and secondary spermatocytes takes place in the acini. In immature lobsters the spermatogonia undergo meiotic division to form spermatocytes and spermatids, which are arranged towards the center. The primary and secondary spermatocytes, spermatids and spermatozoa are observed in the mature testis.

The spermatophoric mass begins to form in the proximal vas deferens (PVD) (Fig.7). As the development of spermatophore progresses they are found as discrete masses filling almost the entire gelatinous matrix of the PVD. The sperms from the seminiferous ducts of the testis are conveyed by the PVD to form the spermatophoric mass. The PVD also secretes the acellular layers around the sperm to form the sperm mass. The sperm mass is compressed by the narrow lumen of the PVD, so that discrete groups of sperms appear in the matrix. Towards the distal vas deferens (DVD), the spermatophoric mass housing the spermatozoa are arranged

(Fig.11), neatly towards the periphery below the typhlosole- the invagination of the glandular epithelium (Fig.13). The typhlosole produces an eosonophilic matrix as a result of which the spermatophoric mass is pushed towards the periphery. The typhlosole is lined with muscles and connective tissue, which is lined with glandular epithelium. The matrix lying below the spermatophoric mass helps in sticking the extruded spermatophore to the female sternum.

The posterior end of the DVD, which is muscular in nature has ejaculatory function. This region by the peristaltic action of the muscle layer extrudes the spermatophoric mass at the time of mating. These emerge through the gonopore located at the base of the 5th walking leg on either side and attach to the ventral sternum of female.

## DISCUSSION

### Morphology

In most of the decapods, the male reproductive tract is situated either in the cephalo-thorax or thorax. In *P.homarus*, the male tract is paired and located in the cephalothoracic region opening at the base of the 5th walking leg, as in *P.argus* where the testis and the ducts are also paired opening at the base of the fifth walking leg (Talbot & Summers, 1978). In the spiny lobster, *J.lalandii* also, the testis are paired organs situated on either side of and dorsal to the alimentary canal. They are joined by a transverse bridge near the heart (Fielder, 1964). In *P.homarus* also, a transverse bridge is visible near the heart. In this species the testis is 'H' shaped. In the crab-*Callinectes sapidus* (Johnson, 1980) and *Chionoecetes opilio* (Beninger et al, 1988) the testis is 'H' shaped. But *P.homarus* differs from the palaemonid shrimp, *M.rosenbergii*, where the testis is united at their anterior ends forming a 'V' shape (Chow et al, 1982). In the male *C. smithii*, the reproductive tract is bilateral and composed of testis, vas deferens, ejaculatory duct and external penis (Balasubramaniam & Suseelan, 2000).

In the male deep water shrimp, *A.antennatus* (Demestre & Fortuno, 1992), the reproductive tract consists of paired testis and vas deferens similar to what is observed in this study, but it is located in the thoracic region. In the crab *Metopograpsus messor*, the paired testis is highly coiled lying in the cephalothroax and fuse distally leading to the vas deferens (Suganthi and Anilkumar, 1999). In *Penaeus setiferus* (King, 1948), the male reproductive system consists of paired testis and vas deferens with petasma as in most shrimps.

In the present study the testis extends up to the telson and is fused by the transverse bridge near the heart. In the mantis shrimp, *Squilla empusa* (Wortham-Neal, 2002), the males have paired testis which fuse at the telson. In the stomatopod, *S.holoschista,* Deecaraman & Subramoniam (1980), has described a pair of elongated testis, fused distally and a pair of accessory reproductive glands. In the anomuran families, Coenobitidae and Paguridae, the male reproductive organs are located in the abdomen (McLaughlin, 1980).

In decapods, the vas deferens is divisible into distinct regions, the number of distinct regions differing among the decapods. In *P.homarus*, the vas deferens consists of two regions- the proximal and distal vas deferens with the ejaculatory duct. King (1948) has distinguished four regions in *P.setiferus* whereas Farmer (1974) has recognized three in *Nephrops norvegicus*. In the hermit crab *Pagurus novaezealandiae,* Greenwood (1972) has distinguished three regions externally and seven internally. Four regions have been identified in several brachyuran species (Cronin, 1947; Ryan, 1967; Hinsch and Walker, 1974).

In all the orders studied, except in the isopoda, the androgenic gland is located between the muscles of the coxopodite of the last walking leg and is attached to the subterminal region of the vas deferens (Charniaux-Cotton, 1960) as has been observed in *P.homarus*. The length of this strand varies with the species and in the same species with the size of the male. An androgenic gland lying in close proximity to vas deferens has been shown in various malacostacans to regulate male morphogenesis and spermatogenic activity (Charniaux-Cotton et al, 1966; Lockwood, 1967; Hoffman, 1968; Nath et al, 1972).

## Histology

The different portion of the male reproductive system of decapods has structural and functional variations. The histological structure of the male reproductive tract was described in several species of decapod crustaceans, such as *Melicertus kerathurus* (Malek & Bawab 1974a), *Marsupenaeus japonicus* (Bizot-Espiard, 1980), *Farfantepeneus notialis* and

*Litopenaeus schmitti* (Guitart et al, 1985) and *Macrobrachium borellii* (Verdi & Delgado, 1998). In general crustaceans have a glandular anterior half and posterior half of the system is ejaculatory.

Many decapods like the shrimps-Sicyonidae, Aristidae and lobsters-*P.pencillatus*, *Enoplometapus occidentalis* and the golden deep sea crab *Greyon fenneri* have testis composed of multiple lobes (Heldt, 1938; Mathews, 1951; Haley, 1984; Hinsch, 1988). The testis of *P.homarus* has multiple lobes, each of which is independent of the other lobes and connects to the vas deferens by a short thin connecting tube. In the crab, *Portunus sanguinolentus* (Ryan, 1967), testicular lobation was only an occasional feature and whenever noticed it was incomplete. In *C.smithii* (Balasubramaniam & Suseelan, 2000), also the testis is with incomplete lobation and divided into many acini. In the present study no lumen was evident and the testis was observed to be filled with numerous follicles or acini separated by the seminiferous ducts with each follicle having cells in one or two stages of development. In *J.lalandii,* Fielder (1964) described the transverse sections of the testis with follicles of varying degrees of maturity. In the deepwater shrimp, *A.antennatus* (Demestre & Fortuno, 1992) all the germinative cells are at the same stage of development.

*P.homarus* has multiple testicular lobes, each of which are independent of the other lobes and connects to the vas deferens by a short, thin connecting tube as reported in penaeid shrimps (Ro et al, 1990). Mathews (1951) described the testis of *P.pencillatus* as racemose or compound glands of freely branching ducts terminating in acini. This convoluted tube finally emerges as vas deferens. In the crab, *C.smithii,* Balasubramaniam and Suseelan (2000) found the testis with numerous acini arranged around a central seminiferous duct or collecting tubule. *P.homarus* does not have muscle layers around the testis and is covered by an outer epithelial layer and an inner connective tissue layer. In shrimps also no muscle layer is apparent in the testis (King, 1948; Bell & Lightner, 1988).

In different species of crustaceans, the vas deferens is divided into distinct parts with varying functions. In *P.homarus,* the proximal vas deferens is highly coiled conducting the sperm cells from the collecting tubules of the testis to the distal vas deferens and produces secretions responsible for the coalescing of the sperm cells into a coherent mass. Haley (1984) observed that in the Hawaiian lobster, *E.occidentalis*, the proximal vas deferens forms a coil lying outside the testicular mesentry. The length and coiling of the vas deferens may serve to increase the surface area for storage, secretory and absorptive functions (Adiyodi & Anilkumar, 1988). The eosonophilic secretions produced by the epithelial lining of the proximal vas deferens was described in *J.lalandei* (Fielder, 1964) and in *P.homarus* from African waters also (Berry & Heydorn, 1970). Deecaraman and Subramoniam (1980) observed the proximal vas deferens of the squilla, *S.holoschista* to be highly glandular and responsible for the synthesis of substances such as protein, carbohydrates and lipid. They suggest that the proximal vas deferens in this species may play a nutritive role in the maturation of the spermatozoa.

This study reports the homogenous secretions of the typhlosole of *P.homarus,* to push the spermatophoric mass towards the periphery as reported in *Procambarus clarkii* (Moses, 1961a), the red swamp crayfish and in the carribean spiny lobster, *P.argus* (Haley, 1984). Most of the spiny lobsters have typhlosole in the distal vas deferens (Berry & Heydorn, 1970). The epithelial lining of the distal vas deferens has undergone extensive modifications giving rise to the glandular areas. The invagination of the glandular epithelium of the distal vas deferens into the lumen was termed as the 'typhlosole' by Mathews (1951) in *P.pencillatus*. It is responsible for secreting the gelatinous matrix of the spermatophoric mass. Fielder (1964) states the sperm mass to be embedded in a homogenous matrix in *J.lalandii* is apparently secreted by the typhlosole. In *Emerita asiatica* (Subramoniam, 1984), the typhlosole corresponds to that of *P.homarus,* in that the typhlosole consists of long columnar cells with multilobation at the peripheral region forming the shape of a leaf. *S.holoschista* too has typhlosole in the distal vas deferens, but they do not form the outer protective layer of the spermatophore as in *P.homarus*. However, in *Albunea symnista* there are two conical shaped dorsolaterally placed typhlosoles (Subramoniam, 1984).

In *P.homarus*, the distal end of the vas deferens is muscular and has ejaculatory function. It is also responsible for the storage of the completely formed spermatophoric mass ready for extrusion to the female, on mating. According to Fielder (1964), the muscular contraction of the walls of the vas deferens expels the spermatophoric mass. The terminal portion of the distal vas deferens in *P.homarus* appears to correspond to the ejaculatory region described for *N.norvegicus* (Farmer, 1974).

In *P.homarus* the distal vas deferens stores the mature spermatozoa after completion of the maturation process here. At the time of mating, the muscular wall of this portion of the distal vas deferens contracts to ejaculate the mature spermatozoa to the female sternum, this region also acting as an ejaculatory duct. In the penaeid shrimp, the enlarged distal vas deferens is termed the terminal ampoule. It has a single layer of tall columnar epithelial cells surrounded by a thick layer of connective tissue and muscle in *P.vannamei* and *P.stylirostris* (Bell and Lightner, 1988). In these species, in the terminal ampoule spermatophores are prepared for placement on the female. In the Indian white prawn, *P.indicus*, the terminal ampoule is believed to have both storage and secretory functions (Champion, 1987). In the brachyuran crabs, the proximal vas deferens is secretory in nature whereas the distal vas deferens has storage and ejaculatory function (Cronin, 1947; Ryan, 1967). In *P.homarus*, the distal vas deferens has secretory, storage and ejaculatory functions.

The cellular structure of the androgenic glands of *P.homarus* is similar to that of other decapod crustaceans (Carlisle, 1959; Hoffman, 1969; Thampi and John, 1972; Fingerman, 1987), consisting of epithelial cells with large oval nuclei and often showing heavy vacuolation. *P.homarus* displays typical crustacean androgenic glands. Androgenic glands are generally observed as cords of epithelial cells folded upon themselves and covered in connective tissue with cells showing oval or round nuclei and often vacuoles (Charniaux-Cotton, 1960). Androgenic glands with similar structures have been observed in various species of crabs (King, 1964; Tcholakian and Reichard, 1964; Adiyodi, 1984; Minagawa et al., 1994), prawn (Carlisle, 1959; Hoffman, 1968, 1969; Thampy and John, 1970; Payen et al., 1982) and crayfish (Puckett, 1964, Warnock, 1975). The androgenic gland secretes the male hormone in the invertebrates. The androgenic gland in *P. homarus* constitutes an example of a separate endocrine sex gland, contrary to that in vertebrates, where the sex hormones are secreted by the interstitial tissues of the gonads. Future studies on the androgenic gland of *P.homarus* are required to establish its control over secondary and possibly primary sexual differentiation in spiny lobsters. The paucity of literature does not permit to draw comparison with other spiny lobster species.

Although the reproductive system shows considerable variation in the Macrura, Anomura and Brachyura, in representatives of each group, spermatogenesis occurs within lobes of sacculi of the testis, masses of spermatozoa are assembled into spermatophores in the distal vas deferens which plays a role of some importance in each species.

## Spermatogenesis

Information on the maturation of a species is very much essential to have a basic knowledge of its biology. Not much attention has been paid to the development of male gametes in spiny lobsters and most attempts at mariculture have been practiced with insufficient information. The upsurge in crustacean aquaculture has stimulated interest in the control of crustacean reproduction in the last few years.

The study of spermatogenesis provides information on the origin and maturation of sperm. During the reproductive cycle, the gonads in crustaceans undergo a sequence of major morphological and physiological transformations. The studies on spermatogenesis on a structural and ultrastructural level carried out on crustacean species of different taxonomic groups (King, 1948; Genthe, 1969, Malek & Bawab, 1974a, b; Aiken & Waddy, 1980) have led to the finding that the process is basically similar in all species. However, the formation of the spermatophore in decapod does not always follow the same pattern nor is the functional structure the same.

In *P.homarus*, the spermatocytes and spermatids move down into the vas deferens and are packaged into spermatophores. In the majority of decapod species, it is thought that spermatozoan formation takes place in the testis. But in *P.homarus*, the formation of the mature spermatozoa continues into the distal vas deferens and is completed here. In *Inachus falangium* (Diesel, 1989), it was found that mature spermatozoa are formed in the medial portion of the vas deferens. Malek and Bawab (1974b) have reported the formation of sperm mass and the role played by the proximal and distal vas deferens in its formation in *P.kerathurus*. In this species the spermatophore acquire final form in the terminal ampoule. In *P.homarus*, as testicular maturation progresses, the spermatogonia undergo mitotic division to produce more spermatogonia and meiotic division to form primary spermatocytes. This has been reported in other crustaceans like *P. clarkii*, the red swamp crayfish and in the carribean spiny lobster, *P.argus* (Lu et al., 1973; Melville Smith, 1987). The proximal vas deferens of the crab, *Libinia emarginata* helps in spermatophore formation and the distal vas deferens forms the seminal secretions and stores spermatophores (Diesel, 1989).

In the present study the testicular epithelium of *P.homarus* was found to always contain at least two cell types. In *J.lalandii*, Fielder (1964) described the transverse sections of the mature testis with follicles of varying degrees of maturity. In this study we could observe only spermatogonia in the immature testis and germinative cells in different stages of development in the mature follicles denoting asynchronous development. Asynchronous development has been reported in the brachyuran crab *Menippe mercenaria* (Binford, 1913) and *Sicyonia ingentis* (Shigekawa and Clark, 1986). The acini encompass the germ cells at various stages of proliferation and maturation in *M.messor* (Suganthi and Anilkumar, 1999). Haley (1984) studied the male reproductive tract of the Hawaiian red lobster, *E.occidentalis* and documented the cyclic nature of spermatogenesis and spermatophore formation. He observed the spermatogenesis in each testicular follicle to be cyclic, characterized by a distinct set of cytological transitions.

In *P.homarus,* the development of spermatozoa continues as the cells are transported towards the vas deferens. Similar observations were reported in *C.smithii* also (Balasubramaniam & Suseelan, 2000). In Calanoid copepods, in general only mature spermatozoa are discharged from the testis into the vas deferens. However *P.homarus* differs from *Labidocera aestiva,* (Blades and Youngbluth, 1991) in showing a notable change in the morphology of the spermatozoa from spherical to a fusiform shape or somewhat spindle shape inside the seminal vesicle. In *P.homarus* spermatogenesis was observed to occur in the testicular tissue and the vas deferens as observed in the pandalid shrimp *Pandalopsis japonica* (Medina, 1994),

In the present study, the glandular epithelium of the distal vas deferens or the 'typhlosole' was observed to be secretory in nature. The typhlosole has columnar epithelium with multiple lobes at the peripheral region forming the shape of a leaf similar to that reported in *P.homarus* from African waters (Berry, 1970). In *A.symnista*, there are two dorsoventrally placed typhlosoles (Subramoniam, 1984). In the brachyurans, the proximal vas deferens is secretory and the distal vas deferens has storage function (Hinsch & Walker, 1974). But in macrurans the distal vas deferens too is highly secretory, producing the accessory mucoid spermatophoric substances (Berry & Heydorn, 1970).

In *P.homarus*, fertilization occurs externally with the spermatophore being deposited on the ventral sternum of the female at mating. In decapod crustaceans, fertilization occurs either externally or internally. When it is external, the spermatophoric mass is deposited on the ventral side of the female and is kept intact pending ovulation. Anomurans and Macrurans are the best known examples for this type of fertilization (Subramoniam, 1977). In majority of crustaceans, the spermtophores are deposited externally on the ventral sternum of the females or inserted into the seminal receptacles. In *Squilla empusa* (Wortham-Neal, 2002) males package sperm and accessory gland material into a sperm cord and is then transferred to the female and stored in the seminal receptacle.

## Spermatophore

The crustacean spermatophores have evolved to protect the sperm during their transfer to the females as the aquatic ancestors moved to dry land (Schaller, 1980). The decapoda, the phylogenetically most advanced among the crustaceans, generally resort to spermatophoric transmission of spermatozoa. They, like insects, seem to have adopted transfer of sperms via spermatohores as a means to circumvent the absence of well organized copulatory organs for the safe transfer of sex cells to the female genital system. The structure of the spermatophores varies within Crustacea according to the method of sperm transfer (Subramoniam, 1984).

Spermatophore morphology has been studied with great interest due to its phyletic importance and also because of the development of the technology for the artificial insemination (Subramoniam, 1993). Decapod Crustaceans produce different types of spermatophores, which is mostly habitat related. The simplest type of spermatophore is spherical in shape, found in the brachyuran crabs (Hinsch & Walker, 1974). Pedunculate type of spermatophore is found in Anormurans (Greenwood, 1972). The macrurans produce the spermatophore as a mass, as found in this study in *P.homarus.*

In *P.homarus*, spermatogenesis continues as the spermatocytes and spermatids move down the vas deferens and the final form is attained in the distal vas deferens. During the course of its formation, several protective layers are formed around the spermatophore. The acellular secretions are produced by the glandular epithelium of the proximal vas deferens as well as distal vas deferens in *P.homarus*. The proximal vas deferens of *J.lalandii* does not secrete granular wall around the spermatophore, but appears to initiate secretion of a fluid matrix. The resultant spermatophore appearing in the distal vas deferens consists of clumps of sperm embedded in the fluid matrix (Fielder, 1964). In *E.occidentalis* there is no evidence of acellular secretions (Haley, 1984). Cells that secrete the spermatophore wall are located in the anterior vas deferens in *L.emarginata* (Hinsch, 1972). In *Cancer borealis*, (Langerth, 1969) the wall of the spermatophore is supplied with epithelial cells of proximal vas deferens. In the present study, the spermatophores are arranged towards the periphery of the distal vas deferens in a gelatinous matrix. The typhlosole is responsible for the arrangement of the spermatophoric mass as packets along the periphery.

The spermatophore of *P.homarus* when removed from the vas deferens is thick and sticky in consistency and hardens and darkens in sea water, but in *P.gilchristi*, the outer protective layer being gelatinous in nature, it does not harden in seawater (Berry, 1970). Palinurid spermatophores undergo hardening and blackening after attachment to the female sternum (Subramaniam, 1984). He states that the spermatophore hardening in these lobsters might have evolved in response to shallow, turbulent water conditions where a soft spermatophoric mass would be liable to be washed off. This condition may imply a trend towards prolonging the interval between mating and oviposition. Many reports have appeared on the occurrence of spermatphores in lobsters belonging to different species (Andrews, 1931). Mathews (1951) was the first to report on the spermatophores in a lobster species- *P.pencillatus* and the mechanism of sperm release during fertilization. He described the spermatohore of this species to be 'putty like' in consistency, which is similar to our observations in *P.homarus*.

In *P.homarus*, the spermatophores remain deposited on the ventral side of the female sternum until fertilization. Crustacean spermatophores once transferred to the female maintain their integrity until spawning and fertilization (Aiken & Waddy, 1980). In palinurid lobsters and scyllarid lobsters, the spermatophoric mass is deposited externally on the ventral sternum of the female, whereas in homarid and nephropsid lobsters, they are stored within the seminal receptacles (thelycum) of the female. The contents of the vas deferens are ejaculated simultaneously from the genital pores of both sides at mating as observed in the present study. The same was reported in the palaemonid shrimp, *M.rosenbergii* (Chow et al, 1982, 1989). In the best of our histological sections of the hardened spermatophoric mass attached to the sternum of the female lobsters, we could not detect the spermatozoa. These were perhaps lost from the spermatophore while sectioning.

This is a comprehensive study of the basic morphological and histological nature of the male reproductive tract of the Indian spiny lobster, *P.homarus*. A better understanding of the reproductive system requires ultrastructural investigation of the reproductive tract.

# Chapter 1.3

# ULTRASTRUCTURE OF SPERMATOGENESIS-MALE MEIOSIS AND SPERMIOGENESIS

## INTRODUCTION

Decapod crustaceans have non flagellate sperm which envisaged lot of interest in the study of their spermatogenesis. They are apparently non motile, as they have not been observed to move when removed from the testis and vas deferens of the male or the seminal receptacle of the female when observed with the light microscope under varying osmotic conditions (Brown, 1966). Among crustaceans, flagellated spermatozoa have so far been reported only in the Mystacocaridae, Cirripedia and Branchiura. But non flagellate male gametes with considerable morphological diversity are common among crustaceans (Pochon-Masson, 1968).

The aflagellate sperms of decapods were first described by Grobben (1878). But significant progress in the understanding of spermatogenesis in decapods was achieved only after the introduction of the electron microscope. The earliest report on the ultrastructure of the reproductive system of male crustaceans is in the crab *Paratelphusa spinigera* (Nath, 1932). Later Hinsch studied the structure of sperm of *Libinia emarginata*

(1969), *Oxyrhyncha* (1973), *Coenobita clypeatus* (1980), in the two golden crabs, *Greyon fenneri* and *G.quinquedens* (1988) and in the anomuran crab, *Pleuroncodes planipes* (1991). He also compared the morphologies, mode of transfer and sperm storage between two species of crab, *Ovalipes ocellatus* and *L.emarginata* (1986). Langerth (1969) explained spermiogenesis in cancer crabs. Ulrastructural studies were conducted in other brachyurans also: *Pinnixia* sp (Reger, 1970), *Callinectes sapidus* (Brown, 1966), *Eriocheir japonicus* (Yasuzumi, 1960), *Rhynchocinetes typus* (Dupre & Barnes, 1983), coconut crab, *Birgus latro* (Tudge & Jamieson, 1991) and *Cervimunida johni* (Lohrmann, 1995). Pochon-Masson (1968) published an account of spermiogenesis in the crab *Carcinus maenas* and in the anomuran *Eupagurus bernhardus*. Spermiogenesis in *Uca tangeri* was investigated by Medina and Rodriguez (1992) and the fine structure of the spermatozoan of *Dromidiopsis edwardsi* by Jamieson & Scheltinga (1993).

Until recently ultrastructural studies on natantians were limited. Now transmission and scanning electron microscopical studies define the developing and mature spermatozoan features of *Sicyonia ingentis*, the grass shrimp, *Palaemonetes kadiakensis*, *Macrobrachium rosenbergii*, *Penaeus setiferus*, *Rhynchounetes typus*, the riverine grass shrimp, *Palaemonetes* *paludosus*, *Procaris ascensionis*, *Penaeus aztecus*, *Palaemon elegans*, Pandalid shrimp *Panddopsis japonica*, *Crangon septemspinosa* and *Parapenaeus longirostris*. (Pochon Masson, 1969; Lu, 1976; Koehler, 1979; Kleve et al, 1980; Dupre & Barnes, 1983; Lynn & Clark, 1983; Shigekawa & Clark, 1986; Felgenhauer et al, 1988; Griffin et al, 1988; Arsenault, 1979 and Medina, 1994; Kim, 2003). In natantian decapods, many studies on sperm ultrastructure have been done in penaeidean shrimps of the suborder Dendrobranchiata (Clark et al., 1973; Kleve et al. 1980; Krol, 1992; Medina, 1994; Medina et al. 1994) and suborder Pleocyemata (Koehler, 1979; Lynn and Clark, 1983)

Compared to the published information available on the ultrastructure of the reproductive tissues and spermatogenesis of crabs and shrimps, studies on lobsters are scarce. Kooda-Cisco and Talbot (1986) described the fine structure of the cells of the proximal vas deferens, in relation to their secretory role in producing the spermatophore layers. In *P.interruptus*, the fine structure of the freshly extruded and hardened spermatophores was investigated (Martin et al, 1987). Talbot and Summers (1978) reported the structure of the sperm from *P.argus* and *P.guttatus* with special reference to acrosome. Besides these only three genera of lobster sperm- *Homarus,* *Nephrops and Jasus* have been described ultrastructurally (Pochon-Masson, 1969; Tudge et al, 1986). Haley (1986) described the ultrastructure of spermatogenesis in the axiid lobster *E. occidentalis*.

The process of spermatogenesis involves both male meiosis and spermiogenesis. The latter is the process of formation of spermatozoa by cytoplasmic changes and condensation of nucleus of spermatids without further division. The current study investigates the process of spermatogenesis in *P.homarus* at the ultrastructure level. This study describes the process of spermatogenesis with emphasis upon the spermatogonia, spermatocytes, spermatids, spikes, lamellar region and mature spermatozoan and relates these observations to the structural features of other decapod sperms.

## MATERIAL AND METHODS

### Tissue samples

*P.homarus* of carapace length ranging from 45-82 mm obtained from lobster fishers off Vizhinjam were used for the study. The carapace was cut open after killing the animals under cold treatment (exposing the lobsters to

–20°C for 15-30 minutes). Few drops of glutaraldehyde were introduced, immediately on cutting open the carapace. The different regions of the reproductive tract- testis, proximal vas deferens and distal vas deferens, were cut into 1mm size and fixed in 3% glutaraldehyde for 4-5 hours (in the refrigerator). These were washed in ice cold caccodylate buffer (working solution).

**Working solution**

Caccodylate buffer stock solution-10 ml
Double distilled water- 10 ml

The tissues were treated to three changes in the working solution each change lasting 30 minutes, in the refrigerator and later stored in fresh working buffer solution in the refrigerator till further processing.

**Post fixation**

The tissues were post fixed in freshly prepared 1% osmium tetroxide in 0.1M caccodylate buffer for 2 hours at 4°C.

### Washing and refrigeration

The tissues were washed five times for 15 minutes each, followed by refrigeration of tissues overnight in fresh buffer.

### Staining

The tissues were stained in fresh 0.5% aqueous uranyl actetate for 3 hours under refrigeration followed by repeated washing in double distilled water.

### Dehydration

The tissues were exposed through graded alcohol series-30%, 50%, 70%, 95% and 100% for 15 minutes each at 4°C followed by two changes in pure acetone for 10 minutes each at room temperature.

## Infiltration

The tissues were treated in 2:1 acetone-Spurr's resin (Spurr, 1969) for 1 hour, 1:1 acetone-spurr's resin for 1 hour, 1:2 acetone-Spurr's resin for 1 hour and in 100% spurr's resin overnight under refrigeration.

## Embedding

Now the tissues were embedded in fresh resin taken in plastic capsules for one hour at room temperature followed by incubation at 70°C for 48 hours.

## Trimming

The resin blocks were trimmed using a glass knife fitted to an ultramicrotome-LKB 2128, ultratome W, Bromma, Sweden.

