HISTOLOGICAL, HISTOCHEMICAL AND BIOCHEMICAL CHARACTERISATION OF MALE MORPHOTYPES OF *MACROBRACHIUM ROSENBERGII* (DE MAN)

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BY

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2002

DECLARATION

*I, Aneykutty Joseph, do hereby declare that the thesis entitled "**Histological, Histochemical and Biochemical characterisation of male morphotypes of Macrobrachium rosenbergii (de Man)**" is a genuine record of research work done by me under the supervision of **Dr.B.Madhusoodana Kurup**, Professor (Fisheries), School of Industrial Fisheries, Cochin University of Science and Technology and has not been previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any university or institution.*

Cochin 682016
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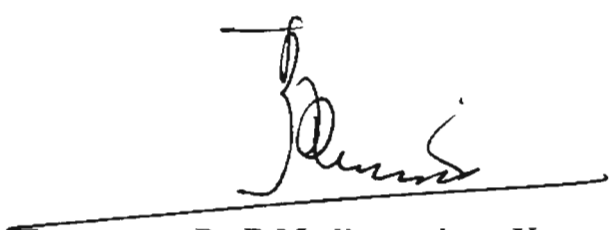
Aneykutty Joseph

CERTIFICATE

This is to certify that this thesis is an authentic record of research work carried out by Smt. Aneykutty Joseph, under my supervision and guidance in the School of Industrial Fisheries, Cochin University of Science and Technology in partial fulfilment of the requirements for the degree of Doctor of Philosophy and no part thereof has been submitted for any other degree.



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CONTENTS

SECTION 1

CHAPTER 1

GENERAL INTRODUCTION

Macrobrachium rosenbergii (de Man), the largest fresh water prawn of the world, is indigenous to the entire South and South-East Asian and Pacific islands (Holthius, 1980). The species has a wide distribution through out the tropics and subtropical regions of the world and are found in most of the inland freshwater bodies (New and Singholka, 1985). In India, it is reported from lower reaches of Narmada and Tapti rivers, Thana creek (Bombay) and backwaters of Kerala along the west coast and nearly all the upper tidal zones and associated regions of the rivers draining in to Bay of Bengal on the East coast (Sebastian *et al.*, 1993). It constitutes a lucrative fishery in Hoogly estuary (Rao,1969), Godavari and Narmada River systems, Kolleru lake (Rao,1992) and Kerala backwaters (Raman, 1967; Kurup *et al.*, 1992 a; Harikrishnan and Kurup, 1997)

Interest in freshwater prawn culture began with the accomplishment of hatchery technology by Ling (1969b). While there are about 125 species of *Macrobrachium*, only *M.rosenbergii* has proved suitable for commercial farming. *Macrobrachium* farming, almost synonymous to freshwater prawn culture, is receiving much attention in the recent years in our country because of its seed availability, high growth rate, large size, hardiness and good market value in domestic and international trade and is becoming an important source of valuable foreign exchange. With the development of economically viable commercial larval rearing techniques during

the past decade and establishment of commercial hatcheries, the seed availability of this species became a reality from the commercial hatcheries and thenceforth interest and enthusiasm have been generated among farmers to undertake farming of *M. rosenbergii* by adopting different systems of culture. Therefore, commercial development of prawn farming based on *M. rosenbergii* has now became widespread, especially in South-East Asian countries.

The global production of farmed *M. rosenbergii* was 23.297 tones in 1991 of which 93% was produced from Asia (New, 1994). Among the non-Asian countries, North America and Caribbean and South America contributed to major part of the production. The global production in 1991 showed a 3.5 fold increase when compared to 1985, and majority of the Asian farmed prawn came from Thailand followed by Thaiwan and Vietnam respectively. India, which was producing hardly 200 tones of Scampi *(M.rosenbergii)* till 1996, had exported 7000 tones of this species during 1999-2000 period as against 1600 tones in 1998-99, registering a 130 percent growth in terms of quantity when compared to the previous year. A record production of 10000 tones had achieved by Thailand during the 1999-2000 period. Thus, the farm raised *M. rosenbergii* from India started occupying a position in the global aquaculture production, however, the quantity was found to be far below when compared to South-East countries like Thailand and Thaiwan. While India is endowed with an estimated 5.4 million hectares of freshwater resources, only 12,000 hectares are prsently utilized for freshwater prawn culture. Nevertheless, an enormous water wealth suitable for

undertaking farming of *M.rosenbergii* remained unutilized and therefore, India is considered as the sleeping giant of freshwater prawn farming. Production to the tune of 600–3000 kg/ha/7-9 months have been invariably reported in India (Raje and Joshi, 1992; Kurup *et al.*, 1998), however, the net revenue from the harvested population is seldom known to reach the level of expectation in view of the fact that there will be preponderance of individual belonging to smaller size groups in the harvested population from the grow outs which are having neither export market nor internal demand.