## Sectioning

Ultrathin ribbons were sectioned in distilled water. Wrinkles were removed by exposing them carefully to chloroform vapours.

## Staining

The sections were mounted on carbon coated copper grids, stained (Hayat, 1970) in fresh filtered 2% uranyl acetate in 50% ethanol for 15 minutes, rinsed in glass distilled water thrice. Then they were treated in fresh filtered 0.4% lead citrate in 0.1N NaOH for 5-10 minutes and later exposed to 0.02N NaOH for 5 seconds.

## Screening and photography

The sections were examined in CARL, ZEISS Electron Microscope, 109 R Transmission EM, under various magnification, photographs were taken using Agfa ortho 25 negative film, and printed in photo-glossy paper. This study was carried out from 2003-2006.

# RESULT

## Spermatogenesis

### Male meiosis

The immature testis has spermatogonia with lamina, nucleus and primary mitochondria like bodies (Fig.1). The lamina was observed only around the spermatogonia and was not found around other germ cell type. The lamina consists of four discontinous layers. The spermatogonia had large round nuclei and globular mitochondria like bodies of varying sizes. The nucleus to cytoplasmic ratio was high. The spermatogonia pass through a period of quick growth (mitotic division) to produce primary and secondary spermatocytes. These two germ cell types were observed from maturing and mature testis and proximal vas deferens. The primary spermatocytes are with nucleus, dense chromatin and vacuolated cytoplasm (Fig.2). The secondary spermatocytes are smaller and round with dense chromatid (Fig.3). The spermatocytes divide to form spermatid, and here acrosome and lamellar region starts formation. The spermatocytes are

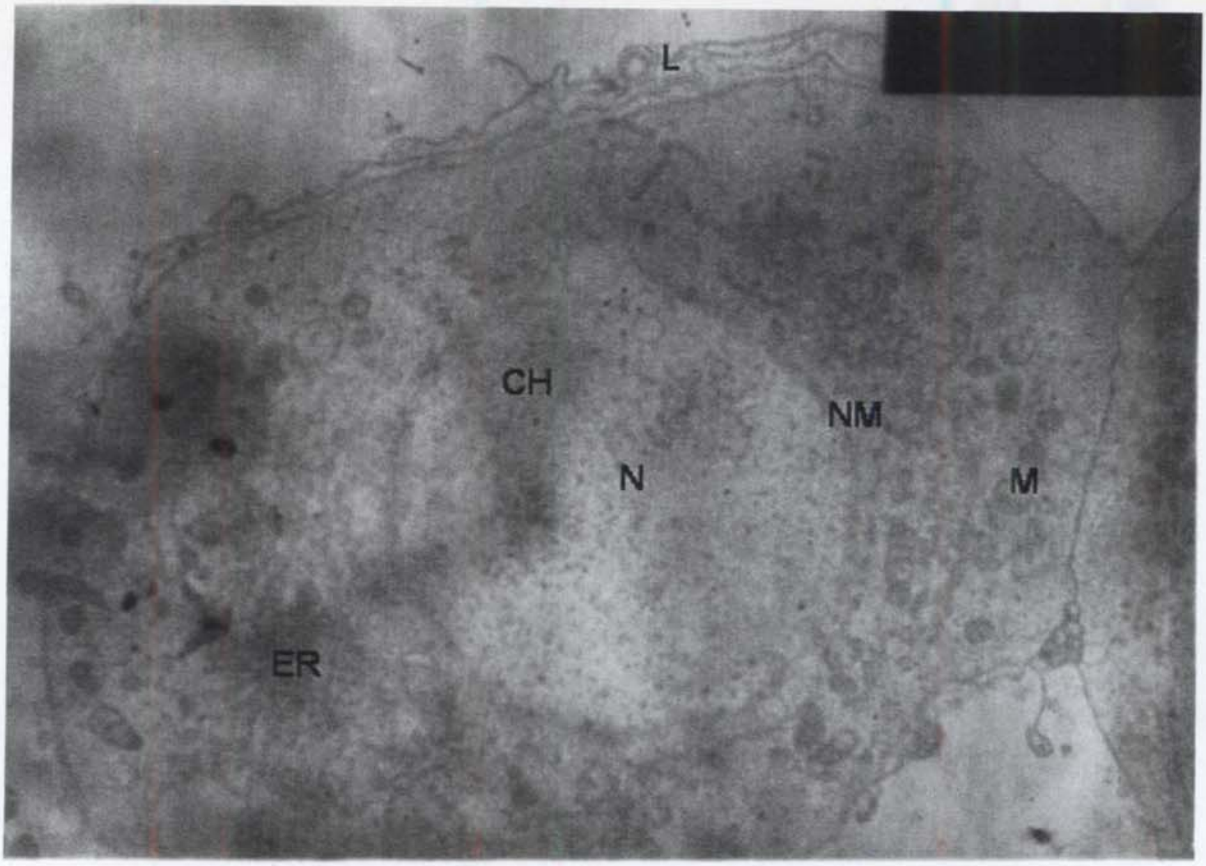


Fig.1. Spermatogonia with nucleus (N), chromatid (CH), lamella (L), mitochondria (M) and nuclear membrane (NM) x 6000
Endoplasmic reticulum (ER)

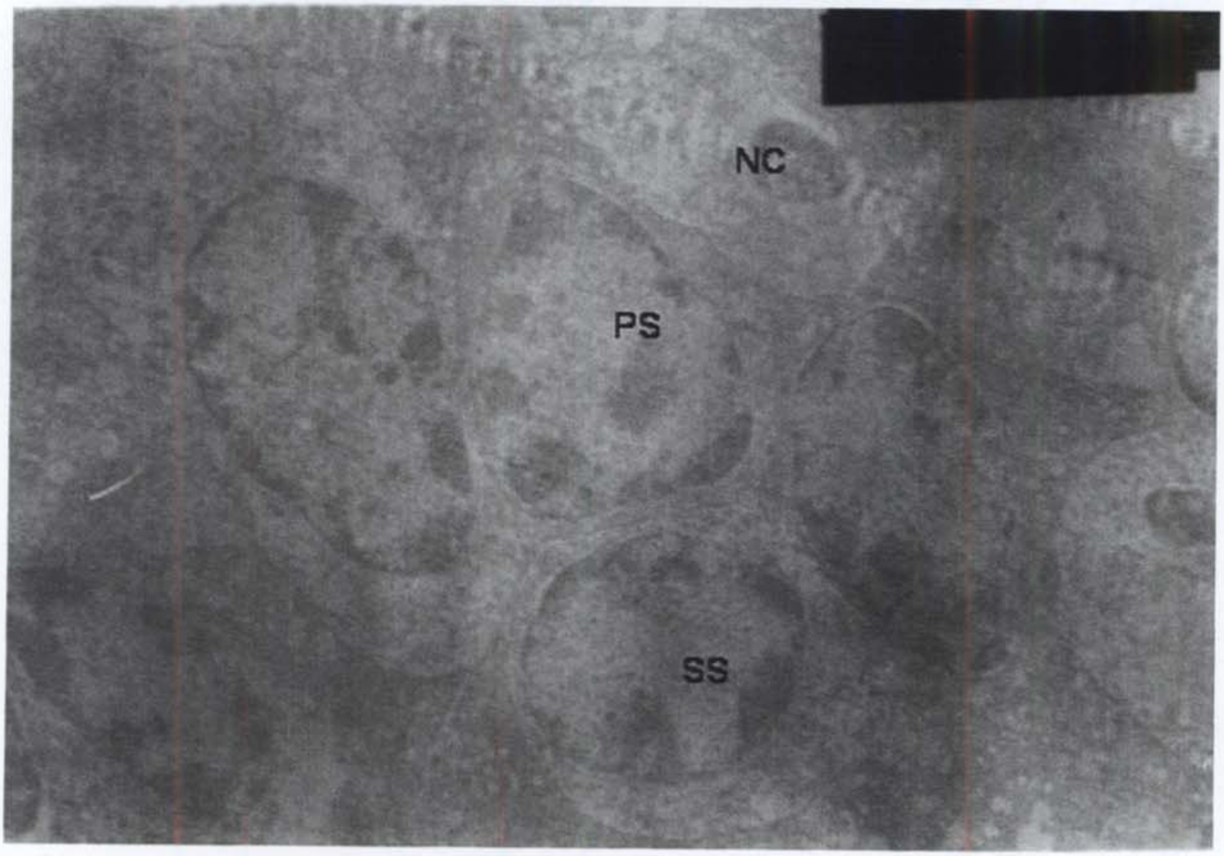


Fig.2. Primary spermatocytes (PS), Secondary Spermatocytes (SS) and nurse cells (NC) x 4000

surrounded by nurse cells. They have irregularly shaped nuclei with prominent chromatin arranged along the periphery (Fig.18).

## Spermiogenesis

### Spermatid

Spermatids in different stages of development were apparent in this study. The spermatids contained mitochondria, endoplasmic reticulum and centrioles (Fig.4). In the early stage of spermiogenesis (Fig.5) the formation of acrosome is evident from the coalescence of ergastoplasmic cisternae, endoplasmic reticulum and nuclear envelope. The inner and outer membranes of the nuclear envelope fuse and become identical to the endoplamic reticulum derivative. As spermatogenesis progresses further, the membrane of the nucleus and the layers of lamellar region become continuous with each other (Fig.6). Later the acrosome become electron opaque and the acrosome and nucleus lie in close contact (Fig.8), with the lamellar region on one side. The nuclear profile differentiates numerous slender arms (Fig.7). With further development, radial arms form with microtubules and the nucleoplasm of spermatids attain homogeneity as the

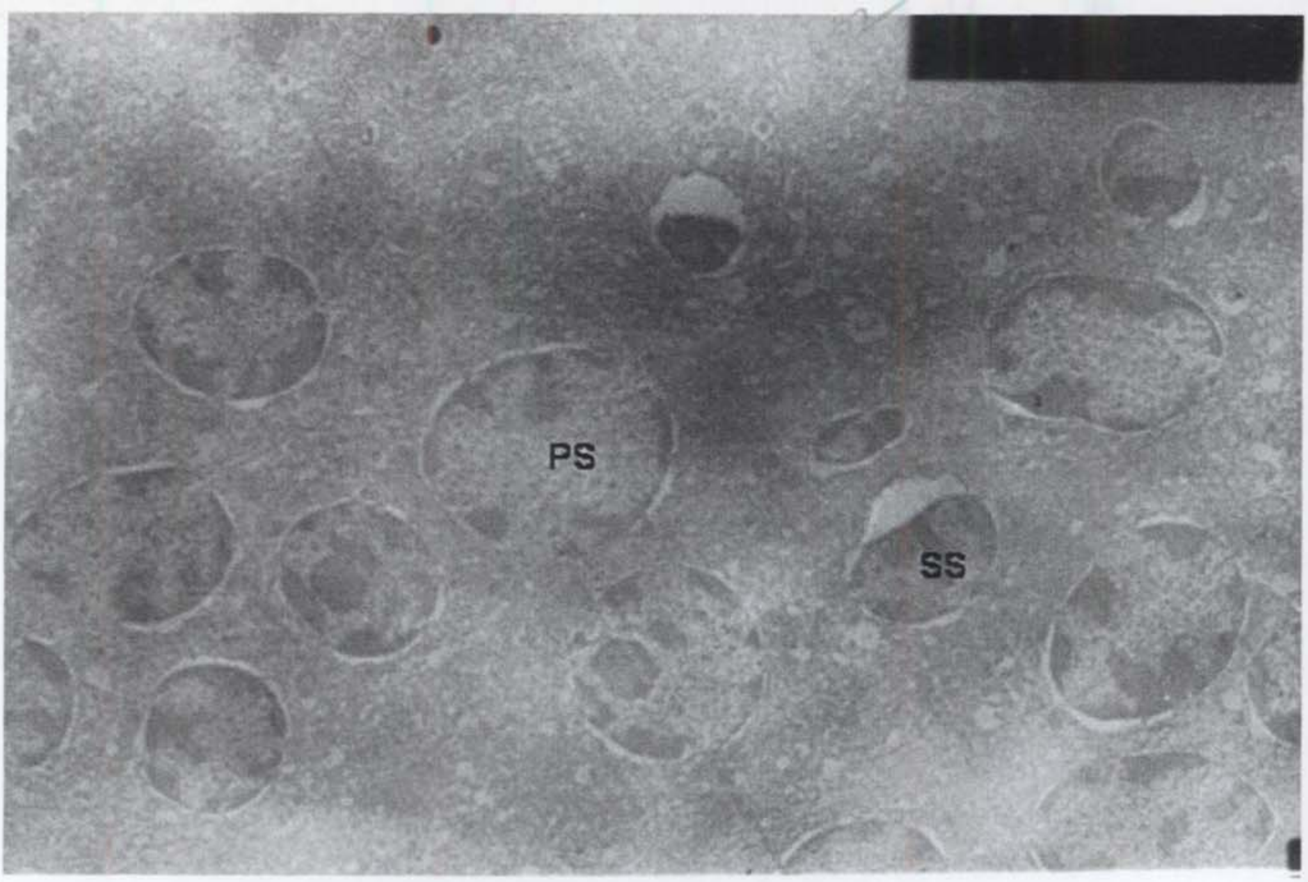


Fig.3. Primary spermatocytes (PS) and Secondary spermatocytes (ss) (SS) x 2500

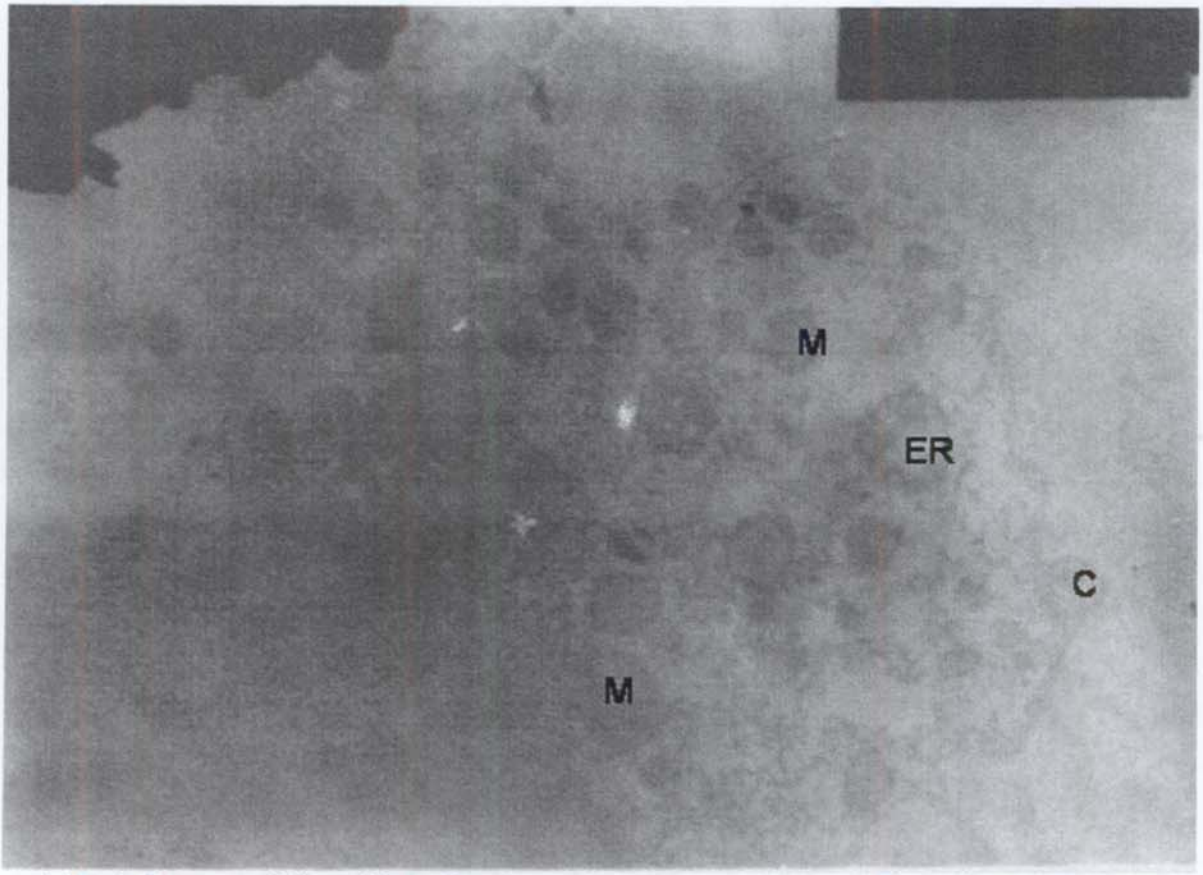


Fig.4. Spermatids with mitochondria (M), centriole (C) and endoplasmic reticulum (ER) x 6000

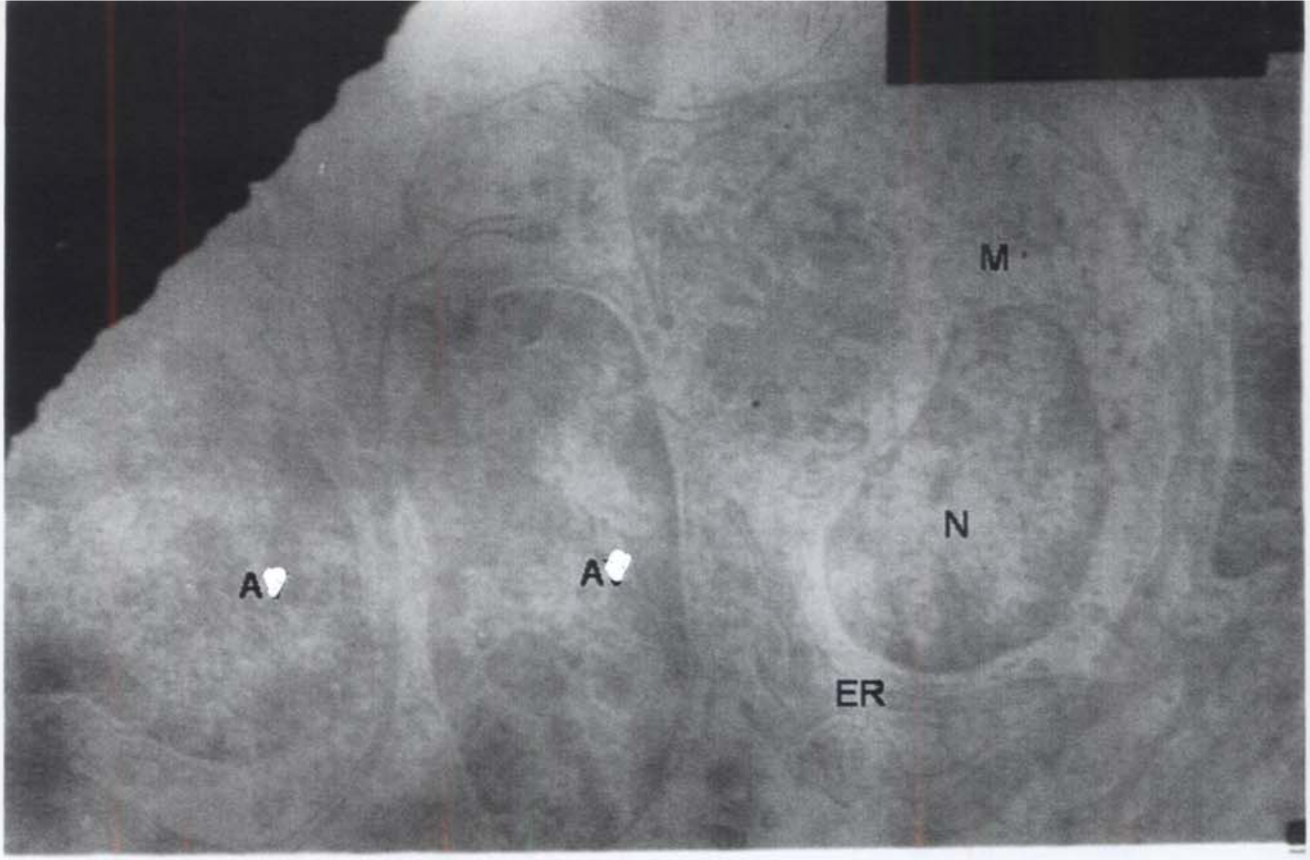


Fig.5. Early spermatid stage with A (acrosome), N (nucleus), ER (Endoplasmic reticulum), M (mitochondria) x 6000

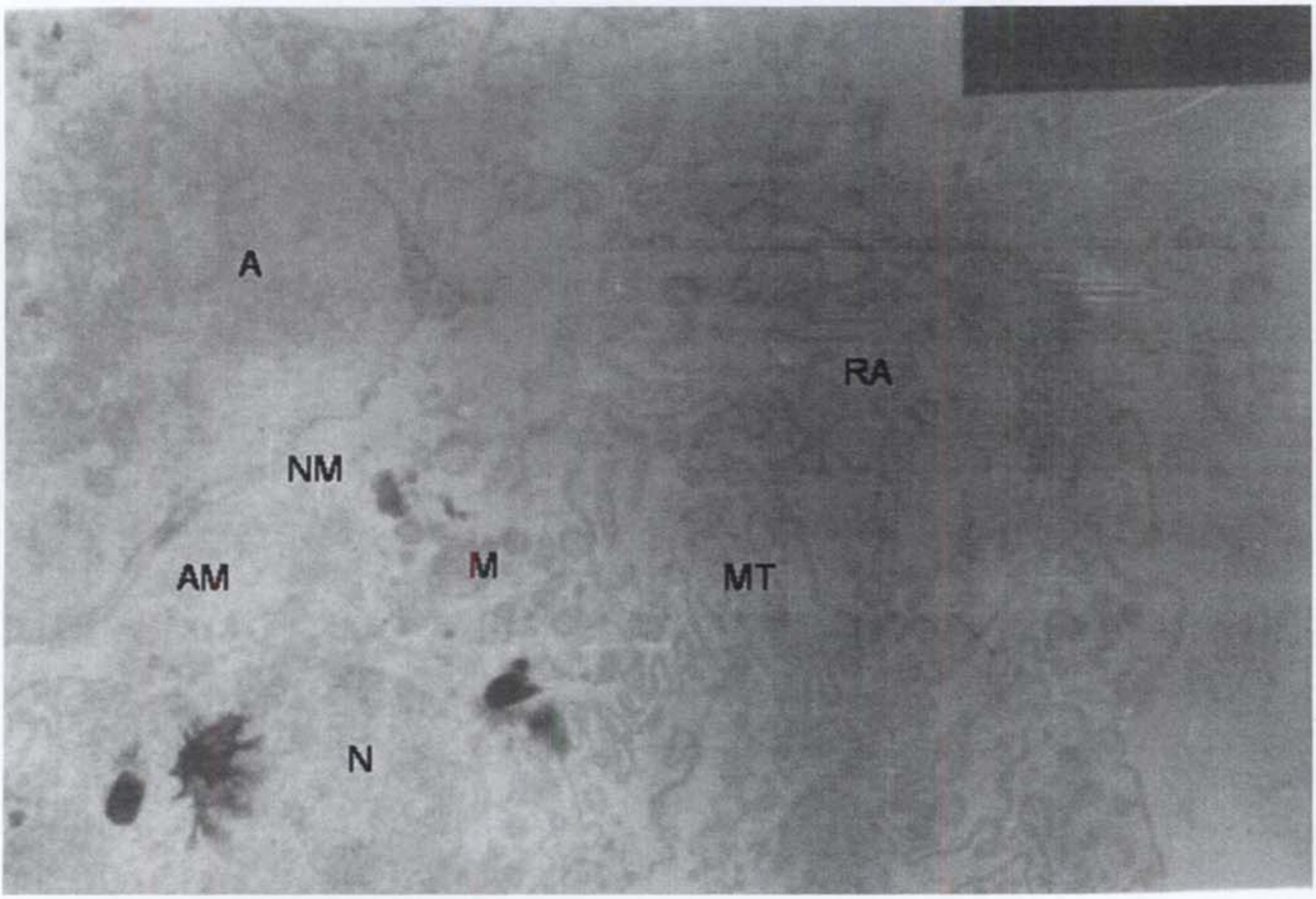


Fig.6. Mid spermatid stage with radial arms (RA) microtubule (MT), acrosome (A), nucleus (N), mitochondria (M), [illegible], nuclear membrane (NM) and acrosomal membrane (AM) x 6000

dense chromatin of the spermatocytes decondenses. The radial arms have large number of microtubules with rounded mitochondria (Fig.7). The radial arms along with microtubules are formed in association with the dense endoplasmic reticulum, near the nucleus (Fig.8). So eventhough the arms lie close to the nucleus, they do not seem to be formed from the nucleus. Later, the radial arms are arranged around the nucleus and the acrosome to form the spikes (Fig.11). The electron micrographs confirm that the radial arms are continuous with the nuclear cup and in the spermatophores are wrapped around the individual sperm. The spermatids form spermatozoa through these gradual changes in the cytoplasm, without further division of the spermatids.

### Immature sperm

The nucleus and acrosome become spherical and the nucleoplasm develops a fine fibrillar consistency. Mitochondria seem to have degenerated. The acrosome loses its granular nature and becomes electron dense. The spikes seem to be continuous with the nuclear cup and in the spermatophores are wrapped around the individual sperm (Fig.9).

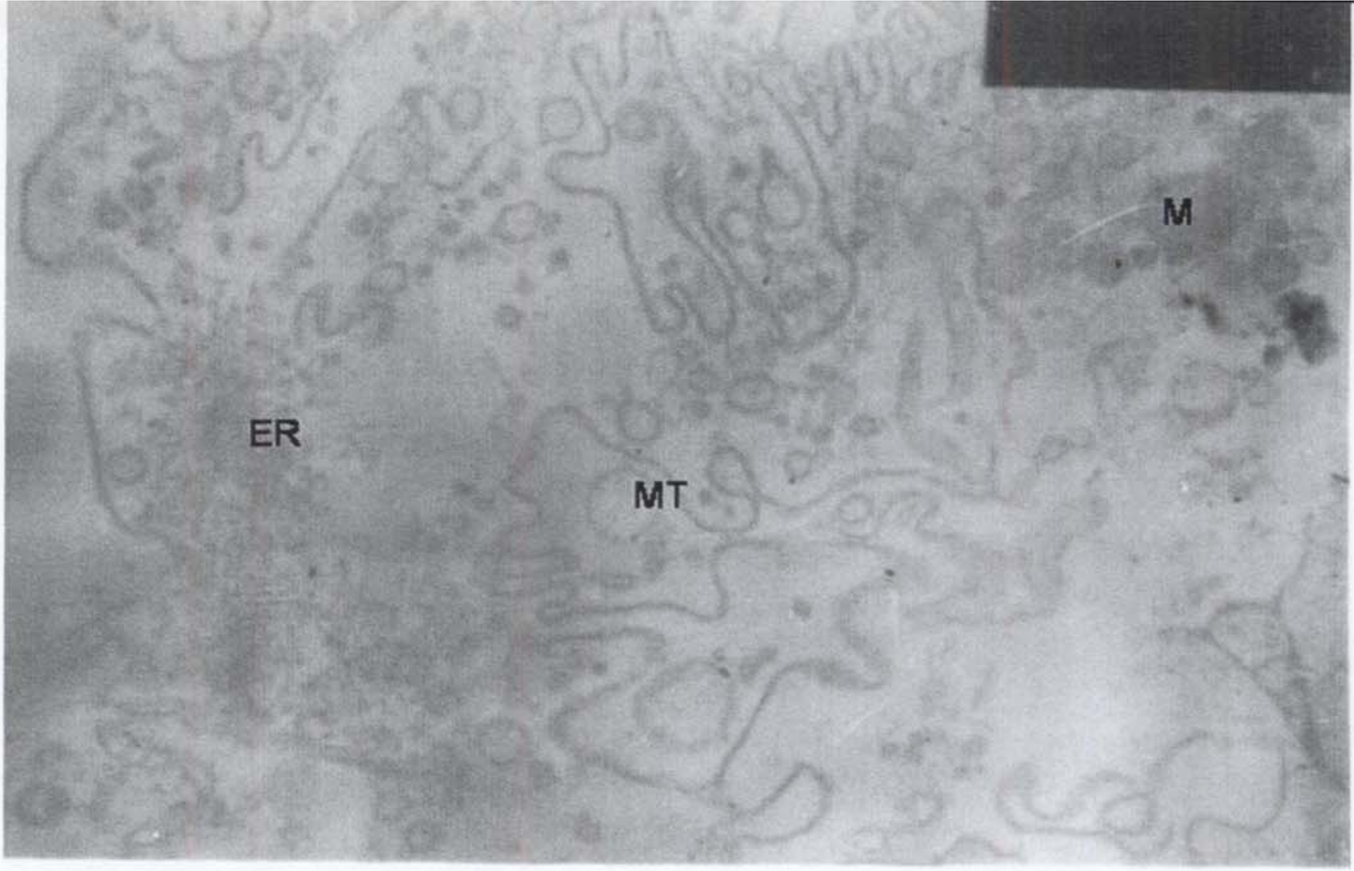


Fig.7. Radial arms with MT( microtubules) and M (mitochondria) x 10,000
Endoplasmic reticulum (ER)

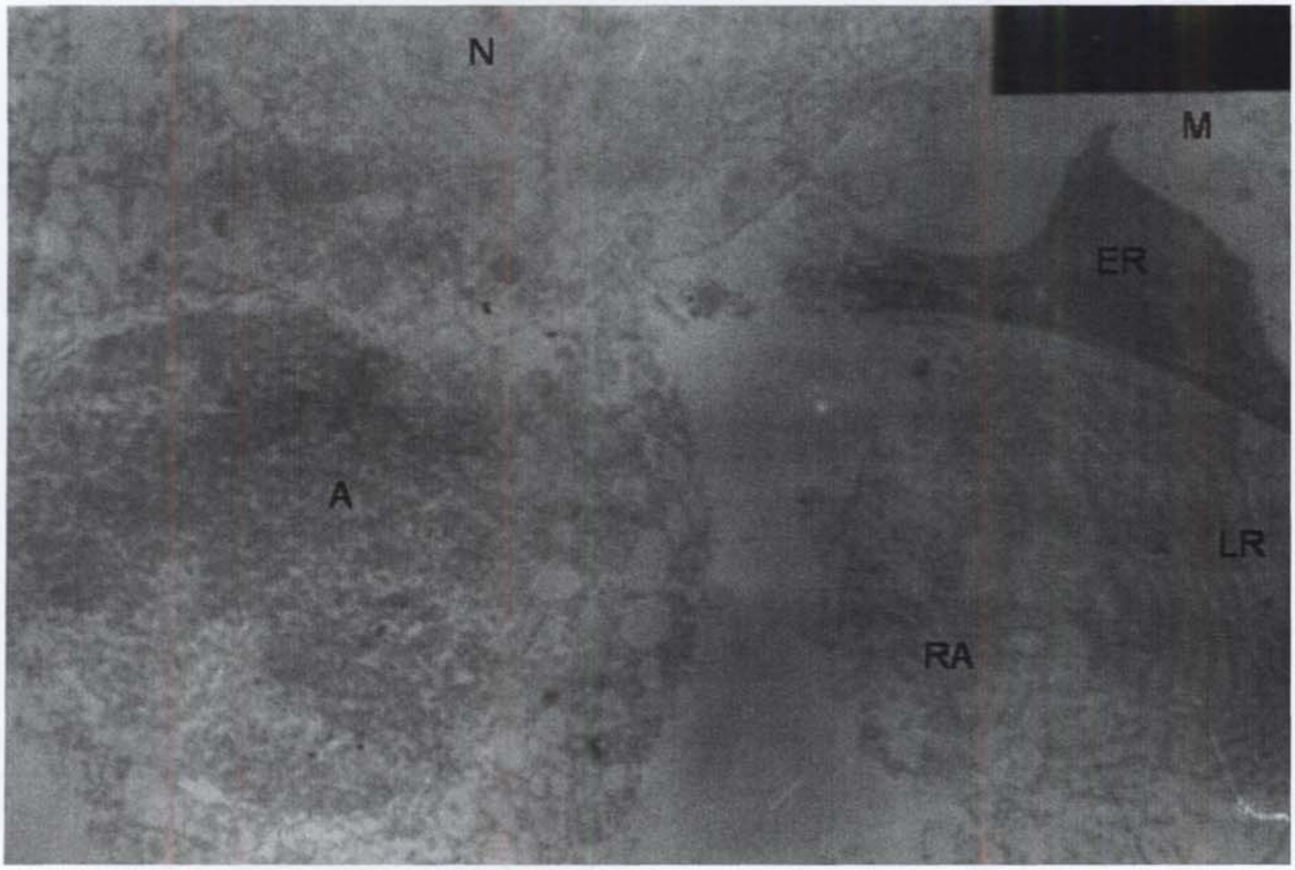


Fig.8. Late spermatid lamellar region (LR), acrosome (A), & radial arms (RA) nucleus (N) and mitochondria (M) x 4000

## Mature sperm

Mature sperms were found within the spermatophores in the distal vas deferens and were not observed in the testis and proximal part of the vas deferens. At the ultrastructural level the sperm consists of nucleus, lamellar region, spikes and an acrosome (Fig.10). The sperm of *P.homarus* is spherical and immotile without a flagellum. The sperm is surrounded externally by a membrane and contains a matrix, granular in nature (Fig.16). The nuclear contents form a network of dense anastamosing fibers (Fig.11), which at low power assume a granular appearance. This granular matrix has electron density similar to the cytoplasm. No distinct membrane separating the cytoplasm from the nucleus is apparent. The nuclear boundary is thickened and is assumed to be a combined nucleoplasm membrane. The nucleus occupies most of the intracellular space within the spermatozoa (Fig.10). The nuclear envelope and plasma membrane are closely apposed along the outer surface of the sperm. The presence of a network like pattern of the nuclear membrane around the acrosome facilitates intermingling of the nuleoplasm with the cell organelles.

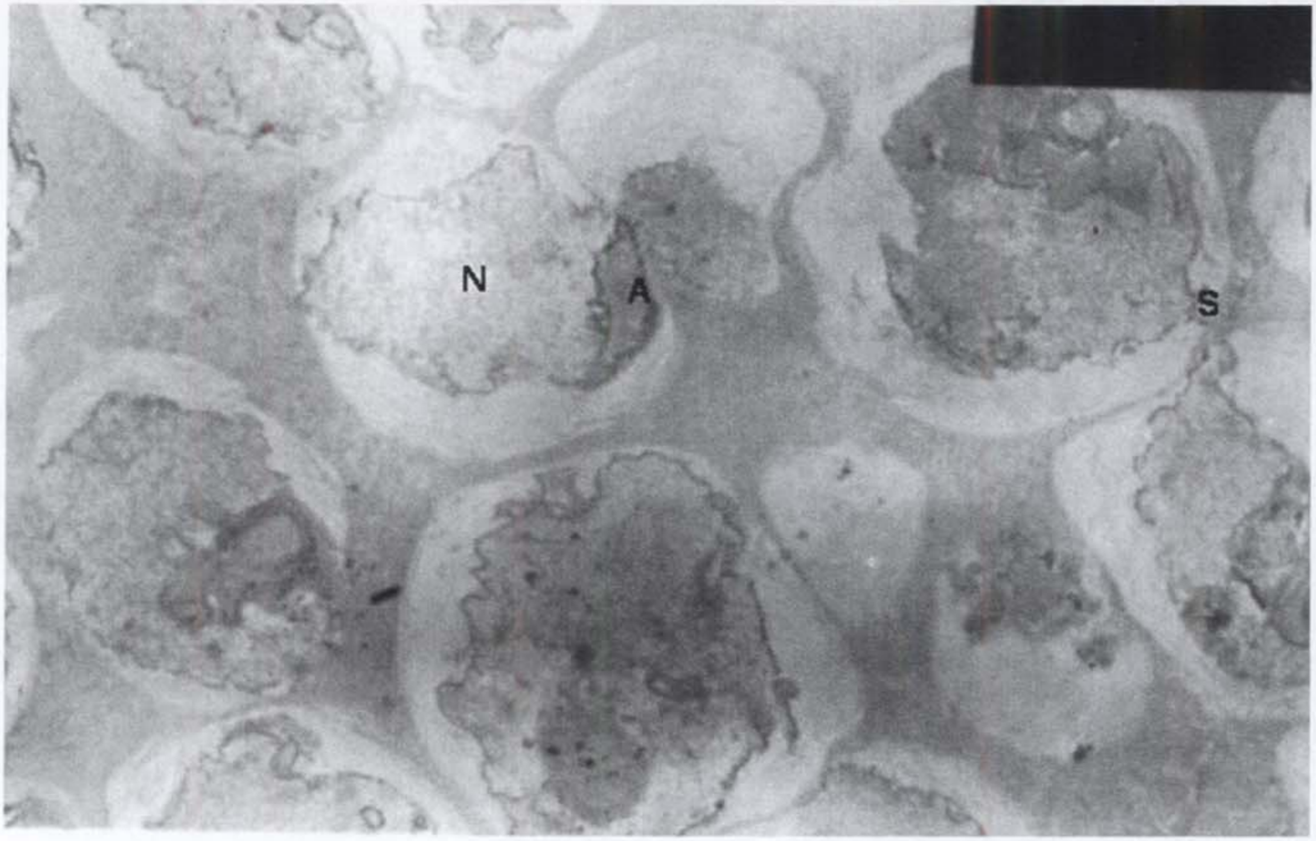


Fig.9. Mature sperm with acrosome (A), spikes (S), nucleus (N) x 4000

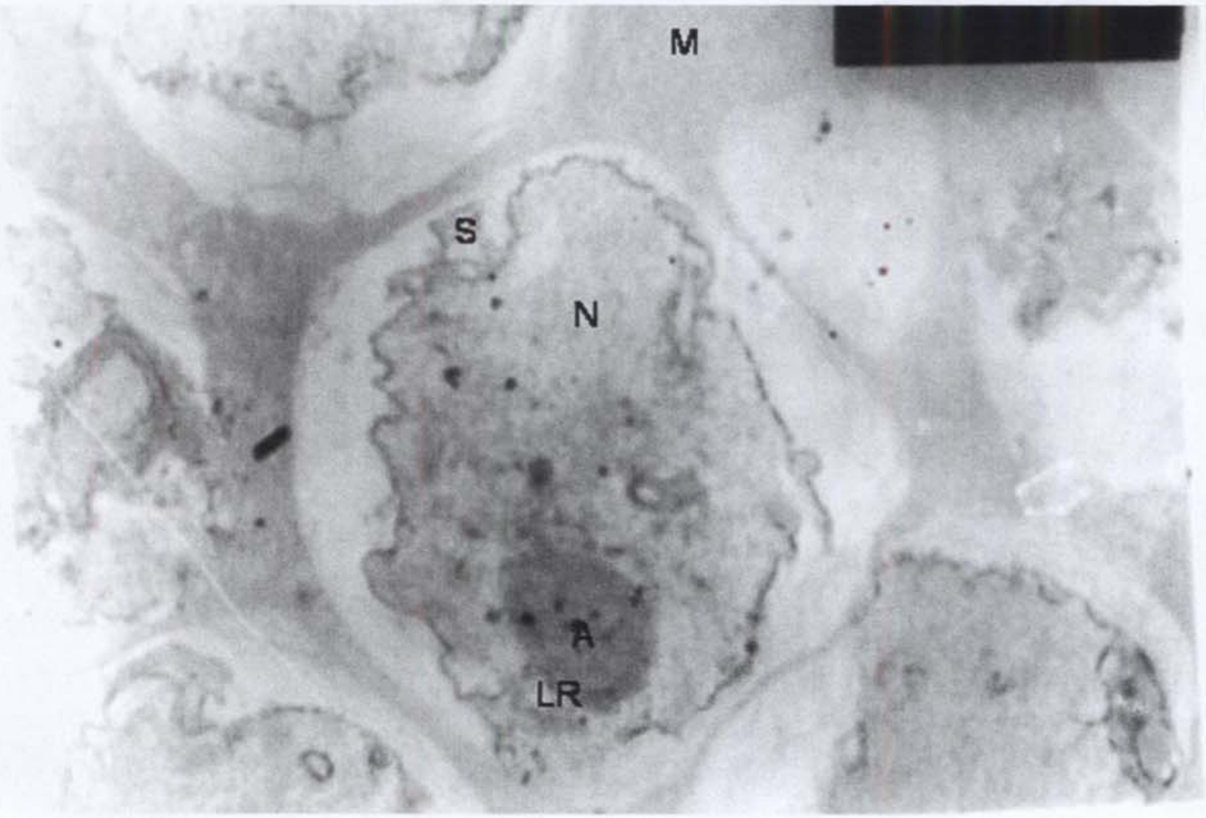


Fig.10. Mature sperm with acrosome (A), nucleus (N), Spike (S) and lamellar region (LR) x 6000
matrix (M)

The acrosome which is lens shaped has four regions of differing electron density- crystalline, scroll, homogenous and flocculent. The acrosome is surrounded by the acrosomal membrane. Perinuclear material is evident near the dense acrosomal vesicle and membranes of the lamellar region are evident near it (Fig.11).

The lamellar region consists of folds of membranes (Fig.15). It lies to one side of the acrosome and outside the nuclear envelope. It is restricted in size and contains remnants of mitochondria. Numerous arms or spikes arise in this area anterior to the nucleus and extend posteriorly (Fig.12). The arms contain microtubules that pass through the lamellar region as they extend from one arm to another (Fig.13). The lamellar region is presumed to be formed from the association of the nuclear membranes and endoplasmic reticulum.

Typhlosoles consisting of bundles of striated muscles are evident in the distal vas deferens. The muscle cells are cuboidal in nature (Fig.17). Contractions of the muscles surrounding the vas deferens and associated with the typhlosole appear to play a role in shaping the spermatophore.

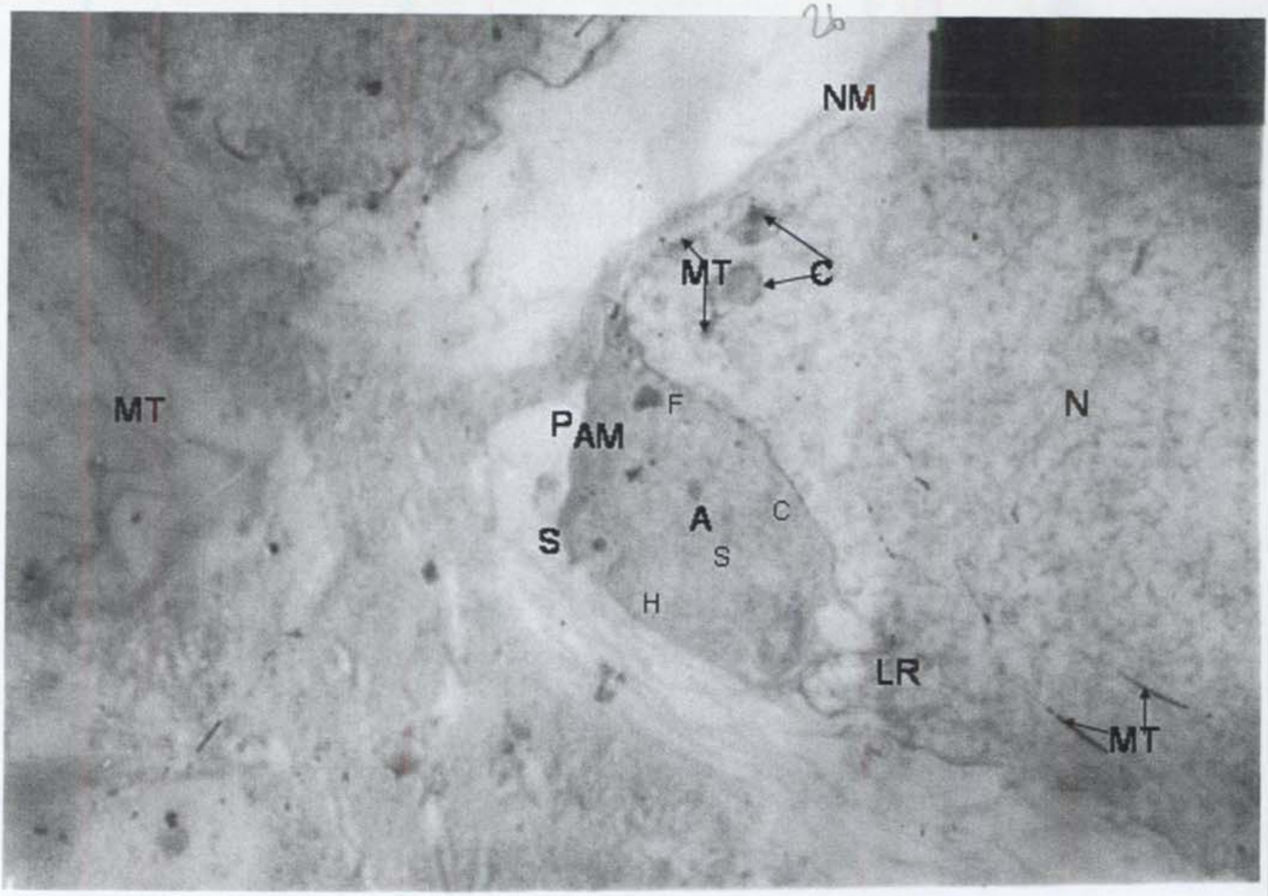


Fig.11. Mature sperm with acrosome (A), nucleus (N), centriole (C), microtubule (MT), lamellar region (LR), nuclear membrane (NM), acrosomal membrane (AM spikes (S), homogenous region (H), scroll (S), flocculent (F), crystal (C) x 10,00
*Perinuclear space (P)*

Fig.12. Spikes (S) showing association with nucleus (N) x 10,000

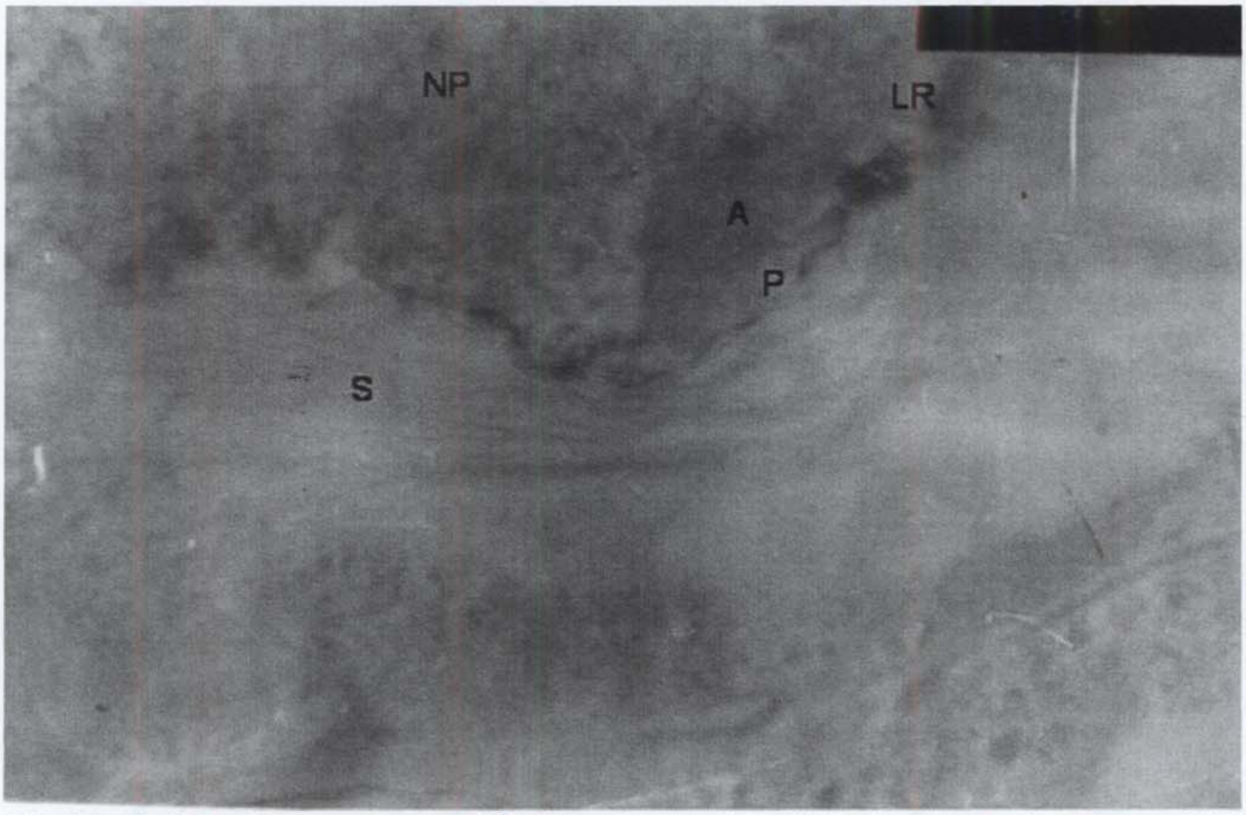


Fig.13. Spikes (S), acrosome (A), lamellar region (LR), dense nucleoplasm (NP) perinuclear space (P) in mature sperm x 20,000

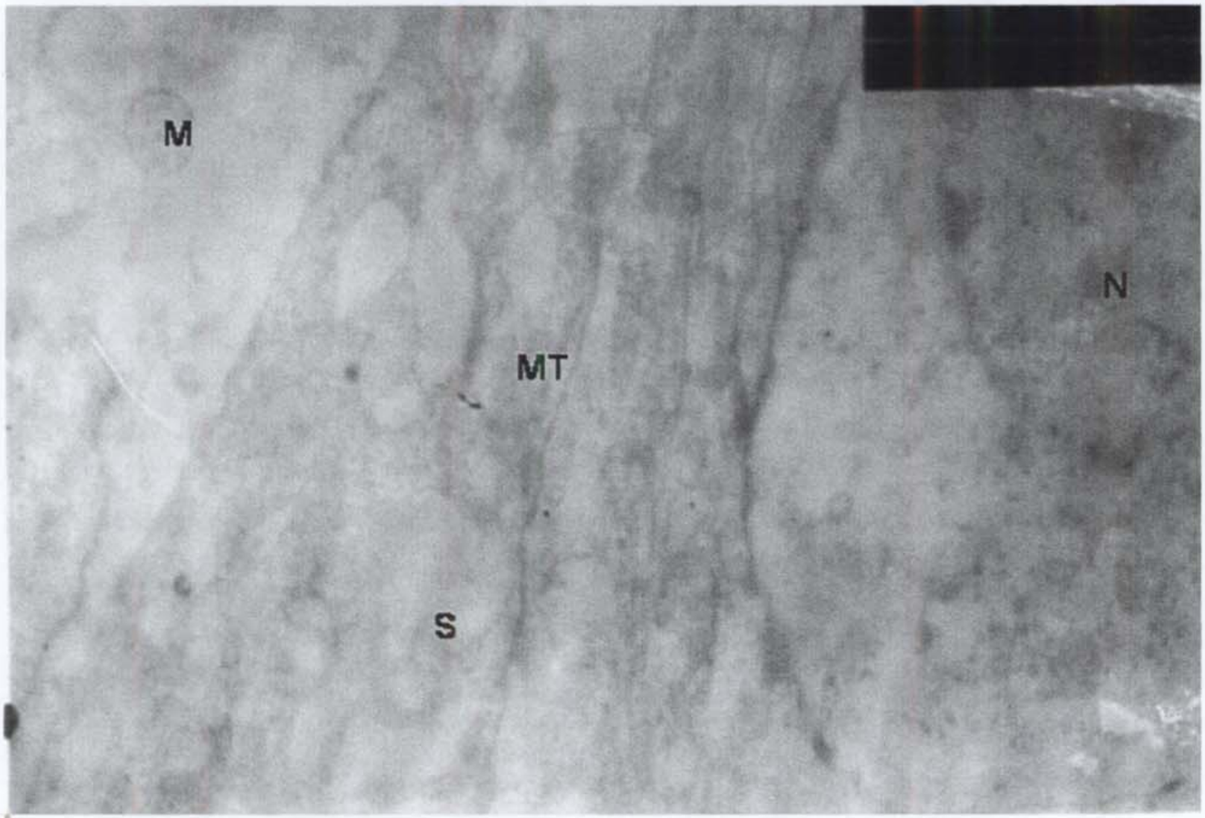


Fig.14. Spikes with microtubules (MT) and M (mitochondria) x 8000
(S) nucleus (N)

Centrioles were observed in the mature sperm at the base of the acromsome (Fig.11). They form the microtubules, which are evident in the nucleus, radial arms and spikes (Fig.11, 14 & 15).

## DISCUSSION

Significant progress has been made in the study of spermatogenesis in decapod crustaceans by light microscopy and in recent times, a new dimension has been added to our knowledge of the fine structure of the spermatozoa and spermatogenesis through electron microscopy. Ultrastructure of spermatogenesis has hitherto not been reported in spiny lobsters so far, though the process was studied in detail in the axiid lobster *E. occidentalis* (Haley, 1986).