Males of *M.rosenbergii* are characterized by a condition termed heterogeneous individual growth (HIG) and this acts as the major biological limiting factor in the raising of theis species in aquaculture ponds (Daniels and Abramo, 1992). *M. rosenbergii* shows a wide range of size variation. The post larvae of this species have a normal size distribution (Sandifer and Smith, 1975; Ra'anam and Cohen, 1984 a), while the growth of juvenile is variable (Malecha, 1980) and with time, the variation in size increases more rapidly. It is reported that half of the population grows rapidly and variably, while the other half grows slowly and relatively uniformly, leading to a markedly positively skewed size distribution (Wickins, 1972; Forster and Beard, 1974; Sandifer and Smith, 1975; Malecha *et al.*,1977; Willis and Barrigan, 1977; Ra'anan and Cohen, 1982,1984a,1985). As the prawns mature, the size distribution becomes quite heterogenous for males and females. Mature females grow slowly than males of similar size and age and the size distribution of female regains approximately normal pattern (Cohen *et al.*, 1981). Unlike in

shrimps, the economic yield of *M.rosenbergii* is a very complex and non-linear function of total biomass of different population that reach marketable size. The wide disparity in the size structure of the cultured stock and the skewness in their weight distribution is profoundly influenced by the relative occurrence of various male morphotypes in the harvested population and this appears to be the greatest commercial disadvantage of *M.rosenbergii.*

The sexually mature adult male population of *M.rosenbergii* belonging to same age group has been differentiated into three distinct morphologically distinguishable forms such as Small males (SM), Orange Clawed males (OC) and Blue Clawed males (BC) representing three phases in the developmental pathway of male (Brody *et al.*, 1980; Cohen *et al.*, 1981). Besides, two transitional stages of Orange clawed males viz. Weak Orange clawed males (WOC) and pre-transforming Orange Clawed males (t-SOC) were also distinguishable from OC, of which the former being an intermediate stage between SM and OC and therefore, the fully differentiated OC are known as strong Orange Clawed males (SOC) (Kuris *et al.*, 1987). Among the different forms of Blue Clawed males, the Weak Blue Clawed males are the transitional stage of t-SOC and Strong Blue Clawed males (SBC) while Old Blue Clawed males (OBC), the terminal stage is characterized by relatively small body in terms of carapace length and body weight disproportionate to claw length (Harikrishnan and Kurup, 1997). Each morphotype develops in sequence in the adult male population from Small males through Orange Clawed males to dominant Blue Clawed males. These morphotypes are known to exhibit a complex social and

organizational hierarchy comprising dominant, subdominant and subordinate groups of animals, which have distinct morphologically distinguishable characteristics such as relative body size, spination on chela, claw characteristics, hierarchial dominance, differential growth patterns and relative alternative mating strategies. In spite of the fact that the male morphotypes are well characterized morphologically from grow outs as well as from natural habitats, in India and abroad, no concerted attempt has so far been made to unravel the puzzling biological phenomenon involved in the morphogenesis to establish the role of various internal organs intrinsically associated with morphotypic differentiation and to elucidate the structural variation of internal organs responsible for morphotypic expression, difference in growth and reproductive activity.

Against this background, the present study was undertaken with the following objectives.

1. To study the histological variations, if any, among the male morphotypes and their transitional stages from grow outs in order to address the structural and functional differences of different body organs responsible for difference in growth and reproduction.
2. To study the histochemical variations, if any, among the male morphotypes and their transitional stages from grow outs.
3. Biochemical characterization of male morphotypes in order to find out biochemical evidence of morphotypic differentiation.
4. With the help of biological characterization of male morphotypes the quantitative difference exist among the morphotypes could be

brought out which would be useful in establishing the phenotypic variations seen in male morphotypes. Thus the role of structural and functional variations of internal organs in phenotypic appearance of male morphotypes can be quantified with the help of biological characterization.

The results of the present study are presented in thirteen chapters which are organised under four sections. The first section encompases four chapters. First chapter gives a general introduction to the topic with details of research aim and approach where second chapter embodies a description about the population profile development of the *Macrobrachium rosenbergii* (de Man). In the third chapter, a brief review of the relevant literature is attempted. In the fourth chapter, the materials and methods followed in the study are described adequately.