The ultrastructure of lobster spermatozoan has been studied in three genera- *Homarus* (Pochon-Masson, 1965a, b; 1968; Talbot & Chanmanon, 1980 a, b), *Panulirus* (Talbot & Summers, 1978) and *Nephrops* (Chevaillier, 1966; Chevaillier & Maillet, 1965). In the genus *Panulirus*, spermatozoan structure has been investigated in two species- *P.argus* and *P.guttatus* (Talbot & Summers, 1978). Among the macrurans, the ultrastructure of the

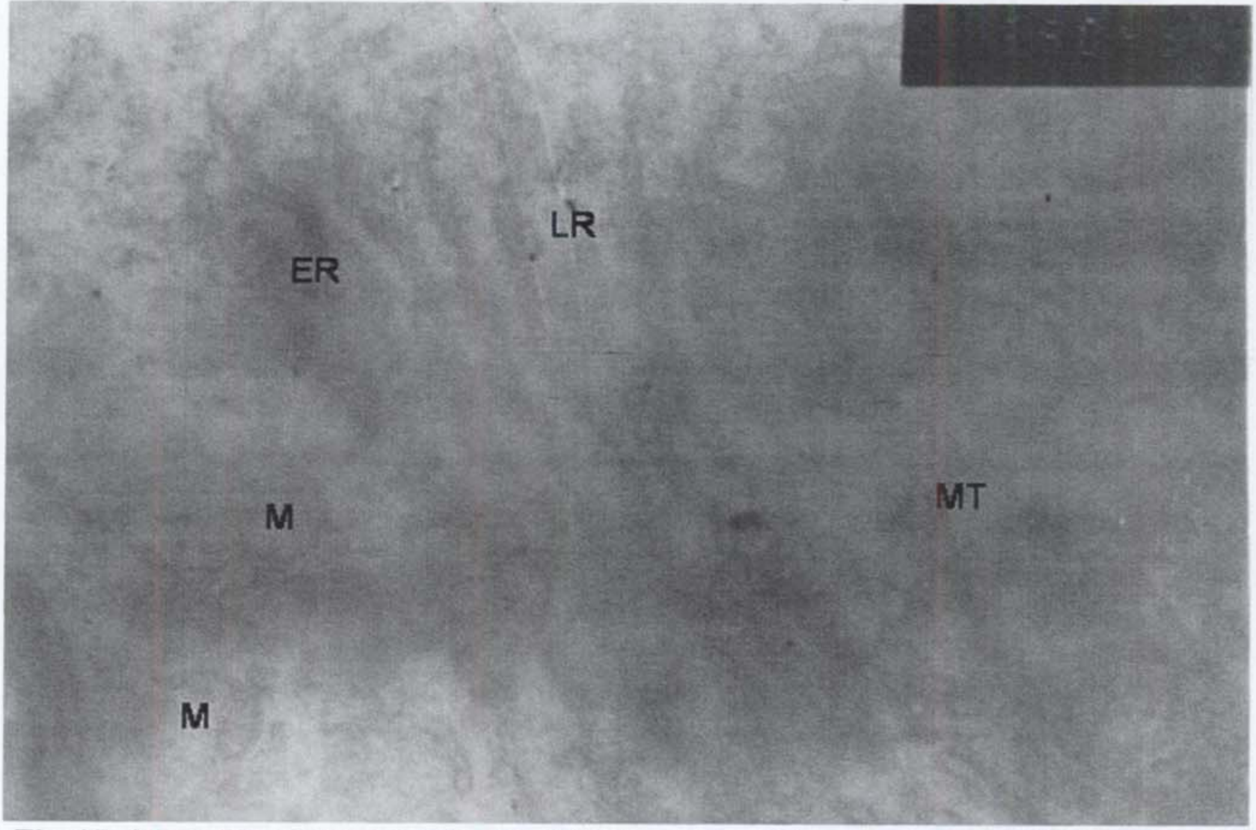


Fig.15. Lamellar region (LR) with mitochondria (M), microtubule (MT) and dense endoplasmic reticulum (ER) x 15,000

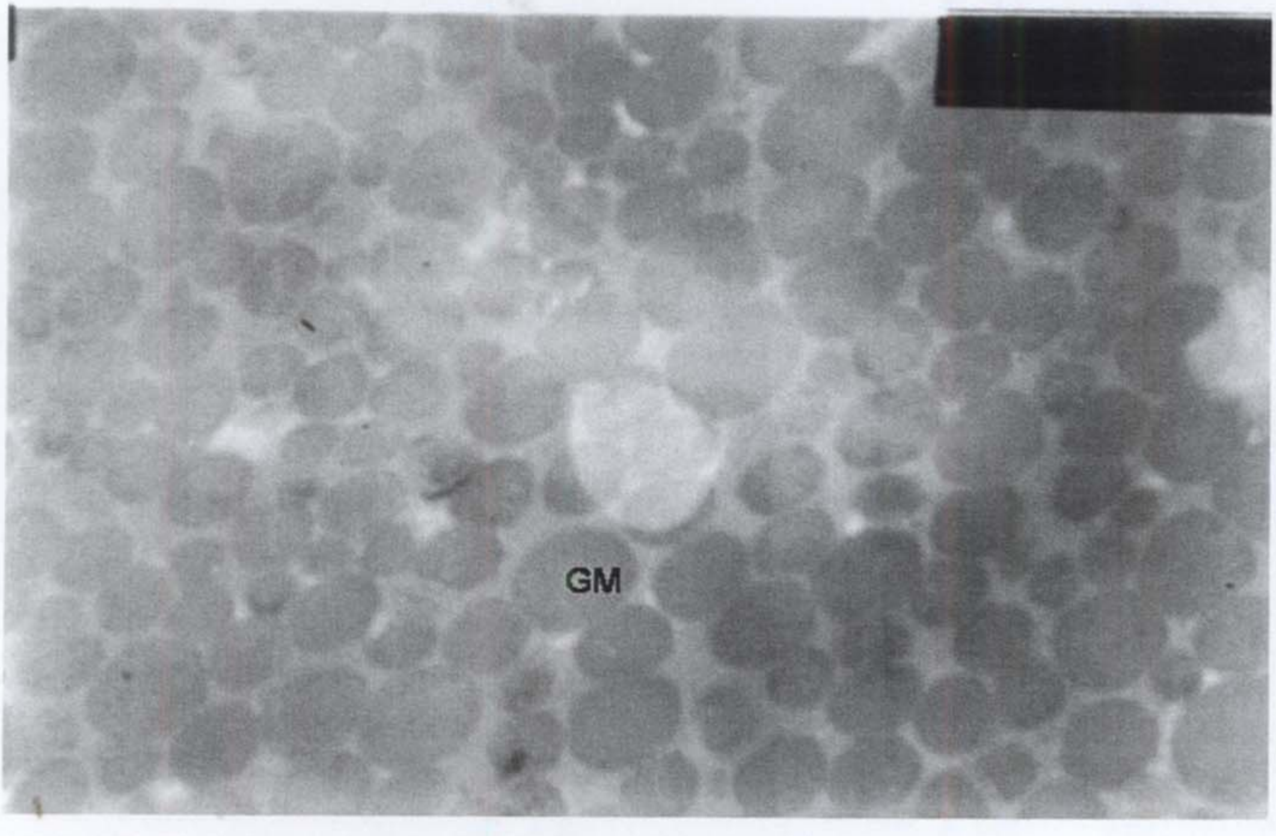


Fig.16. Gelatinous matrix (GM) surrounding the sperm in the spermatophore x 8000

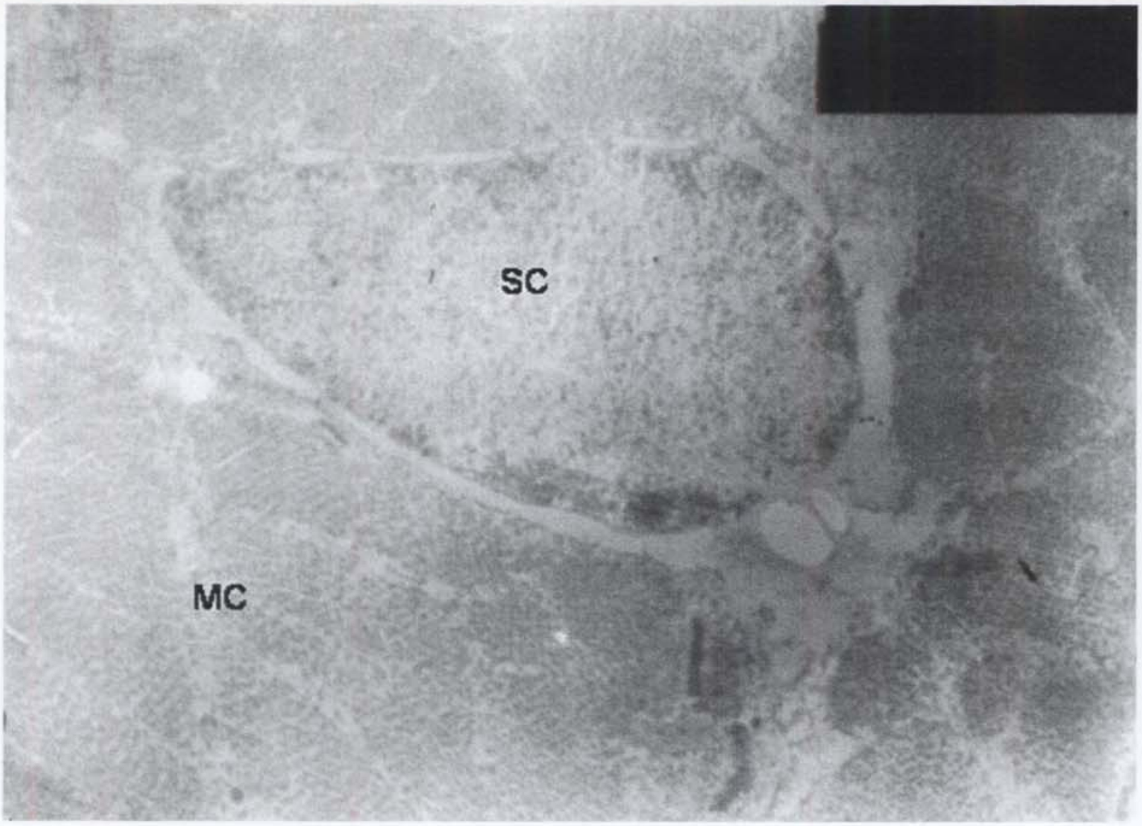


Fig.17. Typhlosole with muscle cells (MC) and secretory cell (SC) x 6000

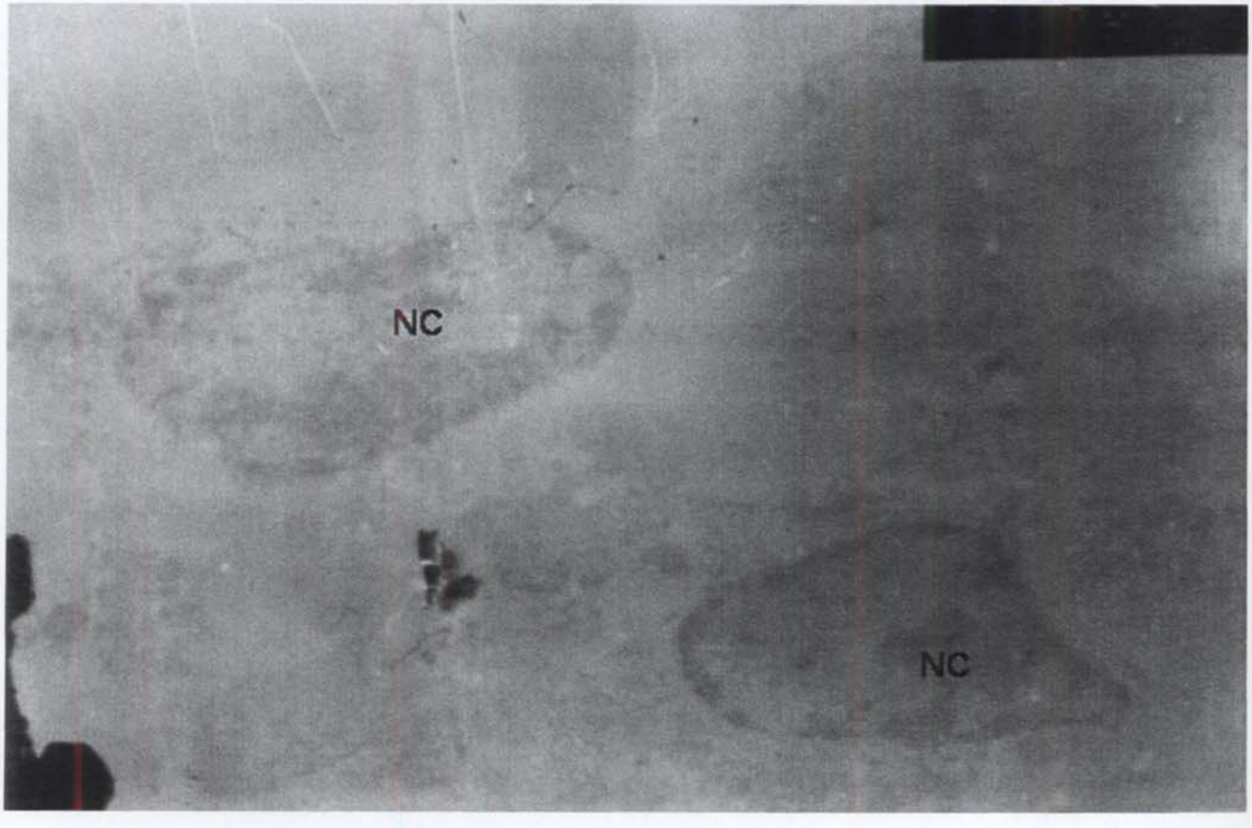


Fig. 18. Nurse cells (NC) in the testis x 4000

spermatozoa and spermatogenesis of the spiny lobster, *P.homarus* from Indian waters has not previously been described. The characteristics of reproductive biology, including sperm structure in this species is little known. Sperm of *P.homarus* follow the basic structural plan of reptantian sperm and morphologically resemble sperm of *P.argus* with certain variations in structure.

In this study mature sperm were observed only in the distal vas deferens whereas in *P.argus* and *P.guttatus* mature sperm were seen in the testis and proximal vas deferens. (Talbot & Summers, 1978). The spermatogonia and spermatocytes (primary and secondary) were observed in the testis and proximal vas deferens and their ultrastructure is described for the first time in a spiny lobster. Haley (1986) has reported the structure of spermatogonia and primary spermatocytes in the lobster, *E.occidentalis*, but could not observe the secondary spermatocytes. He states that the spermatogonia is surrounded by 2-14 laminae, which in *P.homarus* was observed to be four and discontinous.

In *P.homarus*, the nuclear material has very little structural pattern as compared to certain conventional sperms. The nucleus is electron translucent in the mature sperm, the nucleoplasm containing fine filaments which are transformed from the uncondensed chromatin of the spermatids. This is an exception to that of lobsters *Homarus vulgaris* (Pochon-Masson, 1965a) and *N.norvegicus* (Chevaillier, 1966), which have electron opaque and granular nucleoplasm. In the present study the number of layers limiting the boundary of the mature sperm could not be distinguished although layers could be discerned around the spermatogonia. Moses (1961b) was uncertain of the number of layers in the nuclear boundary of *Procambarus clarkii* sperm and Chevaillier (1966) observed tight limiting boundary in *Eupagurus bernhardus* which he presumed to be tripartite. Haley (1986) distinguished several membranous layers around the spermatogonia of *E.occidentalis* and opined that these might serve to limit the steroid producing meiotic events in spermatogenesis from spermatogonia.

The nucleus of *P.homarus* is spherical and occupies most of the space within the spermatophore as in other Palinurids (Talbot & Summers, 1978). But differs from the brachyuran crab *Coenobita,* in which the nucleus is short and thickened and in *Pleuroncodes*, it is elongate (Pochon-Masson, 1968). In *P.homarus*, the nuclear envelope fuses with the plasmalemma along the outer surfaces of the sperm and appears to degenerate where the nucleus and lamellar region meet as in other palinurid sperms. In the natantians (Medina, 1994) and in the shrimp *Pandalopsis japonica* the nucleus is decondensed and does not have a nuclear envelope (Kim, 2003).

The radial arms observed in the *P.homarus* sperm has not been described earlier in other Palinurids reported so far – *P.argus* and *P.guttatus* (Talbot & Summers, 1978), although it has been reported in the lobster *E.occidentalis* (Haley, 1986). The spermatozoa of *P.homarus* has extensions in the form of radial arms which appear to extend from the nucleus. The microtubular arms in *P.homarus* develop from the association of nuclear and cytoplasmic membranes and endoplasmic reticulum and is later associated with the nucleus. This agrees with the observations of Moses (1961a) in the cray fish *P.clarkii.* Hence a part of the radial arms are nuclear in origin with the presence of cytoplasmic component derived from the endoplasmic reticulum. In *Scyllarus,* the radial arms are cytoplasmic in origin (Tudge et al. 1986) and in *C.sapidus*, the radial arms extend from the nuclear cup (Brown, 1966). In the sperm of hermit crab *Coenobita clypeatus*, other anomurans, *Eupagurus* sp. (Pochon-Masson, 1968) and *Pleuroncodes planipes* (Hinsch, 1986) the arms are of cytoplasmic origin.

The spike of *P.homarus* is similar to that of other palinurids studied so far- *P.argus* and *P.guttatus* (Talbot and Summers, 1978) whereas the natantian sperm has only a single spike that may contain microfilaments (Brown et al, 1976; Kleve et al, 1980). Reptantian sperm possess a main body and multiple spikes that mostly consist of microtubules. *P.homarus* has microtubules in the spikes which become fibrillar as in *Homarus* (Talbot and summers, 1978) and spider crab *L.emarginata* (Hinsch, 1969). In the case of the golden crab, *Greyon fenneri*, nuclear arms vary in length and lack microtubules of any type (Hinsch, 1988). In the sperm of the lobster, *Homarus americanus* (Talbot & Summers, 1978), the hermit crab *Coenobita clypeatus* (Hinsch, 1980) and another anomuran *P.planipes* (Hinsch, 1969) the inner acrosomal mass, which is comparable to the acrosomal tubule lacks microtubules. Although *P.homarus* posess spikes with microtubules, these differ from the typical flagellated sperm of other animals in the lack of true axoneme (Hinsch, 1969; Talbot & Summers, 1978; Dudenhausen and Talbot, 1982; Tudge & Jamieson, 1991). The role of these microtubules may be to maintain the shape of the spike or to serve as structural support element (Hinsch, 1969).

*P.homarus* sperm have an acrosome which is lens shaped, which is structurally complex and has several different components. The acrosomal vesicle has four zones-flocculent, crystal, scroll and homogenous in *P.homarus* but in the lobster, *J.novaehollandeae* (Tudge et al. 1986), the crystalline zone is lacking. The acrosome in *P.homarus* is lens shaped and dense, lying to one side of the spermatozoa which agrees with that of the crab *C.sapidus* (Cronin, 1947) and lobster, *P.argus* (Talbot & Summers, 1978). But it differs from that of the lobsters, *Homarus* (Kooda Cisco & Talbot, 1982) and *Nephrops* (Farmer, 1974), which have elongate acrosome as that found in the anomurans, *P.planipes* and *C.clypeatus* (Hinsch, 1969). *P.homarus* sperm also lacks an acrosomal tubule like those in *Homarus* and brachyuran crabs (Jamieson, 1989).

Although the role of golgi body in acrosome formation has been observed in animals like cat (Burgos & Fawcett, 1956), the toad (Burgos & Fawcett, 1955) and the Cricket (Kaye, 1962), it has not been reported in decapod crustaceans so far (Pochon-Masson, 1963). Pochon-Masson (1963) and Chevaillier (1966) have opined that the acrosome is formed from ergastoplasmic elements which are its substitute in the absence of golgi elements. But Moses (1961a) and Langreth (1965) observed the acrosome to be formed from the cytoplasmic vesicles. In *P.homarus*, acrosome is formed from the association of endoplasmic reticulum, ergasoplasmic elements and nuclear envelope.

Prominent laminated regions were observed surrounding the acrosomal region in *P.homarus*, similar to that of crayfish, *P.clarkii* (Moses, 1961b), but this differs from the lobster *E.occidentalis* (Haley, 1986) where the membranes are not complete. These lamellar regions were reported to be formed from cisternae of endoplasmic reticulum in *Carcinus* (Langreth, 1969). In *P.homarus*, the lamellar region is formed from the nucleus and endoplasmic reticulum. In *E.occidentalis*, Haley (1986) states that the electron dense bodies associated with the nucleus and annulate lamellae may organize the developing lamellar region. The laminar region is non existent in the crayfish *Astacus astacus* (Lopez et al., 1981). The lamellar region has been designated as 'membrane complex' by the earlier investigators of decapod crustaceans (Moses, 1956; Pochon-Masson, 1962; Langreth, 1969).

In spiny lobsters, although typhlosoles have been described by light microscopy, its fine structure has not so far been reported. In this study, the typhlosole was observed to have striated muscle bundles. Contraction of the muscles surrounding the vas deferens and those associated with the typhlosole appear to play a role in shaping the spermatophore. The epithelial lining of the different regions of the vas deferens has synthetic and secretory function. Secreting cells are evident in the typhlosole in this species. The secretory cells distributed on the wall of the vas deferens facing the lumen are responsible for producing the sac like protective envelope or spermatophore. In *Homarus*, a typhlosole has not been reported and spermatophore shaping appears to result from a variation in height of the epithelium lining the lumen (Talbot & Summers, 1980b). In *Scyllarus*, although a typhlosole is present, its role in shaping the spermtophore is not definite (Hinsch and Mcknight, 1988).

In the mature sperm of many brachyurans, the mitochondria degenerate during spermiogenesis and mature brachyuran sperm does not contain the enzymes normally incorporated in the mitochondrial memberane capable of oxidative phosphorylation (Pearson and Walker, 1975). In *P.homarus*, mitochondria could not be observed in mature sperms although they were evident in the spermatogonia and spermatids. Mitochondria were observed in the mature sperm of Palinurids (Talbot and Summer, 1978). Mitochondria are degenerate or considerably altered in the mature sperm of decapods (Yasuzumi, 1960; Moses, 1961a, Kaye et al, 1961; Anderson & Ellis, 1967).

In *P.homarus*, centrioles were observed below the acrosome anterior to the nucleus and they form the microtubules. Centrioles have been reported from the mature sperm of palinurid lobster, *P.argus* (Talbot & Summers, 1978) and Cancer crabs (Langreth, 1969). But they are absent or degenerate in some decapods like *P.clarkii* (Moses, 1961b), *Eriocheir japonicus* (Yasuzumi, 1960), *H.vulgaris* (Pochon-Masson, 1965), *Cancer borealis* (Langreth, 1965), *N. norvegicus* (Chevaillier & Maillet, 1965) and *Carcinus maenas* (Pochon-Masson, 1968). Centrioles are unknown in dromiid sperm (Jamieson et al, 1993). But even in decapods-Branchiura, Cirripedia and Mystacocarida, which have flagellate spermatozoa, the centrioles are located anterior to the nucleus (Pocho-Masson, 1965b).

The present study reveals the sperm mass of *P.homarus* to be embedded in a layer of granular matrix. The sperms of *P.gilchristi* are arranged in a ribbon like pattern (Berry, 1969), whereas in *Homarus*, the sperm are packed into a continous tube contained within the ventral region of the trifoil spermatophore (Kooda-Cisco & Talbot, 1982).

In *P.homarus*, the spermatozoa was observed to have a nucleus, lens shaped acrosome, spikes and microtubules. Studies show the decapod sperm to consist of an acrosome, a cup shaped nucleus with several radiating processes and varying quantities of such cellular organelles as centrioles, mitochondria and microtubules (Moses, 1960; Brown, 1966; Hinsch, 1969). Numerous studies of crustacean sperm (Brown, 1966; Anderson & Ellis, 1967; Reger, 1970) also indicate that species differences occur regarding the presence of these organelles.

This study reveals that the structure of the spermatozoa of *P.homarus* differs in its morphology from other decapod crustaceans but with certain similarities. It is difficult to designate sperm features that characterize the entire class because of certain differences in the individual species. Nevertheless, sperm data are extremely useful in determining relationships between crustacean taxa. Some researchers have reviewed the sperm structure of decapod crustaceans, noting that some parts of the sperm structure including the spikes and the nucleus are markedly different enough to be used in taxonomic keys. The ultrastructures of sperm have been extensively used for recognizing phylogenetic linkages in decapod taxa. (Jamieson, 1989; Krol et al., 1992; Tudge, 1992; Tudge and Jamieson, 1991;

Medina, 1994; Tudge, 1999; Medina et al., 1998). Therefore, it appears that knowledge of sperm morphology and components might be very useful for estimating phylogeny of decapod crustaceans. Ultrastructural variations in sperm morphology are so significant in certain groups that some classifications have been questioned on this ground. For example the appearance of microtubule bundles in the spermatozoan of *P.japonicus* is an exception among dendrobranchiate, which is of phylogenetic interest (Medina, 1994). Similarly the differences in the spermatophore wall and ridge morphology between the various taxa in the anomura are of phylogenetic significance (Tudge, 1999). Each crustacean subclass contains a unique type of spermatozoon whose structure departs in varying degrees from that of the fundamental type. In general, decapod sperm are divided into two groups of characteristic natantian and reptantian morphological types on the basis of the number and structure of sperm spikes. Medina (1994) has suggested the division of decapod sperm into three classes- natantian, reptantian and caridean, instead of the traditionally accepted two- natantian and reptantian. It is stated that the dendrobranchiate and reptant sperm types share a number of spermiogenic and functional features, while the caridean sperm type appears to represent an independent evolutive line with regard to sperm development and function. Among the palinuridae, sperm morphology provides preliminary support of the hypothesis of two independent lines of evolution, but investigation into additional taxa within this group is required (Tudge, 1999).

# Chapter 1.4

# SIZE AT MATURITY

## INTRODUCTION

Size at maturity is an important tool in studies of population dynamics, resource management and aquaculture. Estimates of size at first maturity - the size at which males and females begin to change from the juvenile into the adult reproductive phase are important in many biological investigations involving stock assessment of commercially exploited species. It helps to set the Minimum Legal Size (MLS) of a species for its capture and export. If the legal size limit is set below the size at maturity, minimal protection of maturing and mature animals is afforded.

In decapod crustaceans, some authors have used external morphological characters to estimate size at first maturity; in shrimps : *Plesiopenaeus edwardsianus* (Burdkovskij, 1980); *Halioporoides sibogae* (Baelde, 1992); crabs: *Paralithodes camtschatica* (Powel & Nickerson, 1965); Konar crab, *Raina vanina* (Haley, 1969); Ghost crab, *Ocypoda quadrata* (Fielding & Haley, 1976); *Barytelphusa guarini* ( Gangotri et al., 1971); *Portunus sanguinolentus* (Reeby et al., 1990); lobsters: *Panulirus versicolor* (George & Morgan, 1979) and *P.cygnus* (Grey, 1979).

Female spiny lobsters are considered as mature when they are capable of producing eggs, with maturity readily determined from external secondary sexual characteristics (Aiken & Waddy, 1980) and the microscopic and macroscopic appearance of the ovaries. The presence of developed pleopodal setae has been regarded widely as indicating the attainment of functional maturity in females of many species of spiny lobsters (Nakamura, 1940; George, 1958; Kensler, 1967; Booth, 1984; Pollock, 1991). Internal indices have been used to estimate maturity in female decapods, where ovary weight, size and colour are used. The ovary changes colour from creamy white to light orange, then darker orange and finally brick red in *Jasus* and *Panulirus* (Fielder, 1964; Berry, 1971).

Maturity in male spiny lobsters may be either physiological or functional:- Physiological- where they are capable of producing spermatozoa but are incapable of copulation and functional maturity i.e. having the capability to be actively involved in breeding (Aiken & Waddy, 1980). The commonly used terms, size at maturity and size at onset of breeding refer to functional maturity. In male spiny lobsters, it is not possible to determine maturity from the examination of external characters (Lindberg, 1955; Fielder, 1964; Heydorn, 1965; Berry, 1971; Chittleborough, 1974; MacFarlane & Moore, 1986). Several studies have also shown that it is not possible to determine maturity from the macroscopic appearance of the testes and vas deferens: *Jasus lalandii* (Booth et al., 1990), *Panulirus ornatus* (Fielder, 1964; Heydorn, 1965; MacFarlane & Moore, 1986). Maturity in male palinurids has been an infrequent subject of investigation, perhaps due to the need for gonad examination, although histological section of gonads has been used to estimate sexual maturity in *P.interruptus* (Lindberg, 1955) and *J.lalandii* (Hickman, 1946; Bradstock, 1950). In the male lobster species, the increased weight of the testes including accessory genital organs and the vas deferens is useful to determine maturity. Estimates have also been made by plotting the relationship of a morphological character that alters with maturity against length of the animal assessing the 'point of upturn' of a curve fitted by eye (Templeman, 1935).

Functional maturity has been estimated for a small number of spiny lobsters; *P.versicolor* (George and Morgan, 1979) and *P.cygnus* (Grey,

1979) by intersect analysis. Several palinurid species are known to exhibit extraordinary growth of the anterior legs in males (Crawford & De Smidt, 1922; Kubo 1938; Gordon, 1960; Berry 1971). Taking this aspect into consideration, attempt has been made to estimate size at physical maturity of *P.homarus*.

## MATERIAL AND METHODS

### Physical maturity

In this study, size at physical maturity is estimated by the linear intersect method of George and Morgan (1979). Eighty five numbers of male lobsters ranging in carapace length from 36 mm-90 mm were measured with vernier calipers to the nearest millimeter. The leg length was measured along the ventral surface from the tip of the dactyl to the proximal margin of the ischium. Lobsters with second and third walking legs intact were only measured and regenerating legs were not taken into consideration.

The carapace length was plotted against the length of the second and third walking leg. Least square regression was fitted separately to the selected data. Students ‘t’ test was used to determine whether the slopes of the two regression lines for each leg was significantly different. The value of the intersect of the two regression lines was calculated for which a significant difference in slope of the two regression lines was found.

### Gonad maturity

Size at gonad maturity was determined by the histological examination of mature gonads (Baelde, 1992). Minimum and average sizes at maturity were determined from the relative abundance of mature and immature lobsters based on histological sections plotted against 5 mm size classes. The percentage of mature gonads found in each 5mm carapace length interval was plotted against the mid point of the carapace length interval. The size at first sexual maturity was then estimated graphically as the carapace length corresponding to a 50% maturity level.

The vas deferens of lobsters ranging in size from 37-75 mm was weighed to the nearest milligram in a Sartorious balance. The vas deferens weight was plotted against the mid point of the carapace length interval and the graph was visually plotted to the data. The size at 50% maturity was then estimated. This study was carried out during 2003-2006.

## RESULT

### Size at sexual maturity

Fig (1) shows the relationship between the percentage of mature gonads in each 5mm size class interval and mid point of the carapace length interval. Males reached maturity at an average carapace length of 46-50 mm (Fig.2). The smallest male recorded was 35 mm in carapace length in which gonads were discernable. They reached 50% maturity at a size class of 61-65 mm (Fig.2).

Fig (5) shows the relationship between vas deferens weight and carapace length. The estimate of size at 50% maturity from the graph which has been fitted visually to the data is 65 mm for the lobsters.

67 Ⓐ

31

**Fig.1. Percentage of mature gonads**

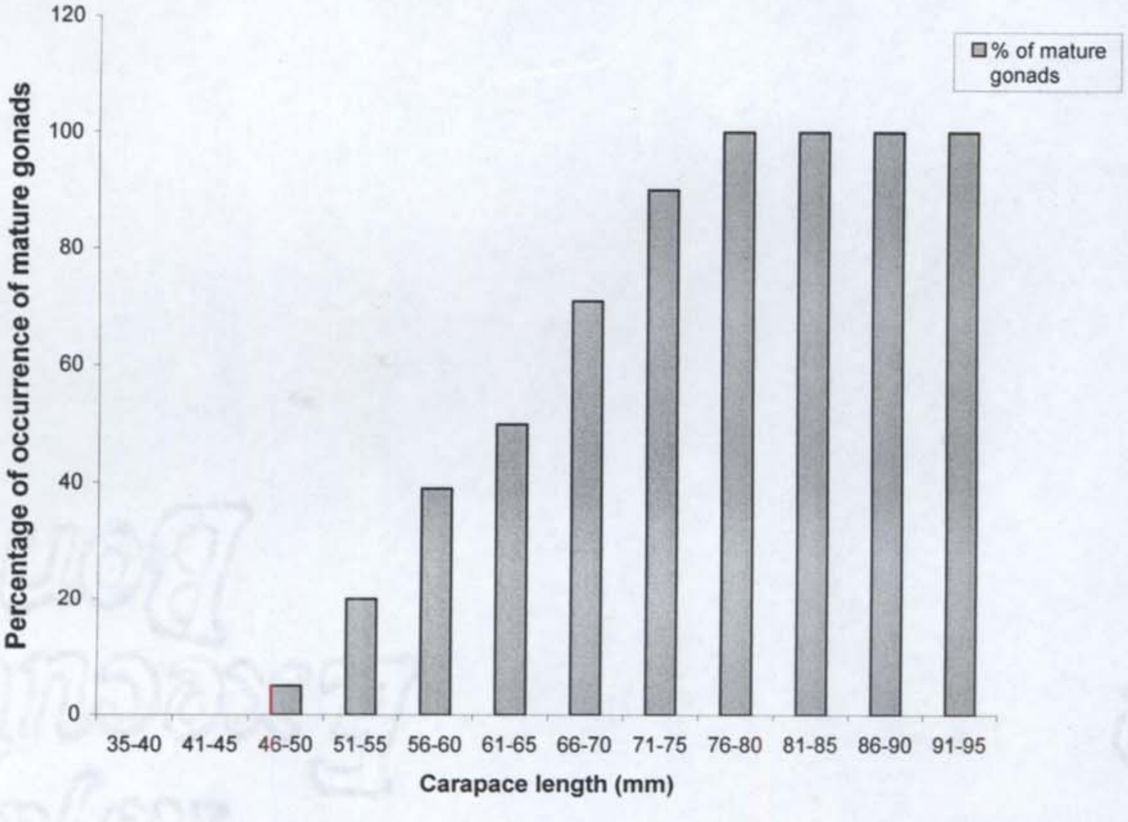
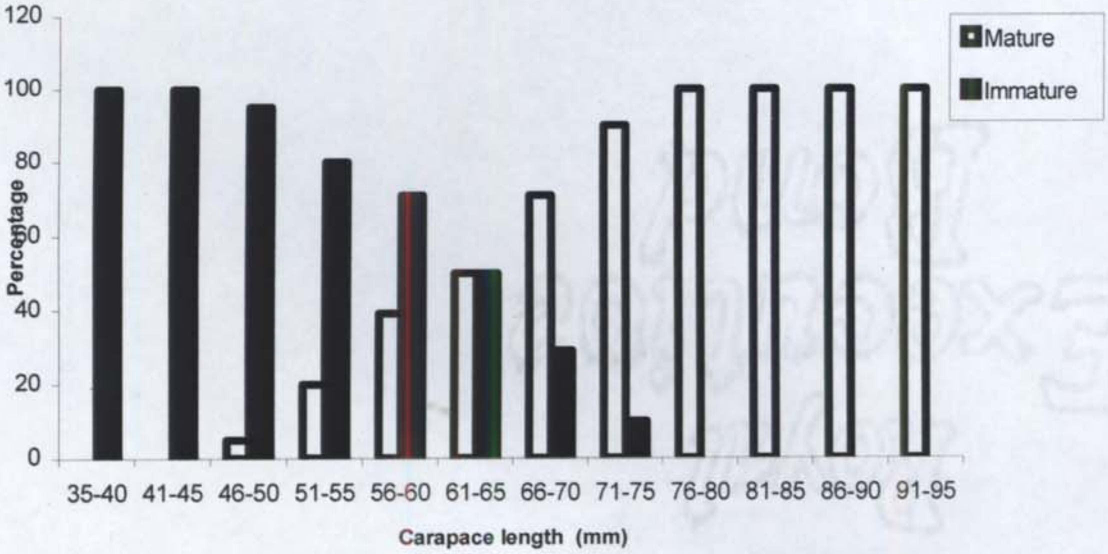


**Fig.2. Percentage of mature and immature animals**



## Size at physical maturity

The regression lines for data of fig (3) ie., for leg 2 were

$Y= -1.88 + 0.203x$

and

$Y=-14.06 + 0.360x$

for males below 44 mm and above 66 mm carapace length respectively and the regression lines for data of fig (4) i.e. for leg 3 were

$Y= -0.566 + 0.255x$

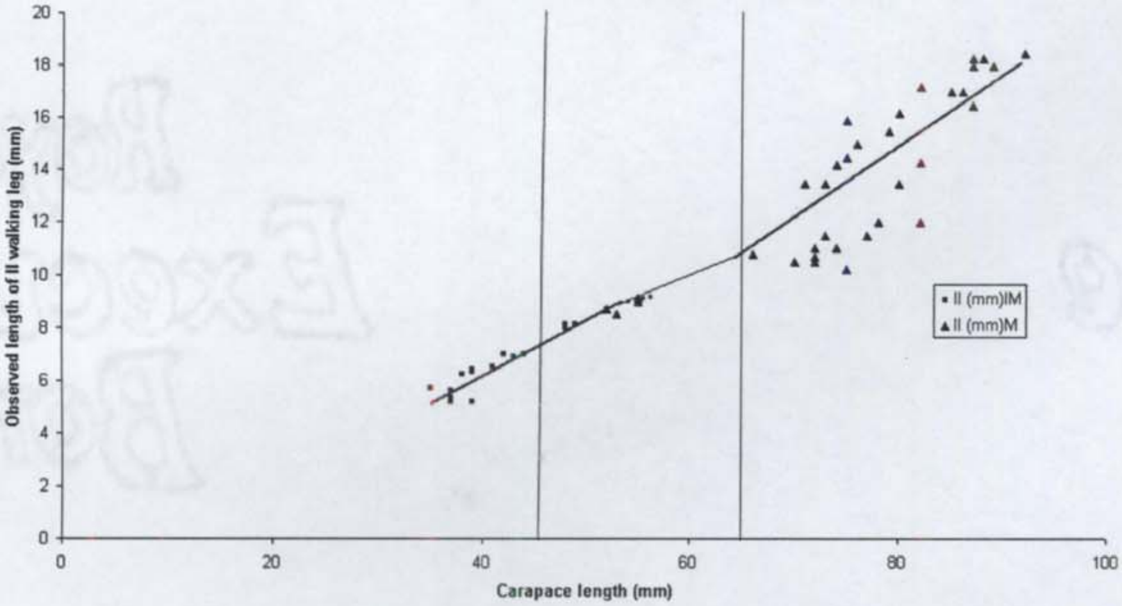
and

$Y=-22.20 + 0.576x$

for males below 44 mm and above 66 mm carapace length respectively.

From the graphs (3 & 4), the intersect values for leg II is 65 mm and for leg III is 66 mm. The size at maturity is taken as the mean of the intercept values of the two legs, ie., 65 mm.

Fig.3. Fitted regression for II walking leg of P.homarus



G8B

*Fig.4.Fitted regression for III walking leg of P.homarus*

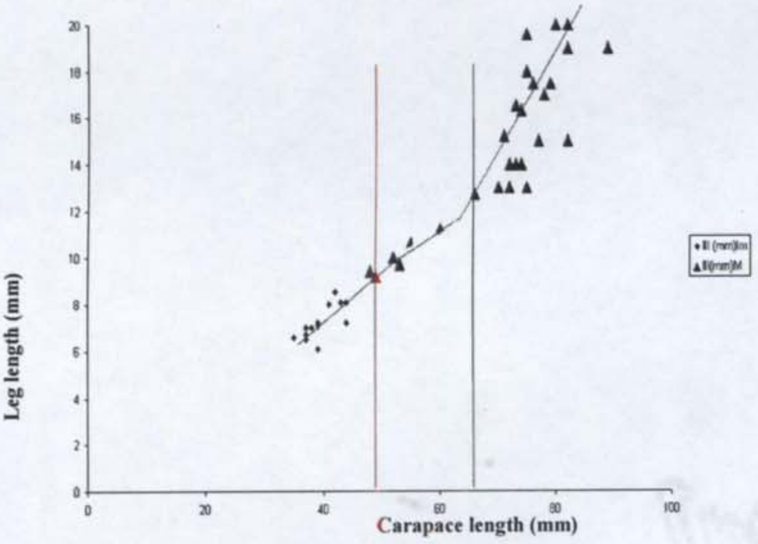
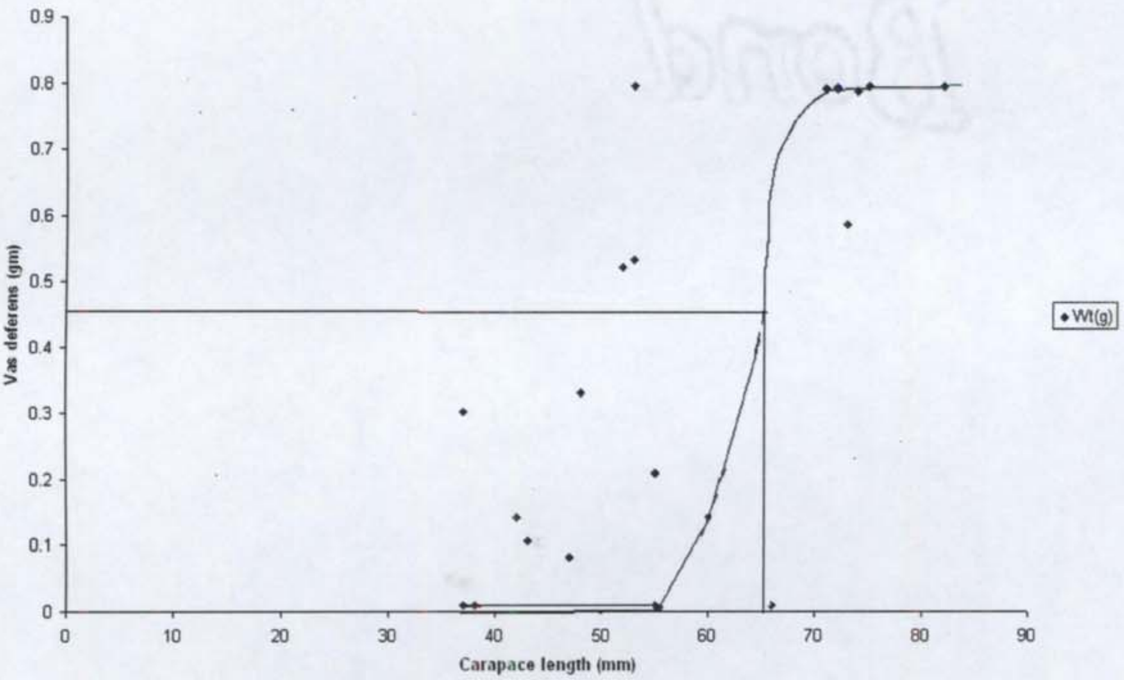


*Fig.5.Relationship between carapace length and vas deferens weight in P.homarus*



## DISCUSSION

The carapace length at which male *P.homarus* matures functionally is estimated to be 65 mm in this study and the carapace length range at which they become sexually or physiologically mature is 61-65 mm. Although in this case both physical and gonadal maturation are attained by *P.homarus* at a more or less similar carapace length, size at physical maturity rather than at gonad maturity is usually preferred for management purposes. This is because palinurid lobsters of small size can have mature gonads but may not be capable of performing the mating act because the vas deferens has not developed (Heydorn, 1965). They may also be unable to mate, until they reach a size equivalent to or larger than female. Copulation probably occurs only when the male is of equal size, or larger than the female. It is doubtful whether small males are strong enough to mate with larger females and the amount of sperms in the vas deferens of such males is always small (Fielder, 1964).

The anterior legs of *P.homarus* are used for grasping the female prior to mating. They are used to establish the male hierarchy. These legs were observed to show marked difference in growth on attainment of physical maturity. The front legs are very important in the establishment of reproductive dominance of males in rock lobsters and are essential for reproductive success (Berry, 1970). In other lobster species also similar observations were reported. Templeman (1935) found that the size of both claws of male lobsters relative to body length indicated maturity. In *P.versicolor*, George and Morgan (1979) has reported on the marked increase in the leg length/carapace length ratio of the front legs of large males.

In male crabs also, Hartnoll (1974) found that there was some differential growth pattern in the chela allometry during the ontogeny. He observed a precise transition from pre puberty to the post puberty phase bringing about abrupt changes in certain morphological characters such as chela measurements in male crabs at pubertal moult. Many investigations have encompassed one pre puberty and post puberty phase in decapod crustaceans (Watson, 1970; Brown and Powel, 1972; Hartnoll, 1968). In the present study, two linear relationships of leg length to carapace length were found to fit the data suggesting that two clear cut growth phases occur during the life of rock lobsters i.e. an early steady growth of immature animals followed by an increased but again steady growth of the secondary sexual characters after gonad maturity. Huxley (1924) termed this phenomenon as “constant differential growth ratio”.

The method of determining size at first physical maturity by intersect analysis should be applicable to those species that show measurable morphological changes after maturity. The present study shows that in male *P.homarus,* the second and third legs show the greatest difference in slopes of the regressions of the selected data. These legs are presumably of major reproductive importance as shown in other male lobsters- *P.versicolor* (George & Morgan, 1979), in *P.japonicus* (Kubo, 1938), *P.ornatus* (Gordon, 1960) and *P. homarus rubellus* (Berry, 1971).

According to George and Morgan (1979), none of the attempts made to calculate the confidence limits for the intersection points of regression lines by previous workers (Kastenbaum, 1959; Robison, 1964; Hudson, 1966) is entirely satisfactory, therefore the best estimate for the intersect is given by those regression lines which have difference in slopes.

Size at maturity varies in different species depending on the geographical location. In this study *P.homarus* was estimated to attain sexual maturity at a carapace length range of 61-65 mm and physical maturity at 65 mm carapace length, whereas in *P.homarus* from the African waters, Berry (1971) recorded the size at maturity to be between 50-60 mm carapace length. Heydorn (1965) placed size at maturity for male *J.lalandii* at 60-65 mm carapace length. Much earlier, Von bonde (1936) observed that the South African stocks of *J.lalandii* were not mature below about 82 mm carapace length. Lyons et al., (1981) and Lyons (1986) demonstrated considerable variation in size at maturity for *P.argus* throughout the Caribbean Sea. In *P.versicolor* (George & Morgan, 1979) provide an estimate of 72 mm as the size at which immature specimens first begin to develop mature morphological features. In the western rock lobster, *P.cygnus* (Grey, 1979) estimated size at maturity as 10.5 mm and 9.8 mm for Freemantle and Geraldton samples. Tropical species generally appear to reach sexual maturity at a relatively smaller size than temperate species and temperature effects may also account for geographical variations in the size at first maturity within a single species (Cobb & Phillips, 1980).

Lobsters should not be commercially exploited below their size of first physical maturity, as this can result in the decrease in breeding stock, which may affect the recruitment in the following seasons. In the long term this might result in the collapse of the fishery. As per government of India gazette notification in 2003, MLS has been set for the export of lobsters. As per this notification, *P.homarus* below 200 g is banned from export (Radhakrishnan et al, 2005). But as yet there is no restriction on the fishing of undersized lobsters based on the length at maturity. In Australia, fishermen carry a scale while fishing and return the undersized lobsters to the sea (Cobb & Phillips, 1994). The lobsters should be allowed to undergo at least two spawning moults before their capture. 'V' marking of the tail of lobsters can be helpful to prevent the capture of maturing lobsters. Spiny lobsters being lucrative and a major foreign exchange earner for India are being fished indiscriminately which has lead to the decline in their catch. The goal of fishery management is sustainable exploitation of a resource for social and economic benefit. Exploitation will be sustainable only if certain biological features of the stock are maintained within limits that allow sufficient reproductive capacity and growth. It is high time that we realized the importance of this valuable resource and took measures to conserve the animals for the future generations.

# Chapter 1.5

# BIOCHEMICAL STUDIES

## INTRODUCTION

The decapod crustaceans which produce the specialized sperm packets or spermatophores require efficient biochemical machinery for the formation, passage and storage of the spermatozoa. Studies on the biochemical aspects of the reproductive tissues and accessory secretions are very few in decapod crustaceans.

Earliest studies on the biochemistry of the spermatophores and seminal secretions were conducted in *Balanus balanus* (Barnes, 1962; Barnes & Blackstock, 1974). Biochemical studies are meager in brachyuran crabs, although they produce copious quantity of seminal plasma (Mann, 1984). Jayalectumie and Subramoniam (1987 & 1991), investigated the biochemical nature of the seminal secretions in *Paratelphusa hydrodomous* and *S.serrata* and Rao et al., (1989) in *Portunus pelagicus.* Pillay and Nair (1973) observed the biochemical changes in the gonads of three decapod crustaceans namely, *Uca annulipes*, *Portunus pelagicus* and *Metapenaeus affinis* during the reproductive cycle. They found that the changes in the biochemical constituents in the male gonads are not so pronounced as in the ovary.

The maturity of males in decapod crustaceans depends on the production of sperm and secretion of accessory materials to form spermatophores. The vas deferens plays a major role in the secretion of materials that form the spermatophore layers. Previous studies on the male reproductive physiology of crustaceans were concerned with descriptive morphology, histology and histochemistry of the reproductive organs (Hinsch & Walker, 1974; Pochon-Masson, 1983; Haley, 1984; Subramoniom, 1991). Studies on the biochemical nature of the reproductive tract in correlation to maturation are not many in decapod crustaceans and unknown for any spiny lobster. This study provides information on the biochemical nature of the immature and mature reproductive tract of the male spiny lobster *P.homarus*.

## MATERIAL AND METHODS

The study was conducted during 2003-2006. Total proteins, carbohydrates and lipids were estimated in UV visible double spectrophotometer.

## Estimation of protein

Total protein was estimated by Folin-Ciocalteau method (Lowry et al., 1951).

## Sample preparation

10 mg of dried (hot air oven at 60°C) testis, proximal vas deferens and distal vas deferens were weighed to the nearest milligram in an electronic balance (Sartorious). These were precipitated with 2 ml of deproteinising agent, 10% Tri Chloro Acetic acid (TCA) by keeping the tubes in ice. All the samples were centrifuged at 3000 rpm for 15 minutes. The supernatant obtained in the individual tube was used for carbohydrate estimation. The protein precipitate in each tube was dissolved in 5 ml of 1N NaOH. Three aliquots each with 0.1 ml solution were used as samples. To this 0.4 ml of double distilled water was added and each sample was made up to 0.5 ml. To this 0.5 ml solution, freshly prepared 5 ml alkaline mixture (48 ml of 2% $Na_2Co_3$ in 0.1N NaOH+1 ml of 0.5% copper sulphate in 1% of sodium potassium tartarate) was added and kept at room temperature for 10 minutes. After 10 minutes, 0.5 ml of Folin reagent (diluted the 2N stock solution with double distilled water) was added and mixed well immediately.

## Preparation of standard

A standard stock solution was prepared using bovine serum albumin crystals at a concentration of 25 mg/ ml 1N NaOH. Different dilutions in the range of 0.25 to 2.5 mg/ml were prepared from this stock solution and the alkaline mixture and Folin-phenol reagent were added as in the case of tissue samples. A blank was prepared with 0.5ml double distilled water and treated the same as above.

All the test tubes were kept at room temperature for 30 minutes. The samples were then read for the optical density of the blue colour developed, in a spectrophotometer at 660 nm against a blank. The protein content of the tissue sample was expressed as mg protein/100 mg dry tissue.

## Estimation of carbohydrate

The phenol sulphuric acid method of Dubois et al (1956) was followed to estimate the total carbohydrate in the samples.

## Sample preparation

The supernatant obtained during protein estimation was used for carbohydrate analysis. From the above supernatant, 0.1 ml was taken and made up to 1 ml with saturated solution of benzoic acid in double distilled water and to this solution; 1 ml of sulphuric acid was added rapidly and carefully to each tube and mixed well using a cyclomixer.

## Standard Carbohydrate solution

A standard solution was prepared using D-glucose (Concentration-20mg/100 ml saturated solution of benzoic acid). Different dilutions of the working solution with the concentration of glucose ranging from 10 to 100 g/ml were prepared and the procedure adopted for the tissue was followed. A blank solution with 2 ml 5% phenol was prepared and the above procedure followed.

All the tubes were kept for 30 minutes at 30°C and the optical density of the orange colour developed was measured at a wavelength of 490 nm.

## Estimation of lipids

The total lipids were quantitatively determined by the sulphophospho-vanillin method of Barnes and Blackstock (1973).

About 10 mg of the tissues-testis, proximal vas deferens and distal vas deferens, were separately homogenized well in 2 ml of chloroform: methanol (2:1V/V) and kept overnight at 4°C for complete extraction. The mixture taken in glass stoppered centrifuge tubes was then centrifuged for 15 minutes at 300 rpm. The clear supernatant containing all lipids was transferred to clean dry glass tubes. 0.5 ml of the lipid extract of all the tissues were taken separately in clean glass tubes and dried in vaccuo over silica gel in a dessicator. To each dried sample, 0.5 ml concentrated sulphuric acid was added and shaken well. The tubes were then plugged with non-absorbent cotton wool and heated at 100°C in a boiling water bath exactly for 10 minutes. The tubes were rapidly cooled to room temperature under running tap water. To 0.1 ml of this acid digest, 2.5 ml of phosphovanillin reagent were added and mixed well by dissolving 80mg of cholesterol in 100 ml of Chloroform : methanol (2:1V/V) mixture (equivalent to 100 mg of total lipid in 100 ml (2:1V/V) Chloroform : methanol mixture). Working solutions of different concentrations were prepared from the stock solution in the range 50-500 g/0.5 ml and the procedure adopted for the tissue samples were followed). 5 ml of 2:1V/V chloroform : methanol mixture was treated as blank. All the tubes were kept at room temperature for 30 minutes. The intensity of the pinkish red colour developed was measured against the blank at 520 nm.

## Preparation of standard graph

Standard graphs were plotted with the concentration of each biochemical parameter in different dilutions of the working standard solution in the 'X'axis and the optical density in the 'Y'axis. The concentration of different parameters in samples were calculated (in mg %) by comparing the OD obtained for the sample with the values in the standard graph and using the formula

Concentration in mg/100 mg dry tissue =

$$\frac{\text{(OD of sample-OD of the blank)}}{\text{(OD of standard-OD of the blank)}} \text{ X Concentration of standard X } \frac{100}{\text{wt of sample in mg}}$$

These experimental data on protein, carbohydrate and lipids with respect to mature and immature lobsters, were subjected to statistical analysis namely, one way ANOVA and students 't' test (Snedecor & Cochran, 1967). The levels of significance were observed at 0.005 and 0.001.

## Characterisation of Protein by Electrophoresis

The reproductive tract was excised out and different regions namely-testis, proximal vas deferens and distal vas deferens were taken for electrophoresis. Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) was performed following the method of Laemelli (1970). Proteins in the gel were stained with Coomassie Brilliant Blue.

## Preparation of reagent

Stock reagent

1. Acrylamide-30g

   N'-N'-bis methylene acrylamide-0.8g

   Distilled water-100ml

The solution was filtered through Whatman No.1 filter paper and stored at 4°C in dark glass bottles.

2. Tris-HCl (1.5M, pH 8.8)

Tris base-18.15g

Distilled water-50ml

The pH was adjusted to 8.8 with 1N HCl, stored in glass bottles (4°C)

3. Tris-HCl (0.5M, pH 6.8)

Tris base-6g

Distilled water-60ml

Adjusted pH to 6.8 with 1N HCl, stored at 4°C in a glass bottle

4. SDS 10% (W/V)

10g of SDS was added to 100ml of distilled water and stirred gently. Stored at room temperature.

5. Ammonium persulphate 10% (APS)

Dissolved 100mg of APS in 1ml distilled water. This is always prepared fresh before use.

6. TEMED-Tetra Methylene Ethylene Diamine catalyst
7. Resolving gel buffer
8. Stacking gel buffer
9. Electrode buffer

**Extraction of sample**

0.1mg of tissue sample (each testis, proximal vas deferens and distal vas deferens) was homogenized in 40% sucrose, in a glass homogeniser. Then it was centrifuged at 10,000 rpm for 15minutes at 4°C.