Result of the histological characterisation of the reproductive system ie. testes, vas deferens including androgenic gland, hepatopancreas and the neurosecretory system viz., eye stalk, brain and thoracic ganglion of various male morphotypes and their transitional stages of *M.rosenbergii* are incorporated under section two. This section consists of five chapters. Histology of the testes, vas deferens and the androgenic gland of various male morphotypes and their transitional stages of *M. rosenbergii* are presented in chapters five, six and seven respectively. The eighth chapter deals with the histology of the hepatopancreas of various male morphotypes and their transitional stages of *M. rosenbergii* while the nineth chapter

consists of similar information in respect of neurosecretory system viz., eye stalk, brain and thoracic ganglion.

Third section deals with histochemical characterisation of the reproductive system and hepatopancreas of various male morphotypes and their transitional stages of *M. rosenbergii* and consists of two chapters. Tenth and eleventh chapters explain the histochemical localization of various metabolites viz., protein, lipid, glycogen and RNA in the reproductive system and hepatopancreas respectively of various male morphotypes and their transitional stages of *M. rosenbergii.*

Section four consists of two chapters describing the biochemical characterization of various male morphotypes and their transitional stages of *M. rosenbergii.* Twelveth chapter describes the biochemical constituents such as protein, lipid, carbohydrate, moisture and ash content in the different organ tissues such as muscle tissue and hepatopancreas and aminoacid profiling of muscle tissue. Variations in the lipid fractions such as cholesterol, phospholipid and triglyceride contents in the muscle tissue and hepatopancreas are discussed in chapter thirteen. This is followed by summary, references and list of publications.

CHAPTER 2

POPULATION PROFILE DEVELOPMENT IN *MACROBRACHIUM ROSENBERGII* (DE MAN)

Macrobrachium rosenbergii (de Man) is a sexually dimorphic fresh water prawn, in which males are easily distinguishable from females by noticing their relatively large body sizes and cephalothorax, longer and stouter second chelipeds and also by the presence of appendix masculina on the endopod of second pairs of swimmerets. During the process of domestication of *M.rosenbergii* under different levels of scientific management, a major difference in the growth strategy of males and of the females became very apparent (Smith *et al.*, 1978; Cohen *et al.*, 1981). In single aged population, the size distribution of female population is reported to be rather homogenous in grow outs, on the contrary, a clear demarcation of the male on the basis of morphotypes having distinct size variation is possible. The differential growth pattern so evidenced by the male population is one of the major obstacles in increasing profitability in the culture of *M. rosenbergii*. The size heterogeneity in males became evident in the post larval population itself and thenceforth the phenomenon progresses very rapidly which will eventually results in wide disparity in the size structure of the harvested population.

Newly metamorphosed post-larvae of *M.rosenbergii* demonstrate a homogenous size distribution and as time progresses the homogenous distribution gradually changes to a positively skewed size distribution (Wickins, 1972; Forster and Beard, 1974; Sandifer and Smith, 1975; Malecha *et al.*, 1977; Willis and Berrigan, 1977;

Ra'anan, 1982; Ra'anan and Cohen, 1984a,b). According to Willis and Berrigan (1977) there are two types of juveniles on the basis of their relative growth rate viz. jumpers and laggards. Jumpers are exceptionally fast growing individuals which are 15 times larger than the population mode within a period of 60 days from metamorphosis (Willis and Berrigan, 1977; Ra'anan, 1987). On the other hand, the laggards are exceptionally slow growing individual and their growth is suppressed by jumpers. According to Ra'anan and Cohen (1984a) the hierarchy is formed within two weeks from metamorphosis.

Ra'anan and Cohen (1984 b) found that jumpers and laggards did not develop when prawns were held individually indicating the importance of social interactions in the development of size variation and hierarchy. They further observed the continuation of differential growth pattern established during communal rearing even when jumpers and laggards were segregated and reared separately.

In the immature population of *M. rosenbergii* the pattern of size distribution is irrespective of sex. In the mature population the size distribution becomes quite different for males and females, mature females growing more slowly than males of similar size. Among the mature females there is a normal size distribution, whereas among the mature males there is a skewed size distribution. According to Ra'anan (1982) growth of female prawns nearly retard after maturation leading to a unimodel, symmetrical size distribution.