**Gel casting**

Gel plates were assembled as per the instructions of the manufacturers (GENEI, Bangalore). The acrylamide mixture was prepared for 10% gel and mixed gently. The components were mixed without delay. The acrylamide mixture (Resolving gel) was poured into the space between the glass plate and overlayed with distilled water. The gel was allowed to polymerize at room temperature for one hour.

Distilled water overlaid on the resolving gel was poured off completely by tilting the assembly. The area above the gel was dried with filter paper (Whatman No.1) without touching the gel surface. A 12 teeth comb was placed in the gel sandwich and the 10% stacking gel was introduced.

After polymerization for 45 minutes to one hour, the gel sandwich with the casting stand was placed in the refrigerator overnight to cool the gel before loading the sample and starting the run.

**Sample loading**

The prepared samples were heated in a boiling water bath for 20 minutes to denature the proteins. The comb was removed from the gel and the wells washed with distilled water. To 30 μl samples, was added the marker dye and these in turn were loaded into wells. Disposable plastic tips were used for each sample to avoid cross contamination. The LMW calibration kit (Sigma) was thawed and 10 μl charged into one of the sample wells. The wells on either side of the molecular weight marker were left unused and filled with an equivalent volume of blank sample buffer.

The electrode of reservoir buffer was diluted in 1:10 ratio by adding double distilled water. The buffer was poured into the upper and lower tanks without forming froth. The gel was run until the Bromophenol Blue dye reached the bottom of the resolving gel. Power supply (250mV) was switched off and the sandwich plates were removed from the apparatus by using spatula. The orientation was marked.

### Staining and destaining

The gel was immersed in 0.2% Coomassie Brilliant Blue in 45:45:10 % methanol, water, acetic acid in a plastic box. It was left overnight. Next day, the gel was destained with 25%, 65%, 10% methanol, water, acetic acid mix. The gel was stored in 7% acetic acid; the bands were visualized under UV trans-illuminator.

### Calculation

$$\text{Rf value} = \frac{\text{distance traveled by the protein (A)}}{\text{distance traveled by the dye marker (B)}}$$

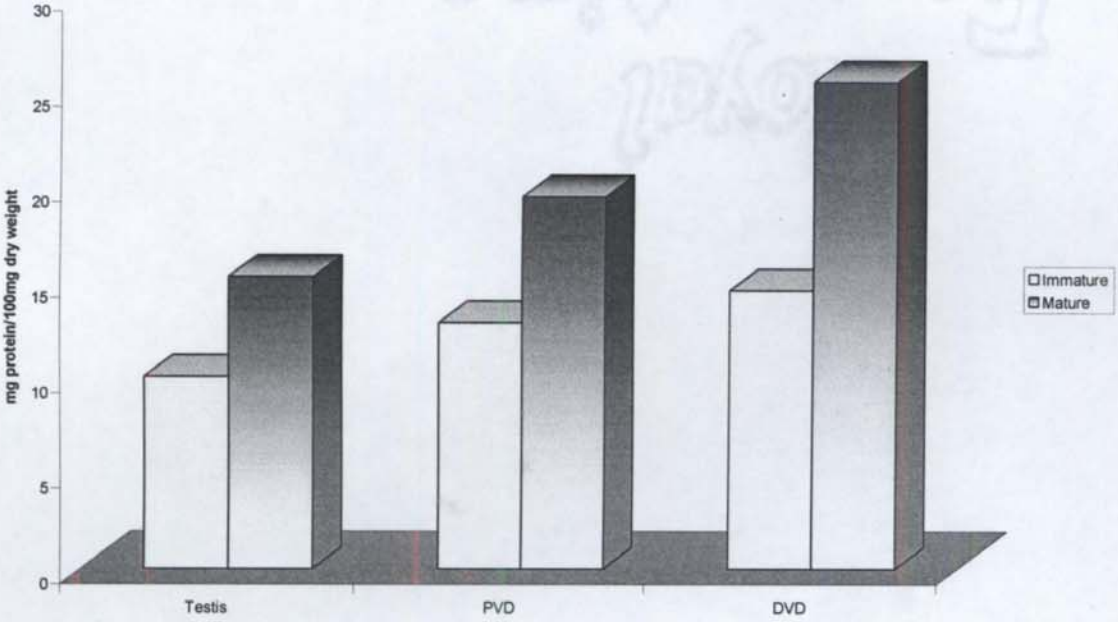
The molecular weight of the sample was calculated by plotting the molecular weight of the standard protein in the Y axis and their corresponding relative frequency (Rf) on the X axis (Table.1). The molecular weight of the different protein bands of the samples were calculated by comparing the Rf values obtained for the sample with the standard graph (Fig.7).

## RESULT

### Protein

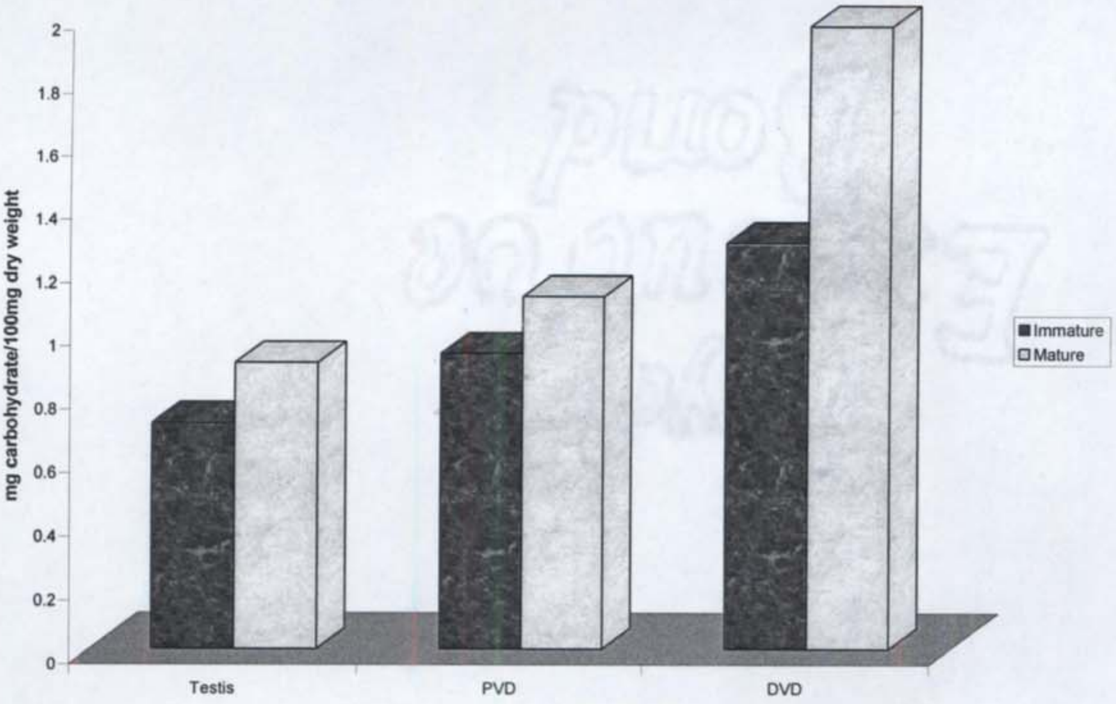
The protein content was observed to increase significantly from the testis to the proximal and distal vas deferens in both immature and mature lobsters (Table 1a, b, c & d). Higher amount of protein was recorded in the proximal vas deferens and distal vas deferens (Fig.1) in the immature and mature lobsters. The protein content in the immature lobsters (Fig.1) was comparatively lower when compared to that of mature lobsters. A significant difference was observed in the protein content between the mature and immature lobsters (Table 2a, b & c).

Fig.1. Biochemical composition of male reproductive tissue of *P.homarus* -Protein



PVD=Proximal Vas Deferens
DVD=Distal Vas Deferens

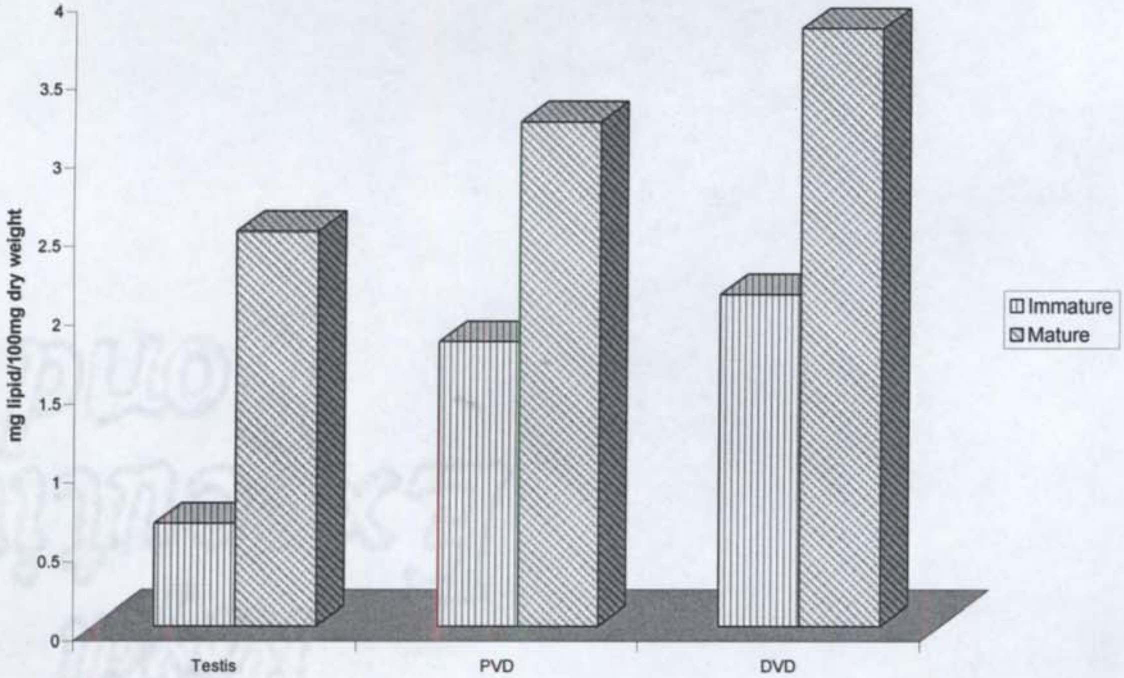
**Fig.2. Biochemical composition of male reproductive tissue of *P.homarus* -Carbohydrate**



PVD=Proximal Vas Deferens
DVD=Distal Vas Deferens

86 C

**Fig.3. Biochemical composition of male reproductive tissue of *P.homarus* -Lipid**



PVD=Proximal vas deferens
DVD=Distal vas deferens

**Table.1a .Anova between the protein content in testis and PVD of immature lobsters**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 17.956 | 1 | 17.956 | 13.62367 | 0.006121 | 5.317645 |
| Within Groups | 10.544 | 8 | 1.318 | | | |
| Total | 28.5 | 9 | | | | |

**Table.1b. Anova between the protein content in PVD and DVD of immature lobsters**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 12.1 | 1 | 12.1 | 7.86736 | 0.023024 | 5.317645 |
| Within Groups | 12.304 | 8 | 1.538 | | | |
| Total | 24.404 | 9 | | | | |

**Table.1c. Anova between the protein content in testis and PVD of mature lobsters**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 43.264 | 1 | 43.264 | 7.537282 | 0.025237 | 5.317645 |
| Within Groups | 45.92 | 8 | 5.74 | | | |
| Total | 89.184 | 9 | | | | |

**Table.1d. Anova between the protein content in PVD and DVD of mature lobsters**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 85.264 | 1 | 85.264 | 7.851197 | 0.023126 | 5.317645 |
| Within Groups | 86.88 | 8 | 10.86 | | | |
| Total | 172.144 | 9 | | | | |

**Table.2a. Anova between the protein content in testis of immature and mature lobsters**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 70.756 | 1 | 70.756 | 217.7108 | 4.38E-07 | 5.317645 |
| Within Groups | 2.6 | 8 | 0.325 | | | |
| Total | 73.356 | 9 | | | | |

**Table.2b.Anova between the protein content in PVD of immature and mature lobsters**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 115.6 | 1 | 115.6 | 17.16917 | 0.003237 | 5.317645 |
| Within Groups | 53.864 | 8 | 6.733 | | | |
| | | | | | | |
| Total | 169.464 | 9 | | | | |

**Table.2c. Anova between the protein content in DVD of immature and mature lobsters**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 272.484 | 1 | 272.484 | 48.09956 | 0.00012 | 5.317645 |
| Within Groups | 45.32 | 8 | 5.665 | | | |
| | | | | | | |
| Total | 317.804 | 9 | | | | |

**Table.3a. Anova between the carbohydrate content in testis and PVD of immature lobsters**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.0968 | 1 | 0.0968 | 14.16585 | 0.009357 | 5.987374 |
| Within Groups | 0.041 | 6 | 0.006833 | | | |
| | | | | | | |
| Total | 0.1378 | 7 | | | | |

**Table.3b. Anova between the carbohydrate content in PVD and DVD of immature lobsters**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.25205 | 1 | 0.25205 | 29.36505 | 0.001634 | 5.987374 |
| Within Groups | 0.0515 | 6 | 0.008583 | | | |
| | | | | | | |
| Total | 0.30355 | 7 | | | | |

### Table.3c. Anova between the carbohydrate content in testis and PVD of mature lobsters

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.090312 | 1 | 0.090312 | 39.05405 | 0.000778 | 5.987374 |
| Within Groups | 0.013875 | 6 | 0.002312 | | | |
| | | | | | | |
| Total | 0.104187 | 7 | | | | |

### Table.3d. Anova between the carbohydrate content in PVD and DVD of mature lobsters

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.690312 | 1 | 0.690312 | 34.78375 | 0.001055 | 5.987374 |
| Within Groups | 0.119075 | 6 | 0.019846 | | | |
| | | | | | | |
| Total | 0.809388 | 7 | | | | |

### Table.4a. Anova between the carbohydrate content in testis of immature and mature lobsters

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.0722 | 1 | 0.0722 | 18.35593 | 0.005181 | 5.987374 |
| Within Groups | 0.0236 | 6 | 0.003933 | | | |
| | | | | | | |
| Total | 0.0958 | 7 | | | | |

### Table.4b. Anova between the carbohydrate content in PVD of immature and mature lobsters

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.066613 | 1 | 0.066613 | 12.77938 | 0.011716 | 5.987374 |
| Within Groups | 0.031275 | 6 | 0.005212 | | | |
| | | | | | | |
| Total | 0.097888 | 7 | | | | |

## Table.4c. Anova between the carbohydrate content in DVD of immature and mature lobsters

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.34445 | 1 | 0.34445 | 14.83632 | 0.008442 | 5.987374 |
| Within Groups | 0.1393 | 6 | 0.023217 | | | |
| | | | | | | |
| Total | 0.48375 | 7 | | | | |

## Table.5a. Anova between the lipid content in testis and PVD of immature lobsters

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 2.645 | 1 | 2.645 | 54.72414 | 0.000313 | 5.987374 |
| Within Groups | 0.29 | 6 | 0.048333 | | | |
| | | | | | | |
| Total | 2.935 | 7 | | | | |

## Table.5b. .Anova between the lipid content in PVD and DVD of immature lobsters

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.245 | 1 | 0.245 | 7 | 0.038245 | 5.987374 |
| Within Groups | 0.21 | 6 | 0.035 | | | |
| | | | | | | |
| Total | 0.455 | 7 | | | | |

## Table.5c. Anova between the lipid content in testis and PVD of mature lobsters

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 1.125 | 1 | 1.125 | 96.42857 | 6.42E-05 | 5.987374 |
| Within Groups | 0.07 | 6 | 0.011667 | | | |
| | | | | | | |
| Total | 1.195 | 7 | | | | |

## Table.5d. Anova between the lipid content in PVD and DVD of mature lobsters

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.605 | 1 | 0.605 | 17.28571 | 0.00596 | 5.987374 |
| Within Groups | 0.21 | 6 | 0.035 | | | |
| | | | | | | |
| Total | 0.815 | 7 | | | | |

## Table.6a. Anova between the lipid content in testis of immature and mature lobsters

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 6.845 | 1 | 6.845 | 273.8 | 3.11E-06 | 5.987374 |
| Within Groups | 0.15 | 6 | 0.025 | | | |
| | | | | | | |
| Total | 6.995 | 7 | | | | |

## Table.6b. Anova between the lipid content in PVD of immature and mature lobsters

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 4.205 | 1 | 4.205 | 120.1429 | 3.42E-05 | 5.987374 |
| Within Groups | 0.21 | 6 | 0.035 | | | |
| | | | | | | |
| Total | 4.415 | 7 | | | | |

## Table.6c. Anova between the lipid content in DVD of immature and mature lobsters

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 5.445 | 1 | 5.445 | 155.5714 | 1.62E-05 | 5.987374 |
| Within Groups | 0.21 | 6 | 0.035 | | | |
| | | | | | | |
| Total | 5.655 | 7 | | | | |

### Carbohydrate

The concentration of carbohydrates increased significantly from the testis towards the proximal and distal vas deferens in both mature and immature lobsters (Table 3a, b, c & d). In the testis, proximal and distal vas deferens, the concentration of carbohydrate was significantly different between the immature and mature lobsters (Table 4a, b & c). The content of carbohydrate was lower in immature lobsters when compared to the mature animals (Fig. 2).

### Lipid

Lipid content also increased steeply from the testis towards the distal vas deferens (Table 5a, b, c & d). The lipid content was higher in the mature than in the immature lobsters (Fig.3).Significant difference in the lipid content in the testis, proximal and distal vas deferens was recorded between the immature and mature lobsters (Table 6a, b & c).

## Characterisation of protein by electrophoresis

The composite gonado-protein pattern specific to *P.homarus*, obtained after SDS PAGE of the gonads of immature and mature males is represented in Fig .6 . Generally the protein fractions ranged from 9-25 in the gonads of mature and immature lobsters.

## Testis

In the immature and mature testis, 9-12 protein fractions were observed. The Rf value of the fractions ranged from 0.10 to 0.86 in immature testis (Fig.4, Table. 2) and from 0.07 to 0.78 in mature testis (Fig.5). There were altogether 12 fast moving fractions. The slowest moving fraction had an Rf value of 0.07 with a molecular weight of 1,20,000 Daltons. The fastest moving fraction had Rf value of 0.86, with a molecular weight of 4000 Daltons.

Fig.7. Standard graph

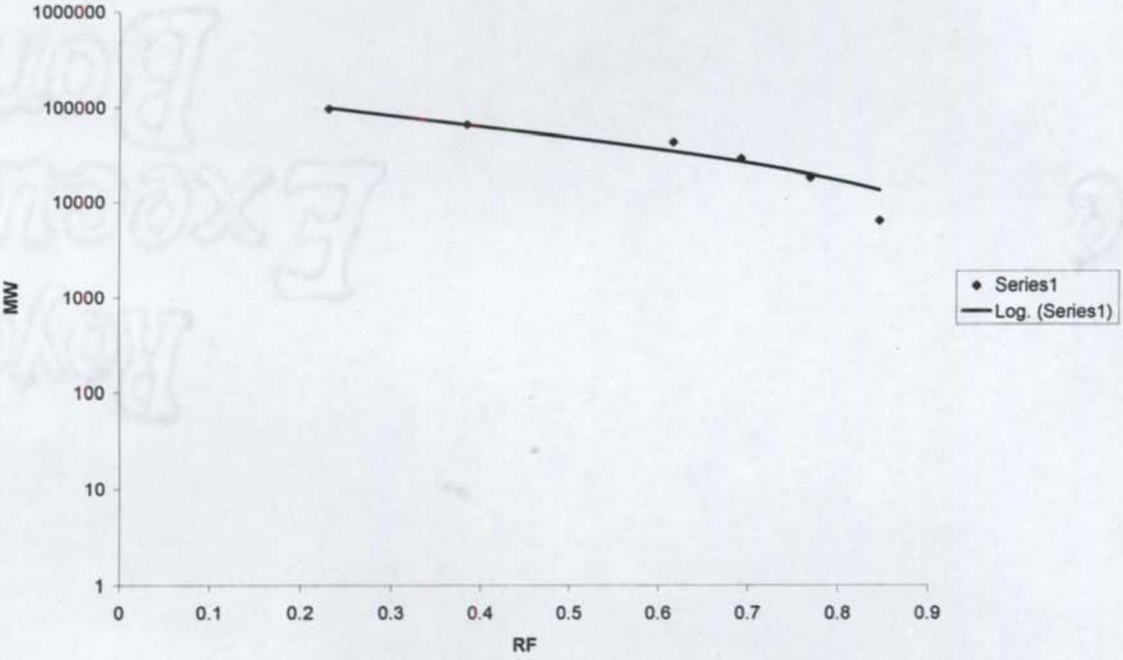


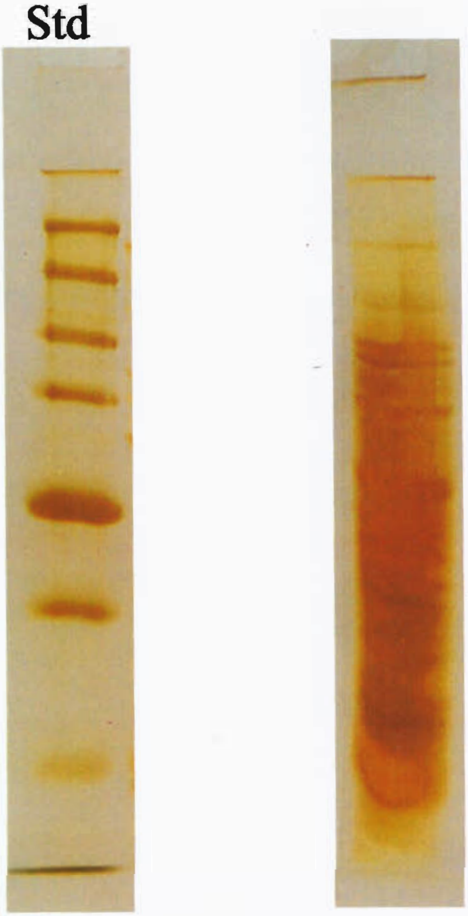
**Table1. The molecular weight and their corresponding Relative frequency of the standard proteins**

| **Standard protein** | **Molecular weight of standard proteins** | **Rf values of the standard proteins** |
|---|---|---|
| Phosphorylase b | 97,400 | 0.23 |
| Bovine Serum Albumin | 66,000 | 0.38 |
| Ovalbumin | 43,000 | 0.61 |
| Carbonic anhydrase | 29,000 | 0.69 |
| Lactalbumin | 18,400 | 0.76 |
| Aprotinin | 6,500 | 0.84 |

**Table 2. The Rf values of the protein fractions in the testis, proximal vas deferens and distal vas deferens of immature and mature *P.homarus*.**

| Rf value of immature DVD | Rf value of mature DVD | Rf value of immature PVD | Rf value of mature PVD | Rf value of immature testis | Rf value of mature testis |
|---|---|---|---|---|---|
| 0.09 | 0.11 | 0.08 | 0.09 | 0.10 | 0.07 |
| 0.156 | 0.13 | 0.12 | 0.12 | 0.17 | 0.10 |
| 0.25 | 0.18 | 0.19 | 0.19 | 0.31 | 0.18 |
| 0.26 | 0.19 | 0.24 | 0.24 | 0.36 | 0.21 |
| 0.27 | 0.21 | 0.27 | 0.27 | 0.37 | 0.31 |
| 0.28 | 0.22 | 0.29 | 0.29 | 0.44 | 0.36 |
| 0.31 | 0.27 | 0.32 | 0.32 | 0.55 | 0.42 |
| 0.34 | 0.28 | 0.35 | 0.35 | 0.72 | 0.47 |
| 0.37 | 0.31 | 0.38 | 0.38 | 0.86 | 0.55 |
| 0.40 | 0.34 | 0.4 | 0.4 |  | 0.57 |
| 0.43 | 0.37 | 0.48 | 0.48 |  | 0.63 |
| 0.5 | 0.40 | 0.54 | 0.54 |  | 0.78 |
| 0.56 | 0.45 | 0.56 | 0.56 |  |  |
| 0.62 | 0.47 | 0.58 | 0.58 |  |  |
| 0.65 | 0.52 | 0.6 | 0.6 |  |  |
| 0.71 | 0.54 | 0.62 | 0.62 |  |  |
| 0.75 | 0.59 | 0.64 | 0.64 |  |  |
| 0.76 | 0.62 | 0.70 | 0.70 |  |  |
| 0.78 | 0.71 | 0.72 | 0.72 |  |  |
| 0.87 | 0.75 | 0.74 | 0.74 |  |  |
|  | 0.77 | 0.76 | 0.76 |  |  |
|  | 0.78 | 0.77 | 0.77 |  |  |
|  | 0.81 | 0.79 | 0.79 |  |  |
|  | 0.84 | 0.84 | 0.84 |  |  |
|  | 0.90 |  |  |  |  |

Fig.6. Composite protein banding pattern of immature & mature *P.homarus*



## Proximal vas deferens

The protein fractions ranged from 12-24 in the mature and immature proximal vas deferens (Fig.4 & 5). The protein fractions had Rf value ranging from 0.08 to 0.84 in immature proximal vas deferens and 0.096 to 0.84 in mature proximal vas deferens (Table.2), the Rf value of the slowest fraction was 0.08 with a molecular weight of 1,30,000 Daltons and that of the fastest fraction, 0.870, its molecular weight was 5500 Daltons. Altogether, 11 fast moving fractions were recorded.

## Distal vas deferens

20-25 protein fractions were observed in the immature and mature distal vas deferens of which 11 were fast moving (Fig.4 & 5). The Rf value of the immature distal vas deferens ranged from 0.09 to 0.87 and in the mature distal vas deferens from 0.11 to 0.90 (Table.2). The Rf value of the slowest fraction was recorded to be 0.09 with a molecular weight of 1,40,000 Daltons. The Rf value of the fastest fraction was 0.90 with a molecular weight of 5,750 Daltons.

Fig.4. Electrophoretic protein patterns of the reproductive tissues of immature *P.homarus*.