Available information on population profile development, morphotypic differentiation and their transformation, description of

morphotypes based on histology, morphology, morphometry and allometric characterization are only based on harvested male population of *M. rosenbergii* from the grow outs, mostly from Israel (Brody *et al.*, 1980; Cohen *et al.*, 1981; Kuris *et al.*, 1987; Sagi et al., 1988) and from India (Kurup *et al.*, 1998; suresh kumar and Kurup, 1998; Kurup and Harikrishnan 1999; Suresh kumar and Kurup, 1999; Hari and Kurup, 2001; Joseph and Kurup, 2001; Kurup and Ranjeet, 2001; Ranjeet and Kurup, 2001; Kurup and Ranjeet, 2002 (in press); Ranjeet and Kurup, 2002;) and from the natural waters, from India (Harikrishnan and Kurup, 1997; Harikrishnan and Kurup, 1997a; Harikrishnan et al., 1998; Kurup *et al.*, 1998; Kurup and Harikrishnan, 2000). A single aged adult male population of the *Macrobrachium rosenbergii* is known to exhibit remarkable variations in their morphological characteristics. Sexually matured male population of *M. rosenbergii* reared in ponds are differentiated into three morphologically distinguishable forms such as Small males (SM), Orange Clawed males (OC) and Blue Clawed males (BC) based on differential growth pattern, relative body size, second cheliped characteristics, differential reproductive behaviour and social hierarchial dominance (Cohen *et al.*, 1981; Ra'anan, 1982, Sagi, 1984; Telecky, 1984; Ra'anan and Cohen, 1985; Harikrishnan and Kurup, 1997; Kurup *et al.*, 1998; Suresh kumar and Kurup, 1998; Kurup and Harikrishnan, 1999). It is also known that males reared in culture systems follow a complex social hierarchy as manifested by the presence of dominant, subdominant and subordinate individuals which are characterized themselves into distinct morphotypes. Small males occupy the initial stage of developmental pathway (Cohen *et*

al., 1981). They are subordinate, not territorial, sexually competent and are known to fertilize females by using "Sneak mating strategy in the absence of large dominant males" (Ra'anan, 1982; Sagi, 1984; Telecky, 1984; Ra'anan and Sagi, 1985; Suresh kumar and Kurup, 1998). In contrast, Orange Clawed males are subdominant, not territorial, less reproductively active when compared to other two morphotypes and represent stage of high somatic growth (Ra'anan, 1982, Ra'anan and sagi, 1985; Suresh kumar and Kurup, 1998). Blue Clawed males, on the contrary, are large dominant, territorial and sexually competent animals which represent the final stage in the course of development (Cohen *et al.*, 1981; Ra'anan, 1982). These males sequestered post molt adult females prawns prior to mating and grew slowly (Ra'anan, 1982; Sagi, 1984; Ra'anan and Sagi, 1985; Suresh kumar and Kurup, 1998). These morphotypes represent three developmental stages of male maturation process and are known to undergo transformation from SM to OC to BC. (Cohen *et al.*, 1981; Ra'anan; 1982; Kuris *et al.*, 1987, Kurup *et al.*, 1998; Kurup and Harikrishnan, 1999). Two transitional stages of OC males viz. WOC (weak Orange Clawed) and t-SOC (Pre-transforming Orange Clawed) were also recognized, former being transitional between SM and SOC (Kuris *et al.*, 1987) and therefore, the fully differentiated OC males are known as SOC (Strong Orange Clawed) and the latter being transitional between SOC males and BC male (Sagi and Ra'anan, 1988; Harikrishnan and Kurup, 1997). Similarly, two transitional stages of SBC such as Weak Blue Clawed males (WBC) and Old Blue Clawed males (OBC) could also be differentiated (Harikrishnan and Kurup, 1997).

Description of male morphotypes and their transitional stages

1) Small Males (SM) (Fig.2.1)

(1st male morphotypic stage)

Individuals with very small body size ranging from 71 to 139 mm in total length and translucent body colour. Second chelipeds also appear translucent, however, propodus may have traces of light blue shades at the sides and on the fixed finger. Most of propodus is translucent white while carpus has a real band on the distal end. A red spot is present on propodus at the point of articulation with dactylus. Dactylus is slightly yellowish.

2) Weak Orange Clawed Males (WOC) (Fig 2.2)

(Transitional stage between SM and SOC)

Possess weak second pereiopodes characterised with feeble spination and first appearance of orange colour on propodus. Inner and medium sides of ischium, merus and carpus whitish with tinges of orange chromatophores whereas outer proximal area is suffused with blue pigments. Most of propodus is orange while dactylus is yellowish orange and naked.

3) Strong Orange Clawed Males (SOC) (Fig.2.3)

(2nd male morphotypic stage)

Large animals with strong second chelipeds, most of them are orange in colour. Colouration of ischium, merus and carpus are similar to that of WOC, however, these podomeres show difference by possessing stout spines on them. Most of propodus is orange with whitish medial face. Spines on propodus are stout with horny tips

and appear as orange in colour. Dactylus is fully covered with greyish brown hairs.