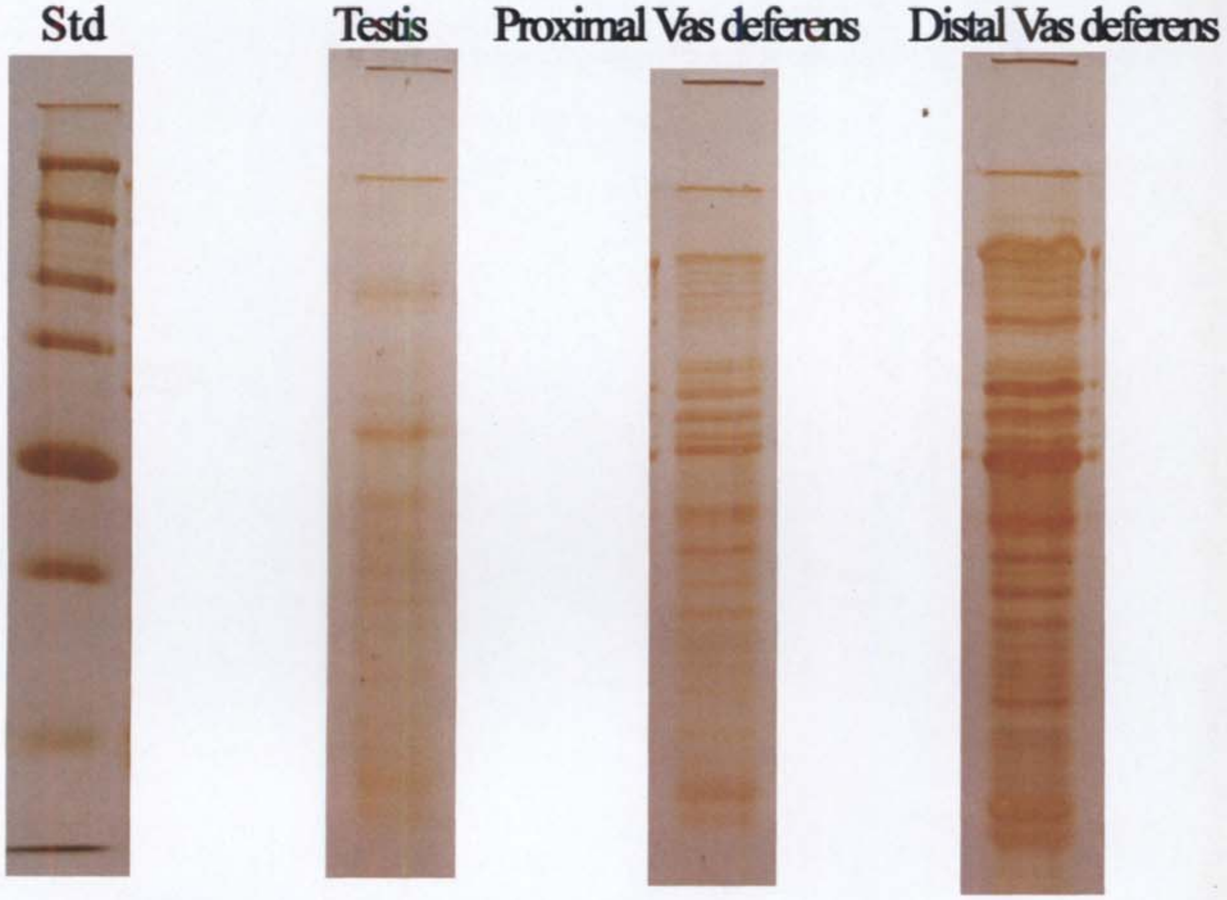
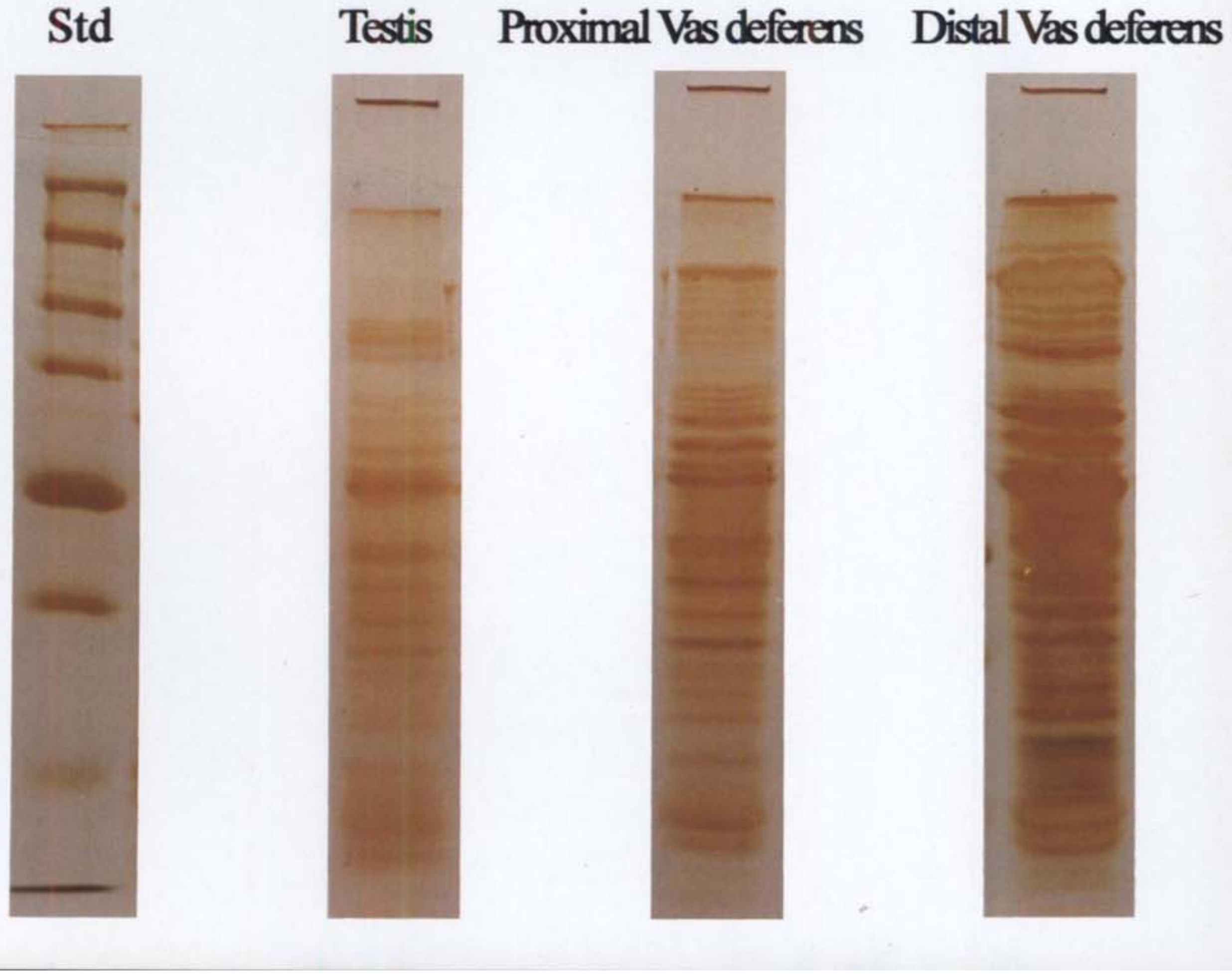


Fig.5. Electrophoretic protein patterns of the reproductive tissues of mature *P.homarus*



## DISCUSSION

The result reported in the present study throw light on the biochemical nature of the different regions of the reproductive tract in immature and mature lobsters. The biochemical components increase in the reproductive tissues during maturation. The distal vas deferens shows the maximum concentration of the biochemical constituents.

As the reproductive tissues mature, there is an increase in their biochemical content - proteins, carbohydrates and lipids were observed to be higher in the proximal and distal vas deferens of both mature and immature lobsters, which shows that these regions of the reproductive tract are secretory in nature. The highest concentration of the biochemical constituents being in the distal vas deferens signifies that it has the storage function also. In *P.homarus*, the final sperm formation and storage prior to ejaculation occurs in the distal vas deferens. Uma (1982) has shown the occurrence of free sugars such as fructose, glucose and mannose within the spermatophores of *Scylla serrata* and suggested that they may be involved in the anaerobic metabolism of spermatozoa. In the horseshoe crab, *Limulus polyphemus*, semen diluted with seawater showed prolonged sperm activity, suggesting that spermatozoa may rely on their carbohydrate for their survival under conditions lacking exogenous substrates (Behlmer & Brown, 1984). A high carbohydrate content of spermatophores was reported in the crab *P.hydrodromous* (Jeyalectumei & Subramoniam, 1987). In cirripedes, endogenous lipids are used for oxidative metabolism (Barnes, 1962).

Earlier workers have reported on the isoenzyme separation of LDH by PAGE in cirripedes (Barnes and Blackstock, 1974), in *S.serrata* (Uma, 1982) and *P.hydrodromous* (Jeyalectumei & Subramoniam, 1987). But studies have not so far reported on the protein fractions in the reproductive tract of any decapod crustaceans. The protein banding pattern of the gonads is reported for the first time in *P.homarus*.

An almost uniform protein banding pattern was observed in the mature and immature *P.homarus*. The maximal number of protein fractions recorded was 25 in the distal vas deferens of mature lobsters and minimum 9 in the testis of immature lobsters. The basic pattern was the same in both mature and immature lobsters. The least number of protein fractions were observed in the testis which increased towards the distal vas deferens. In the proximal and distal vas deferens, the number of fractions is more or less uniform. The increase in the protein content from the testis to the distal vas deferens, is reflected by the increase in the number of protein bands and the intensity of the fast moving fraction and in the increase in the number of simple protein fractions. The slow moving fractions also increased in thickness and intensity towards the distal vas deferens, in both mature and immature lobsters.

# Summary

## SUMMARY

The sexes of *P.homarus* are easily distinguishable externally. The male reproductive tract is located in the thoracic region dorsal to the alimentary canal consisting of paired testis and vas deferens. The testis is 'H' shape, semi transparent closely approximated.

In the immature lobsters, the testis is extremely delicate and transparent structure which could be located and removed only with great difficulty. As the animal mature, the testis change little in appearance except to grow in size and become somewhat opaque.

The reproductive system could be discerned from 35 mm carapace length onwards. In animals with 35-55 mm carapace length, the testicular lobe if left unwound measure 20-22 mm in length and in animals of 55 mm carapace length could measure 35-50 mm.

The vas deferens can be divided into two distinct portions- the highly coiled proximal vas deferens and the distal vas deferens - this is dull white

and the portion leading to the gonopore is transparent. The androgenic gland is located just before the distal vas deferens opens out through the gonopore.

Histological sections revealed the testis to be multilobed or with many follicles separated by seminiferous duct. Each lobule is independent of the other and connects to the proximal vas deferens through the ducts. The immature testis has only spermatogonial cells measuring 6.9-13.3 µm in diameter.

In mature testis, spermatogonia are present towards the periphery and the primary and secondary spermatocytes towards the centre. The primary spermatocytes measure 5.4-5.9 µm and the secondary spermatocytes, 2.8-3 µm in diameter. Muscle layers were not observed around the testis.

The proximal vas deferens is thinner and highly coiled than the distal vas deferens. Spermatophores housing the spermatocytes are found towards the periphery and spermatozoa in the centre. In the juvenile lobsters, the outer walls of the proximal vas deferens are not well differentiated. In the mature lobsters, the outer covering of the proximal vas deferens are well defined with an outermost connective tissue layer followed by epithelial layer. The proximal vas deferens is highly secretory as evidenced by the very thick inner epithelial layer and a very thin outer connective tissue layer.

The distal vas deferens is almost transparent in the juvenile lobsters. They contain the primary and secondary spermatocytes, which are embedded in a gelatinous matrix. The mature lobsters have well defined inner muscular and epithelial walls, the invagination of which forms the 'typhlosole'. This produces a matrix dorsally pushing the spermatophoric mass containing the spermatozoa towards the periphery. The posterior part of the distal vas deferens stores the mature spermatozoa prior to ejaculation. The posterior part of the distal vas deferens has circular muscle fibers that contract to ejaculate the sperm during mating.

The androgenic gland is located between the muscles of the coxopodite of the last walking leg and is attached to the subterminal region of the vas deferens. The gland consists of strands of cells folded back on itself. It is 2-3 cm in length.

In *P.homarus*, spermatogenesis is synchronous, that is only one or two developmental stages occur concurrently in any segment of the seminiferous tubules. In immature lobsters the spermatogonia undergo meiotic division to form spermatocytes and spermatids, which are arranged towards the center. The primary and secondary spermatocytes are observed in the mature testis.

The spermatophoric mass begins to form in the proximal vas deferens. They are found as discrete masses filling almost the entire gelatinous matrix of the PVD. The PVD also secretes the acellular layers around the sperm to form the sperm mass.

The distal vas deferens (DVD) contains the spermatozoa within the spermatophoric mass which is neatly arranged towards the periphery below the typhlosole- the invagination of the glandular epithelium.

At the ultrastructure level the immature testis has spermatogonia with lamina, nucleus and primary mitochondria like bodies. The lamina was observed to be discontinuous and only around the spermatogonia and was not found around other germ cell type. The primary and secondary spermatocytes were observed in maturing and mature testis and proximal vas deferens. The spermatocytes divide to form spermatid, and here acrosome and lamellar region starts formation.

Spermatids in different stages of development were apparent in this study. The spermatids contained mitochondria, endoplasmic reticulum and centrioles. In the early stage of spermiogenesis the formation of acrosome is evident. As spermiogenesis progresses further, the membrane of the nucleus and the layers of lamellar region become continuous with each other. Later the acrosome become electron opaque and the acrosome and nucleus lie in close contact with the lamellar region on one side. With further development, radial arms form with microtubules. The electron micrographs shows that the radial arms are continuous with the nuclear cup and in the spermatophores are wrapped around the individual sperm. The spermatids form spermatozoa through these gradual changes in the cytoplasm, without further division of the spermatids.

Mature sperms were found within the spermatophores in the distal vas deferens and were not observed in the testis and proximal part of the vas deferens. At the ultrastructural level the sperm consists of nucleus, lamellar region, spikes and an acrosome. No distinct membrane separating the cytoplasm from the nucleus is apparent.

The acrosome which is lens shaped has four regions of differing electron density- crystalline, scroll, homogenous and flocculent.

The lamellar region consists of folds of membranes. It lies to one side of the acrosome and outside the nuclear envelope. It is restricted in size and contains remnants of mitochondria. Numerous arms or spikes arise in this area anterior to the nucleus and extend posteriorly.

Typhlosoles consisting of bundles of striated muscles are evident in the distal vas deferens. The muscle cells are cuboidal in nature.

Centrioles were observed in the mature sperm at the base of the acromsome. They form the microtubules, which are evident in the nucleus, radial arms and spikes.

Males reached sexual maturity at an average carapace length of 46-50 mm. The smallest male recorded was 35 mm in carapace length in which gonads were discernable. Histological sections revealed that the lobsters reached 50% maturity at a size class of 61-65 mm. The estimate of size at

50% maturity from the relationship between vas deferens weight and carapace length is 65 mm for the lobsters.

Size at physical maturity was estimated from the slopes of the two regression lines of the II and III walking leg which were 65 mm and 66 mm respectively. The size at maturity is taken as the mean of the intercept values of the two legs, ie. 65 mm.

Biochemical analysis of the reproductive tract revealed the protein, carbohydrate and lipid content to increase significantly from the testis to the proximal and distal vas deferens in both immature and mature lobsters. A significant difference was observed in the protein, carbohydrate and lipid content between the mature and immature lobsters.

The composite gonado-protein pattern specific to *P.homarus*, obtained after SDS PAGE of the gonads of immature and mature males showed the protein fractions to range from 9-25.

In the immature and mature testis, 9-12 protein fractions were observed. The Rf value of the fractions ranged from 0.10 to 0.86 in immature testis and from 0.07 to 0.78 in mature testis. The slowest moving fraction had an Rf value of 0.07 with a molecular weight of 1, 20,000 Daltons. The fastest moving fraction had Rf value of 0.86, with a molecular weight of 4000 Daltons.

The protein fractions ranged from 12-24 in the mature and immature proximal vas deferens. The Rf value of the slowest fraction was 0.08 with a molecular weight of 1,30,000 Daltons and that of the fastest fraction, 0.870, its molecular weight was 5500 Daltons.

20-25 protein fractions were observed in the immature and mature distal vas deferens. The Rf value of the slowest fraction was recorded to be 0.09 with a molecular weight of 1,40,000 Daltons. The Rf value of the fastest fraction was 0.90 with a molecular weight of 5,750 Daltons.

# References

# REFERENCES

Adiyodi, R.G. 1984. Seasonal changes and the role of eyestalks in the activity of the androgenic gland of the crab *Paratelphusa hydrodromous* (Herbst). *Comp.Physiol.Ecol.*, **9**: 427-431.

Adiyodi, K.G. and Anilkumar, G. 1988. Arthropoda-Crustacea. In: Reproductive Biology of Invertebrates: Accessory sex glands, Vol.3, K.G. and R.G.Adiyodi (Eds), Oxford and IBH, New Delhi, pp: 261-318.

Aiken, D.E and Waddy, S.L. 1980. Reproductive Biology. In: The Biology and Management of lobsters. Vol.I. Physiology and behaviour.(Ed:J.S.Cobb and B.F.Phillip). pp: 215-276.Academic Press, New York.

*Anderson, W.A. & Ellis, R.A. 1967. Cytodifferentiation of the crayfish spermatozoon; acrosome formation, transformation of mitochondria and development of microtubules. *Z. Zellforsch. Mikroskop. Anat.*, **77:** 80-94.

*Andrews, E.A. 1931. Spermatophores of an Oregon crayfish. *Am.Nat.,* **65**:.277-280.

Arsenault, A.L., Clattenburg, R.E and Odense, D.H. 1979. Spermiogenesis in the shrimp, *Crangon septimspinosa. Can.J.Zool.*, **51**: 486-498.

Baelde, P .1992. Reproductive biology of commercially exploited deepwater royal red prawns (*Haliporoides sibogae*, Solenoceridae) in the south-east Australia. *Mar. Biol.*, **113**: 447-456.

Balasubramaniam, C.P. and Suseelan, C. 2000. Male reproductive system and spermatogenesis in the deep water crab, *Charybdis smithii* McLeay (Brachyura: Portunidae). *Ind.J.Fish.*, **47(4)**: 275-282.

*Barnes, H. 1962. The composition of the seminal plasma of *Balanus balanus*. *J.Exp.Biol.*, **391**: 345-356.

Barnes, H and Blackstock, J. 1973. Estimation of lipids in marine animals and tissues: Detailed investigation of the sulphovanillin method 'total lipids'. *J.Exp.Mar.Biol.Ecol.*, **12**: 103-118.

Barnes, H and Blackstock, J. 1974. Biochemical composition of the seminal plasma of the cirripede *Balanus balanus* (L) with particular respect to free aminoacids and proteins. *J.Exp.Mar. Biol.Ecol.*, **16**: 47-85.

Behlmer, S.D and Brown, G.1984. Variability of cryopreserved spermatozoa of the horseshoe crab, *Limulus polyphemus* (L). *Int.J.Invert.Reprod.Dev.,* **7**: 193-199.

Bell, T and Lightener, D. 1988. Male reproductive system. In: A handbook of normal penaeid shrimp histology. Aquaculture society. Baton Rouge, Lousiana. pp: 574-583.

Beninger, P.G., Elner, R.W., Foyle, T.P and Odense, P.H. 1988. Functional anatomy of the male reproductive system and the female spermatheca in the snow crab *Chionecetes opilio* (O.Fabricius) (Decapoda:Majidae) and a hypothesis for fertilization. *J.Crust.Biol.,* **8**: 322-332.

Berry, P.F. 1969. Occurrence of an external spermatophoric mass in the spiny lobster *Panulirus gilchristi* (Decapoda, Palinuridae). *Crustaceana,* **17(2)**: 223-234.

Berry, P.F and Heydorn, A.E.F. 1970.A comparison of the spermatophoric masses and mechanisms of fertilization in Southern African spiny lobster (Palinuridae).*Oceanogr.Res.Inst.(Durban).Invest.Rep.,* **25**: 1-18.

Berry, P.F. 1971. The biology of the spiny lobster *Panulirus homarus* (Linnaeus) off the east coast of southern Africa. *Investl.Rep.Oceanography.Res.Inst.,* **28**: 75.

*Binford, R .1913. The germ cells and the process of fertilization in crab *Menippe mercenaria*. *J.Morph.*, **24**: 147-202.

*Bizot-Espiard, A. 1980. Croissance et reproduction de la crevette Penaeus japonicus (Bate) en elevage: Effects de l'eyenlontam histology de l' appareil genital male, These pour porlarat de seme cycle a l' Universite de Bretagne Occidentale.pp: 1-36.

*Blades-Eckelbarger, P.I and Youngbluth. 1991. Functional morphology of spermatophores and sperm transfer in calanoid copepods. In R.T.Bauer and J.W.Martin (Eds), Crustacean sexual biology, Columbia University Press, New York, pp: 246-270.

Booth, J.D. 1984. Size at onset of breeding in female, *Jasus verreauxi* (Decapoda:Palinuridae) in New Zealand. *N.Z.J.Mar.Freshwat.Res.,* **18**: 159-169.

Booth, J.D., Street, R.J and Smith, P.J. 1990. Systematic status of the rock lobsters, *Jasus edwardsii* from New Zealand and *J.navaehollandiae* from Australia. *N.Z.J.Mar.Freshwat.Res.*, **24**: 239-249.

*Bradstock, C.A. 1950. A study of the marine crayfish *Jasus lalandii* (Milne Edwards) including accounts of autotomy and autospasy. Zool.Publ.Vict.Univ.Coll.Wellington. **7:** 1-38.

Brown, G.G. 1966. Ultrastructural studies of sperm morphology and sperm-egg interaction in the decapod, *Callinectes sapidus*. *J. Ultrastruct. Res.*, **14**: 425-440.

*Brown, R.B and Powel, G.C. 1972. *J.Fish.Res.Bd.Canada*. **29**: 423

*Brown, A.Jr., Kleve, M.G and Clark, W.H.Jr. 1976. Evidence for the presence of actin in natantian sperm. *Am.Zool.*, **16**: 180

*Burgos, M.H and Fawcett, D.W. 1955. Studies on the fine structure of the mammalian testis. I. Differentiation of the spermatids in the cat (*Felis domestica)*. *J.Biophys.Biochem.Cytol.*, **1**: 287

*Burgos, M.H and Fawcett, D.W. 1956. An electron microscope study of spermatid differentiation in the toad, *Bufo arenarum* Hensel. *J.Biophys.Biochem.Cytol.*, **2**: 223

Burukovskij, R.N.1980. Some biological aspects of the shrimp *Plesiopenaeus edwardsianus* in the southeast Atlantic. *Sov. J. Mar. Biol.*, **6**: 21-26.

Burton, T.E. 1995. The spermatid pathway and associated reproductive structures of the squat lobster, *Thenus orientalis* (Lund, 1793). *Invertebr.Reprod.Dev.*, **28(1):** 53-61.

Carlisle, D.B. 1959. On the sexual biology of *Pandalus borealis* (Crustacea Decapoda): 1. Histology of incretory elements. *J.Mar.Biol.Ass.U.K.*, **38**: 381-394.

Champion, H.F.B. 1987. The functional anatomy of the male reproductive system in *Penaeus indicus*. *S.Afr.J.Zool.*, **22**: 297-307.

*Charniaux-Cotton, H., Zerbib, C., and Meusy, J.J. 1966. Monographic de la glandi androgene des Crustaces superieurs. *Crustaceana*, **10**: 113-136.

*Charniaux-Cotton, H. 1960. Androgenic gland of crustaceans. *Gen.Comp.Endocrinol.Suppl.*, **1**: 241-247.

*Chevaillier, P. 1966. Structure et constitution cytochimique de la capsule du spermatozoide des Crustaces Decapodes. C.R.Acad.Sci.Paris. **262**: 1546.

*Chevaillier, P. and Maillet, P.L. 1965. Structure fine et constitution cytochimique du spermatozoide de la Langoustine *Nephrops norvegicus* L. (Crustace, Decapode). *J.Microsc.*, **4**: 679.

Chittleborough, R.G. 1974. Western rock lobster reared to maturity. *Aust.J.Mar.Freshwat.Res.*, **25**: 221-225.

Chow, S. 1989. Homologous functional structure and origin of the spermatophores in six palaemonid shrimps (Decapoda, Caridae). *Crustaceana*, **57**: 247-252.

Chow, S., Ogasawara, Y and Take, Y. 1982. Male reproductive system and fertilization of the Palaemonid shrimp, *Macrobrachium rosenbergii*. *Bull.Jap.Soc.Sci.Fish.*, **48**: 177-183.

Clark, W.H.Jr., Talbot, P.T., Neal, R.A., Mock, C.R and Salser, B.R. 1973. In vitro fertilization with nonmotile spermatozoa of the brown shrimp *Penaeus aztecus*. *Mar.Biol.*, **22**: 353-354.

Cobb, J.S and Phillips, B.F. 1994. In: Spiny lobster management. (eds) B.F.Phillips, J.S.Cobb and J.Kitaka. Fishing News Books. pp: 529-535.

*Crawford, D.R and DeSmidt, W.J.J. 1922. The spiny lobster, *Panulirus argus* of Southern Florida-its natural history and utilization. *U.S.Bur.Fish.Bull.,* **38**: 281-310.

*Cronin, L.E. 1947. Anatomy and histology of the male reproductive system of *Callinectes sapidus* (Rathbun). *J.Morph.*, **81**: 209-240.

Diaz, A.C., Fernandez Gimenez, A.V., Petriella, A.M and Fenucci, J.N. 2002. Morphological and functional study of the male reproductive tract in the shrimp, *Pleoticus muelleri* bate (Decapoda, Penaeoidea). *Invert.Rep.Dev.*, 1-6

Deecaraman, M and Subramoniam, T. 1980. Male reproductive tract and accessory glands of a stomatopod, *Squilla holoschista. Int.J.Invert.Rep.*, **2**: 175-188.

Demestre, M. and Fortuno, J.M. 1992. Reproduction of the deepwater shrimp *Aristeus antennatus*. (Decapoda:Dendrobranchiata). *Mar. Ecol. Prog. Ser.*, **84** : 41-51.

Diesel, R. 1989. Structure and function of the reproductive system of the symbiotic spider crab *Inachus phalangium* (Decapoda: Majidae): observations on sperm transfer, sperm storage and spawning. *J.Crust.Biol.*, **9(2)**: 266-277.

Dubois, M.K., Giller, A., Hamilton, J.K., Roberts, R.A and Smith, F. 1956. Calorimetric method for determination of sugars and related substances. *Analyt. Chem.,* **28**: 350-356.

Dudenhausen, E.E and Talbot, P. 1982. An ultrastructural comparison of soft and hardened spermatophores from the crayfish, *Pacifastacus leniusculus*. Dana. *Can.J.Zool.*, **61**: 82-194.

*Dupre, E. and Barnes, C. 1983. Fine structure of the mature spermatozoan of *Rhynchocinetes typus*, Crustacea, Decapoda. *Gamete Research*, **7**: 1-18.

*Farmer, A.S.D. 1974. Reproduction in *Nephrops norvegicus* (Decapoda: Nephropidae). *J.Zool., London,* **174**: 161-183.

Felgenhauer, B.E., Abele, L.G and Kim, W. 1988. Reproductive morphology of shrimp, *Procaris ascensionis* (Decapoda: procarididae). *J.Crust.Biol.*, **8**: 333-339.

Fielder, D.R. 1964. The spiny lobster *Jasus lalandii* (H.Milne Edwards), in south Australia. II.Reproduction. *Aust.J.Mar.Freshwat.Res.,* **15(2):** 133-144.

*Fielding, A and Haley, S.R. 1976. *Pac.Sci.,* **30**: 131.

Fingerman, M. 1987. The endocrine mechanism of crustaceans. *J.Crust.Biol.,* **7**: 1-24.

*Gangothri, M.S; Vasantha, N and Venkatachari, S.A.T. 1971. *Proc.Ind.Acad.Sci.,* **87**: 195.

Genthe, H.C.Jr. 1969. The reproductive biology of the *Sergestes similes* (Decapoda, Natantia). *Mar.Biol.,* **2**: 203-217.

*George, R.W. 1958. The biology of the western Australian commercial crayfish, *Panulirus longipes*. Ph.D thesis, University of western Australia, Perth. pp: 124.

George, R.W and Morgan, G.R. 1979. Linear growth stages in the rock lobster (*Panulirus versicolor*) as a method for determining size at first physical maturity. *Rapp.P.V.Reun.Cons.Int.Explor.Mer.*, **173**: 182-185.

Gordon, I. 1960. On the genus Justitia Holthuis (Decapoda, Palinuridae), with a note on allometric growth in *Panulirus ornatus* (Fabricius). *Crustaceana*, **1(4)**: 295-306.

*Greenwood, J.G. 1972. The male reproductive system and spermatophore formation in *Pagurus novaezealandiae* (Dana) (Anomura:Paguridae). *J.Nat.Hist.*, London. **6**: 561-674.

Grey, K.A. 1979. Estimates of the size of first maturity of the Western Rock lobster, *Panulirus Cygnus*, using secondary sexual characteristics. *Invest.Rep.Oceanogr.Res.Inst.*, **62**: 1-8.

Griffin, F.J., Shigekawa, K and Clark, W.H. Jr. 1988. Formation and structure of the acrosomal filament in the sperm of *Sicyonia ingentis*. *J.Exp.Zool.*, **246**: 94-102.

*Grobben, C. 1878. Beitrage zur Kantness der mannlichen Geschlectsorgane der Dekapoden. Arbeiten aus den Zoologeschen Instituten der universitat wien and der zoologeschen station in Triest. **1**: 57.

*Guitart, B., Gonzalez, E., Reyes, R and Fraga, I. 1995. Descripcion del aparato reproductov masculine de *Penaeus notialis* y *Penaeus schmitti*. *Rev.Cub.Inv.Pesq.,* **10 (1-2):** 41-58.

Haley, S.R. 1969. *Crustaceana*, **17**: 285.

Haley, S.R. 1984. Spermatogenesis and spermatophore production in the Hawaiian red lobster *Enoplometopus occidentalis* (Randall) (Crustacea, Nephropidae). *J.Morph.*, **180**: 181-193.

Haley, S.R. 1986. Ultrastructure of spermatogenesis in the Hawaiian Red lobster, *Enoplometopus occidentalis* (Randall). *J. Morph.*, **190**: 81-92.

*Hartnoll, R.G. 1968. Mating in the Brachyura. *Crustaceana*, **16**: 161-181.

Hartnoll, R.G. 1974. *Crustaceana*, **27**: 131

*Heldt, J.H. 1938. La reproduction chez les crustaces decapods de la famille des Peneides. *Ann.L'Inst.Oceanogr.*, **18**: 31-206.

*Heydorn, A.E.F. 1965. The rock lobster of the south African west coast, *Jasus lalandii* (H.Milne Edwards). 1. Notes on the reproductive biology and the determination of minimum size limits of commercial catches. Investl.Rep.Div.Seafish.S.Afr. **53**: 1-32.

*Hickman, V.V. 1946. Notes on the Tasmanian marine crayfish, *Jasus lalandii* (Milne Edwards). Rap.Roy.Soc.Tasm. pp: 27- 38.

Hinsch, G.W. 1969. Microtubules in the sperm of the spider crab *Libinia emarginata. J.Ultrastruct.Res.*, **29**: 525-534.

Hinsch, G.W. 1972. Some factors controlling reproduction in the spider crab, *Libinia emarginata. Biol.Bull.*, **143**: 358-366.

Hinsch, G.W. 1973. Sperm structure of Oxyrhyncha. *Can.J.Zool.*, **51**: 421-426.

Hinsch, G.W. 1980. Spermiogenesis in *Coenobita clypeatus.* 1. Sperm structure. *Int.J.Invert.Reprod.*, **2**: 189-198.

Hinsch, G.W. 1986. A comparison of sperm morphologies, transfer and sperm mass storage between two species of crab, *Ovalipes ocellatus* and *Libinia emarginata. Int.J.Invert.Rep.Dev.*, **10**: 79-87.

Hinsch, G.W. 1988. Ultrastructure of the sperm and spermatophores of the golden crab, *Greyon fenneri* and a closely related species, the red crab, *G.quinquedens* from the eastern gulf of Mexico. *J.Crust.Biol.*, **8(3):** 340-345.

Hinsch, G.W. 1991. Ultrastructure of the sperm and spermatophores of the anomuran crab *Pleuroncodes planipes. J.Crust.Biol.*, **11(1):** 17-22.

Hinsch, G.W and Mcknight, C.E. 1988. The vas deferens of the Spanish lobster *Scyllarus chacei. Int. J.Invert.Rep.Dev.,* **13(3):** 276-280.

Hinsch, G.W and Walker, M.H. 1974. The vas deferens of the spider crab *Libinia emarginata. J.Morph.*, **143**: 1-20.

Holthuis, L.B. 1991. FAO species catalogue. Marine lobsters of the world. FAO. Fisheries synopsis (Vol.13. No.125). Food and Agriculture Organisation of the United Nations, Rome, Italy. pp. 292.

*Hoffman, D.L. 1968. Seasonal eyestalk inhibition on the androgenic glands of a protrandric shrimp, Nature, **218**: 170-172.

*Hoffman, D.L. 1969. The development of the androgenic glands of a protandric shrimp. *Biol.Bull.*, **137**: 286-296.

*Hudson, D.J. 1966. Fitting segmented curves whose join points have to be estimated. *J.Am.Statist.Ass.*, **61**: 1097-1129.

*Huxley, J.S. 1924. Constant differential growth-ratios and their significance. *Nature*, London. **114**: 895-896.

*Jamieson, B.G.M. 1989. Ultrastructural comparison of spermatozoa of *Ranina ranina* (Oxystomata) and of other crabs exemplified by *Portunus pelagicus* (Brachygnatha) (Crustacea, Brachyura). *Zoomorph.,* **109:** 103-111.

Jamieson, C.C. Tudge and Scheltinga, D. M. 1993. The ultrastructure of the spermatozoon of Dromidiopsis-Edwardsi Rathbun 1919 (Crustacea, Brachyura, Dromidae) - Confirmation of a Dromid sperm type. *Aust. J.Zool.*, **41(6):** 537-548.

Jeyalectumie, C and Subramoniam T. 1987. Biochemical composition of seminal secretions with special reference to LDH activity in the reproductive tissues of the field crab, *Paratelphusa hydrodromous* (Herbst). *Exp.Biol.*, **46**: 231-236.

Jeyalectumie, C and Subramoniam T. 1991. Biochemistry of seminal secretions of the crab *Scylla serrata* with reference to sperm metabolism and storage in the female. *Mol.Rep.Dev.*, **30**: 44-45

*Johnson, P.T. 1980. Histology of the blue crab, *Callinectes sapidus*. A model for the Decapoda. Praeger Publishers, New York. pp: 1- 440.

Kagwade, P.V., Manickaraja, M., Deshmukh, V.D., Rajamani, M., Radhakrishnan, E.V., Suresh, V., Kathirvel, M., Rao, G.S. 1991. Magnitude of lobster resources of India. *J.Mar.Biol.Ass.India*, **33 (1 & 2)**: 150-158.

*Kastenbaum, M. 1959. A confidence interval on the abscissa of the point of intersection of two fitted regressions. *Biometrics*, **15**: 323-324.

Kaye, J.S. 1962. Acrosome formation in the house Cricket. *J.Cell Biol.*, **12**: 411.

Kaye, G.I., Pappas, G.D., Yasuzumi, G and Yamamoto, H. 1961. The distribution and form of the endoplasmic reticulum during spermatogenesis in the crayfish, *Cambaroides japonicus*. *Z.Zellforsch.*, **53**: 159.

Kensler, C.B. 1967. Fecundity in the marine spiny lobster *Jasus verreauxi* (H.Milne Edwards) (Crustacea:Decapoda:Palinuridae). *N.Z.J.Mar.Freshwat.Res.*, **2**: 81-89.

Kim, D.H., Qtae Jo, Jung Hwa choe, Seong Jung yun, Taeg Yun Oh, Byung Ki Kim and Chang-Hee Han. 2003. Sperm structure of the pandalid shrimp, *Pandalopsis japonica* (Decapoda, Pandalidae). *J.Crust.Biol.*, **23(1):** 23-32.

King, J.E. 1948. A study of the reproductive organs of the common marine shrimp, *Penaeus setiferus*. *Biol.Bull.*, **94**: 244-262.

*King, D.S. 1964. Comparitive morphology of the crustacean androgenic gland. Masters Thesis, Zoology Department, University of California.

*Kleve, M.G., yudin, A.I and Clark, W.H. Jr. 1980. Fine structure of the unistellate sperm of the shrimp, *Sicyonia ingentis* (Natantia). *Tissue Cell*, **12**: 29-45.

Koehler, L.D. 1979. A unique case of cytodifferentiation: Spermiogenesis of the prawn, *Palaemonetes paludosus*. *J.Ultrastruct.Res.,* **69**: 109-120.

Kooda-Cisco, M.J and Talbot, P. 1982. Structural analysis of the freshly extruded spermatophore from the lobster, *Homarus americanus*. *J.Morph.*, **172**: 193-207.

Kooda-Cisco, M.J and Talbot, P. 1986. Ultrastructure and role of the lobster vas deferens in spermatophore formation. The proximal segment. *J.Morph.*, **188(1):** 91-103.

Krol, R.M., Hawkins, W.E. and Overstreet, R.M. 1992. Reproductive components. In: Microscopic Anatomy of Invertebrates, Decapoda, Crustacea. F.W.Harrison and A.G.Humes (Eds), Wiley-Liss, New York, pp: 295-343 .

*Kubo, I. 1938. Notes on the sexual dimorphism of the spiny lobster, *Panulirus japonicus* (v.Siebold). *J.imp.Fish.Inst.,* Tokyo, **33(1):** 101-106.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **277**: 680-685.

Langreth, S.G. 1965. Ultrastructural observations on the sperm of the crab *Cancer borealis*. *J.Cell.Biol.*, **27**:56A

Langreth, S.G. 1969. Spermiogenesis in cancer crabs. *J.Cell.Biol.*, **43**: 575-603.

*Lindberg, R.G. 1955. Growth, population dynamics and field behaviour of the spiny lobster, *Panulirus interruptus* (Randall). *Univ.Calif.Publ.Zool.*, **59:** 157-248.

Lockwood, A.P.M. 1967. "Aspects of the physiology of Crustacea". Freeman, San Francisco, California.

Lohrmann, K and Raineri, M. 1995. Ultrastructure of the spermatozoon of the crab, *Cervimunida johni* Porter, 1903 (Galatheidae, Anomura, Crustacea). *Invert.Rep.Dev.*, **28**: 71-76.

*Lopez-Camps, J., Bargalla, R., Bozzo, M.G., Dufort, M and Fontarnau, R. 1981. The spermatogenesis of Crustacea. VII. Review of spermatozoon of the crayfish, *Astacus astacus* (Malacostraca, Decapoda, Macrura, Reptantia). *Gam.Res.*, **4**: 65-82.

Lowry, D.H., Rosebrough, N.J., Fair, A.L., Randall, R.J. 1951. Protein measurement with Folin phenol reagent. *J.Biol.Chem.*, **193**: 265-275.

*Lu, C.C., Clark, W.H. and Franklin, L.E. 1973. Spermatogenesis of the decapod *Penaeus setiferus*. *J.Cell.Biol.*, **59**.

Lynn, J and Clark, W.H. Jr. 1983. The fine structure of the mature sperm of the freshwater prawn, *Macrobrachium rosenbergii. Biol.Bull.*, **164**: 459-470.

*Lyons, W.G., Barber, D.G., Foster, S.M., Kennedy, F.S and Milano, G.R. 1981. The spiny lobster, *Panulirus argus* in the middle and upper Florida keys: Population structure, seasonal dynamics and reproduction. Fla.Mar.Res.Publ. **38**: 45.

*Lyons, W.G. 1986. Problems, perspectives regarding recruitment of spiny lobster, *Panulirus argus* to south Florida fishery. *Can.J.Fish.Aquat.Sc.,* **43**: 2099-2106.

MacFarlane, J.W and Moore, R. 1986. Reproduction of the ornate rock lobster, *Panulirus ornatus* (Fabricius) in Papua New Guinea. *Aust.J.Mar.Freshwat.Res.*, **37**: 55-65.

Malek, S.R.A and F.M.Bawab. 1974a. The formation of the spermatophore in *Penaeus kerathurus* (Forskal, 1775) (Decapoda, Penaeidae). I. the initial formation of sperm mass. *Crustaceana,* **26**: 273-285.

Malek, S.R.A and F.M.Bawab. 1974b. The formation of the spermatophore in *Penaeus kerathurus* (Forskal, 1775) (Decapoda, Penaeidae). II. The deposition of the layers of the body and of the wing. *Crustaceana,* **27**: 73-83.

*Mann, T. 1984. “Spermatophores”. Springer-Verlag, Berlin, pp: 217.

Marine Products Export Review. 2000. *MPEDA.*

Martin, G.G., Herzeg, C and Narimatsu, G. 1987. Fine structure and histochemistry of the freshly extruded and hardened spermatophore of the spiny lobster *Panulirus interruptus. J.Morph.*, **192**: 237-246.

*Mathews, D.C. 1951. The origin, development and nature of the spermatophoric mass of the spiny lobster, *Panulirus pencillatus* (Olivier). *Pacif.Sci.*, **5**: 359-371.

*McLaughlin, P.A. 1980. Comparitive morphology of recent crustacea. Freeman, San Francisco, California.

Medina, A. 1994. Spermiogenesis and sperm structure in the shrimp *Parapenaeus longirostris* (Crustacea: Dendrobranchiata): Comparitive aspects among decapods. *Mar.Biol.*, **119(3)**: 449-460.

*Medina, A. and Rodriguez, A. 1992. Spermiogenesis and sperm structure in the crab, *Uca tangeri* (Crustacea, Brachyura) with special reference to the acrosome differentiation. *Zoomorph.*, **111(3):** 161-165.

*Medina, A., Mourente, G., Lopez de la Rosa, I. Santos, A and Rodriguez., A. 1994. Spermatozoal ultrastructure of *Penaeus kerathurus* and *Penaeus japonicus* (Crustacea, Dendrobranchia). *Zoomorph.*, **14(3):** 161-167.

Medina, A., Vila, Y and Santos, A. 1998. The sperm morphology of the euphasid *Meganyctiphores norvegica* (Crustacea, Eucarida). *Invert.Reprod.Dev.*, **34:** 65-68.

Melville Smith, R. 1987. The reproductive biology of *Greyon maritae* (Decapoda, Brachyura) off south West Africa, Namibia. *Crustaceana,* **53**: 259-275.

Minagawa, M., Chiu, J.R., Kudo, M., Takashima, F. 1994. Male reproductive biology of the red frog crab, *Ranina ranina*, off Hachijojima Izu islands, Japan. *Mar.Biol.,* **118**: 396-401.

*Moses, M.J. 1956. Studies on nuclei using correlation cytochemical, light and electron microscope techniques. *J.Biophys.Biochem.Cytol.Suppl.,* **2**: 397.

*Moses, M.J. 1960. A light and electron microscope study of spermiogenesis in decapod crustacea. *Anat.Rec.,* **130**: 343.

*Moses, M.J. 1961a. Spermiogenesis in the crayfish (*Procambarus clarkii*).1.Structural characterization of the mature sperm. *J.Biophys.Biochem.Cytol.,* **9**: 222-228.

Moses, M.J. 1961b. Spermiogenesis in the crayfish (*Procambarus clarkii*). Description of stages. *J.Biophys.Biochem.Cytol.*, **10**: 301.

*Nakamura, S. 1940. Ecological studies on the spiny lobster, *Panulirus japonicus* (V.Siebold) with special reference to its conservation. *I.J.Imp.Fish.Inst.,* Tokyo. **34** : 101-113.

Nath, V. 1932. The spermatid and sperm of the crab, *Paratelphusa spinigera. Quart.J.Microsc.Sci.*, **75**: 543.

Nath, C.N., Thampy, D.M and Pillai, N.K. 1972. A note on the androgenic gland of a subterranean mysidacean, *Spelaeomysis longipes* (Pillai and Mariamma). *Crustaceana,* suppl.**3:** 354-356.

*Okamura, S. 1956. Anatomy of the Experimental Animals. Kazamo Syobou, Tokyo. pp: 141-171 (in Japanese).

*Ortmann, A.E. 1896. Geschlechtsorgane der Decapods. In "Klassen und ordnungen des Thierreichs", Bd V, Abt.1.Buch II, (Ed.H.G.Bronn), pp: 1042-1073.

*Paterson, N.R. 1968. The anatomy of the cape rock lobster, *Jasus lalandii* (H.Milne Edwards). *Ann.S.Afr.Mus.*, **51**: 1-232.

Payen, G.G., Chim, L., Laubier-Bonichon, A., Charniaux-Cotton, H. 1982. The androgenic gland of the shrimp, *Penaeus japonicus* Bate. Description, role and control of the eyestalks. *Gen.Comp.Endocrinol.*, **46**: 384.

*Pearson, P.J and Walker, M.H. 1975. Alteration of cytochrome c oxidase activity during spermatogenesis in *Carcinus maenas. Cell.Tiss.Res.,* **164**: 401-410.

Pillay, K.K and Nair, N.B. 1973. Observations on biochemical changes in gonads and other organs of *Uca annulipes, Portunus pelagicus* and *Metapenaeus affinis* (Decapoda, Crustacea) during the reproductive cycle. *Mar.Biol.*, **18(3); 167-198.

*Pochon-Masson, J. 1962. Le Chondriofusome des gametes males du crustace decapode *Carcinus maenas.* C.R. Acad. Sci (Paris). **253**: 4076-4078.

*Pochon-Masson, J. 1963. Oringine et formation de la vesicule du spermatozoide d' *Eupagurus bernhardus* (Decapoda, Anomure). C.R.H.Acad.Sci. **261**: 2226-2228.

*Pochon-Masson, J. 1965a. L'ultrastructure des epines du spermatozoide chez les decapods (Macroures, Anomoures, Brachyoures). C.R. Acad. Paris. **260**: 3762-3764.

*Pochon-Masson, J. 1965b. Schema general du spermatozoide vesiculaire des decapods. C.R. Acad.Sci. (D) (Paris). **260:** 5093-5095.

*Pochon-Masson, J. 1968. L'ultrastructure des spermatozoids vesiculaires chez les crustacean decapods avant et au cours de leur devagination experimentale. I. Brachyoures el anomoures. *Ann.Sci.Nat.Zool.Biol.Anim.Ser.*, **10**: 1-100.

*Pochon-Masson, J. 1969. *Arch.Zool.Exp.Gen.*, **110**: 363.

*Pochon-Masson, J. 1983. Arthropoda- Crustacea. In: Adiyodi, K.G, Adiyodi, R.G. (eds). Reproductive biology of invertebrates. Vol.II. Spermatogenesis and sperm function. Wiley and Sons, Chichester, pp: 407-449.

*Pollock, D.E. 1991. Spiny lobsters at Tristan de Cunha, south Atlantic: inter-island variations in growth and population structure. *S.Afr.J.Mar.Sci.*, **10:** 1-12.

*Powel, G.C and Nickerson, R.B. 1965. *J.Fish.Res.Bd.Canada.*, **22**: 101.

*Puckett, D.H. 1964. Experimental studies of the crayfish androgenic gland in relation to testicular function. Doctor of Philosophy Dissertation, University of Virginia.

Radha, T and Subramoniam, T. 1985. Origin and nature of spermatophoric mass of the spiny lobsters, *Panulirus homarus*. *Mar. Biol.*, **86**: 13-19.

Radhakrishnan, E.V and Manisseri, M.K. 2001. Status and management of lobster fishery resources in India. *Mar.Fish.Infor.Serv.T & E.Ser.*, **169**: 1-3.

Radhakrishnan, E.V and Vijayakumaran, M. 1990. An assessment of potential of spiny lobster culture in India. Bulletin of Central Marine Fisheries Research Institute. **44(2)**: 416-427.

Radhakrishnan, E.V., Deshmukh, V.D., Manisseri, M.K., Rajamani, M.,.Kizhakudan, K.K and Thangaraja, R. 2005. Status of major lobster fisheries in India. *N.Z.J.Mar.Freshwat.Res.*, **39**: 723-732.

Rao, K.H., Babu, B.T and Shyamsundari, K. 1989. Chemical nature and mode of stabilization of spermatophore capsule in the crab, *Portunus pelagicus* (Linnaeus). *Int.J.Invert.Zool.Aquat.Biol.*, **1**: 3-6.

Reeby, J., Prasad, P.N and Kusuma, M.S. 1990. Size at maturity in the male crab, *Portunus sanguinolentus* and *P.pelagicus*. *Fish.Tech.*, **27**: 115-119.

Reger, J.F. 1970. Studies on the fine structure of spermatids and spermatozoa of the crab, *Pinmixia* sp. *J.Morph.*, **132**: 89-100.

Ro, S., Talbot, P., Leung-Trijilla, J and Lawrence, A.L. 1990. Structure and function of the vas deferens in the shrimp, *Penaeus setiferus* segments 1-3. *J.Crust.Biol.*, **10(3)**: 455-468.

*Robison, D.E. 1964. Estimates for the points of intersection of two polynominal regressions. *J.Am.Statist.Ass.*, **59**: 214.

Ryan. E.P. 1967. Structure and function of the reproductive system of the crab *Portunus sanguinolentus* (Herbst) (Brachyura: Portunidae). 1. The male system- Proceedings of the symposium on Crustacea, Ernakulam, Marine Biological Association of India. **2**: 506-521.

*Schaller, F. 1980. Significance of sperm transfer and formation of spermatophores in arthropod phylogeny. Pp: 587-607. In Arthropoda phylogeny, A.P.Gupta, ed., Van Nostrand Reinhold Co., NY.

*Shigekawa, K and Clark, W.H . 1986. Spermatogenesis in the marine shrimp *Sicyonia ingentis*. *Dev.Growth Dift.*, **28**: 95-112.

Snedecor, G.W and Cochran, W.G. 1967. Statistical principles and design. McGraw Hill, Inc. pp: 592.

Spurr, A.R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct.Res.*, **26:** 31-43.

Subramoniam, T. 1977. Aspects of sexual biology of the anomuran crab, *Emerita asiatica. Mar.Biol.*, **43**: 369-377

Subramoniam, T. 1984. Spermatophore formation in two intertidal crabs, *Albunea symnista* and *Emerita asiatica* (Decapoda:Anomura). *Biol.Bull.,* **166:** 78-95.

Subramoniam, T. 1991. Chemical composition of spermatophores in decapod crustaceans. pp: 308-321 in RT Baer and J.W.Martin. eds. Crustacean sexual Biology, Columbia university press, New York.

Subramoniam, T. 1993. Spermatophores and sperm transfer in marine Crustaceans. In " Advances in Marine Biology" Vol. 29. Blaxter J.H.S. and Southward A.J. (Eds). Academic Press, New York. pp: 129-214.

Suganthi, A and Anilkumar, G. 1999. Moult-related fluctuation in ecdysteroid titre and spermatogenesis in the crab, *Metopograpsus Messor* (Brachyura:Decapoda). *Zool. Stud.*, **38(3)**: 314-321.

Suseelan, C., Muthu, M.S., Rajan, K.N., Nandakumar, G., Kathirvel, M., Neelakanta Pillai, N., Surendrakurup, A.N and Chellappa, K. 1990. Results of an exclusive survey for deep sea crustaceans off southwest coast of India. Proceedings of the First Workshop on Scietific results of FORV Sagar Sampada. pp: 347-359.

Taketomi, Y., Murata, M and Miyawaki, M. 1990. Androgenic gland and secondary sexual characters in the Crayfish, *Procambarus clarkii. J.Crust.Biol.*, **10(3):** 492-497.

Talbot and Channamon. 1980a. The structure of sperm from the lobster, *Homarus americanus. J.Ultrastruct.Res.*, **70:** 275-286.

Talbot and Channamon. 1980b. Morphological features of the acrosome reactions of lobster (*Homarus*) sperm and the role of the reaction in generating forward sperm movement. *J.Ultrastruct.Res.,* **70**: 287-297.

Talbot, P and Summers, R.G. 1978. The structure of sperm from *Panulirus*, the spiny lobster, with special regard to the acrosome. *J.Ultrastruct. Res.*, **64**: 341-351.

*Tcholakian, R.K and Reichard, S.M. 1964. A possible androgenic gland in *Callinectes sapidus* Rathbun. *Amer.Zool.*, **4**: 20.

*Templeman, W. 1935. Local differences in the body propotions of the lobster, *Homarus americanus*. *J.Fish.Res.Bd.Canada.*, **1**: 213-226.

Thampi, D.M and John, P.A. 1970. On the androgenic gland of the ghost crab, *Ocypoda platytarsis*. M.Edwards (Crustacea:Brachyura). *Acta.Zool.*, **51**: 203-210.

Thampi, D.M and John, P.A. 1972. The androgenic gland of the shrimp *Palaemon dayanus*. *Mar.Biol.,* **12:** 285-288.

Tudge, C.C. 1992. Comparitive ultrastructure of hermit crab spermatozoa (Decapoda:Anomura:Paguridea). *J. Crust. Biol.,* **12:** 397-409.

Tudge, C.C. 1999. Ultrastructure of the spermatophore lateral ridge in hermit crabs (Decapoda, Anomura, Paguroidea). *Crustaceana*, **72(1):** 77-84.

Tudge, C.C and Jamieson, B.G.M. 1991. Ultrastructure of the mature spermatozoan of the coconut crab, *Birgus latro* (Coenobitidae:Paguroidea:Decapoda). *Mar.Biol.*, **108**: 395-402.

Tudge, C.C and Jamieson, B.G.M. 1996. Spermatophore and spermatozoal morphology in the Porcellanidae.II. The genera Petrolisthes and Polyonyx (Decapoda:Anomura). *J.Crust.Biol.*, **16**: 535-546.

Tudge, C.C., Scheltinga, D.M and Jamieson, G.M. 1986. Spermatozoal ultrastructure in the spiny lobster *Jasus novaehollandiae* Holthuis, 1963 (Palinuridae, Palinura, Decapoda). *J. Morph.*, **236 (2):** 117-126.

*Uma, K. 1982. Studies on the seminal secretions of a portunid crab, *Scylla serrata* (Forskal) (Brachyura, Crustacea). Ph.D thesis, University of Madras, India.

*Verdi, A and Delgado, E. 1998. Microestructura del aparato reproductor masculino de *Macrobrachium borellii* (Nobilis, 1896) (Crustacea, Palaemonidae). *Rev.Brasil.Biol.*, **58(2)**: 343-348.

*Von Bonde, C. 1936. The reproduction, embryology and metamorphosis of the cape crayfish. *S.Afr.Dep.Comm.Industr.Invest.Rep.*, **6**: 1-25.

*Watson, J. 1970. *J.Fish.Res.Bd.Canada.*, 27: 1607.

*Warnock, R.W. 1975. Reproductive anatomy, spermatophoric mass formation and early development of the yabbie *Cherax destructor* (Crustacea: Parastacidae). Honours Thesis, School of Biological Sciences, Macquaie University.

Wortham-Neal, J.L. 2002. Reproductive morphology and biology of male and female mantis shrimp (Stomatopoda: Squillidae). *J.Crust.Biol.,* **22(4):** 728-741.

*Yasuzumi, G. 1960. Spermatogenesis in animals as revealed by electron microscopy. VII. Spermatid differentiation in the crab, *Eriocheir japonicus*. *J.Biophys.Biochem.Cyto.*, **7**: 73-78.

* Not referred in original