4) Pre-transforming Orange Clawed Males(t-SOC) (Fig.2.4)

(Transitional stage between SOC and WBC)

Animals having varying body sizes similar to both WOC and SOC.They are transitional stage between Orange Clawed males and Blue Clawed males. The colouration shows resemblance with that of WOC or SOC but can easily be distinguished by the presence of patches of blue colouration on propodus replacing orange colour, which may manifest the first signs of transformation into BC.

5) Weak Blue Clawed Males (WBC) (Fig.2.5)

(Transitional stage between t-SOC and SBC)

Animals with wide range of body sizes ranging from 100-280 mm in total length. Characterized by the presence of blue colouration on all podomeres of second cheliped, ischium, merus and carpus have whitish inner and medial faces, however, deep blue colour persists on major portion. Propodus is blue in colour while dactylus is dark and naked. The spines on podomeres are small and feeble and appear blue in colour.

6) Stong Blue Clawed Males (SBC) (Fig.2.6)

(3rd male morphotypic stage)

Large animals with very strong second chelipeds. All podomeres are dark blue coloured with long, stout and robust spines. Dactylus has a thick covering of short greyish brown hairs.

7) Old Blue Clawed Males (OBC) (Fig.2.7)

(Transitional stage of SBC)

Largest animals occupying the terminal position of the transformation pathway characterized with the presence of exceptionally strong and stout second chelipeds longer than that of total length and disproportionate with the body length. Colouration and spination are almost similar to that of SBC.

Though the SM is capable of transforming into OC males and eventually becoming BC males, especially, while the number of BC males are low in the population, the involvement of gene in the variation of reproduction and grwoth as well as on expression of morphotypic characters can not be ruled out (Ranjeet and Kurup, 2002). Ra'anan and Cohen (1985) opined that some differences in gene which direct the individuals relative growth may be possible which can very much determine the preliminary size hierarchy. The growth and transformation of various morphotypes are also governed by interaction prevalent with the population. Juvenile prawns have a relatively uniform growth rate when raised in isolation. However, when raised in groups, the difference in size became more pronounced. It can reasonably be inferred that the social interaction among the individuals together with some genetical factors may probably play major roles in the development of size disparity in *M.rosenbergii.*

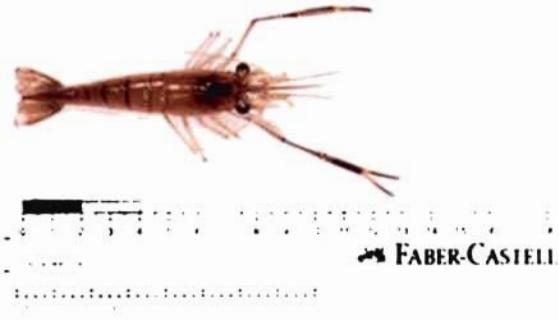


Fig 2.1 : *Macrobrachium rosenbergii* (de Man)
Small Males (SM)

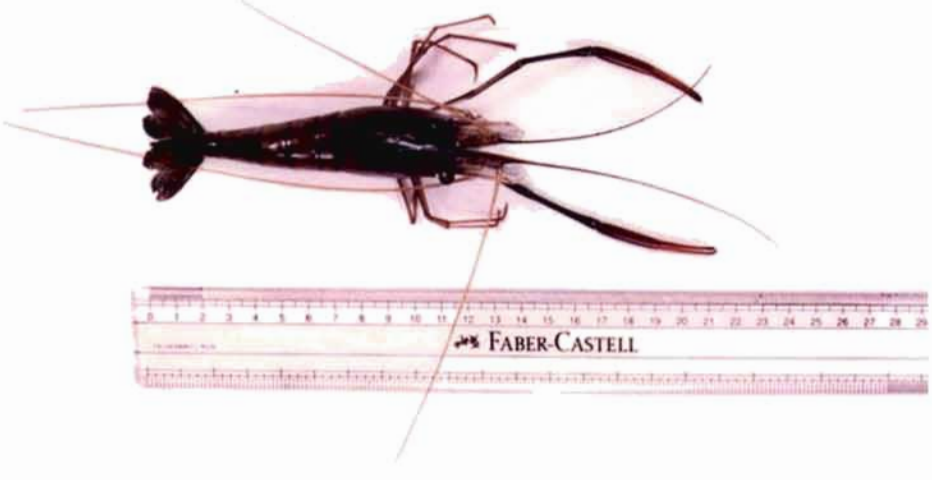


Fig 2.2 : *Macrobrachium rosenbergii* (de Man)
Weak Orange Clawed Male (WOC)

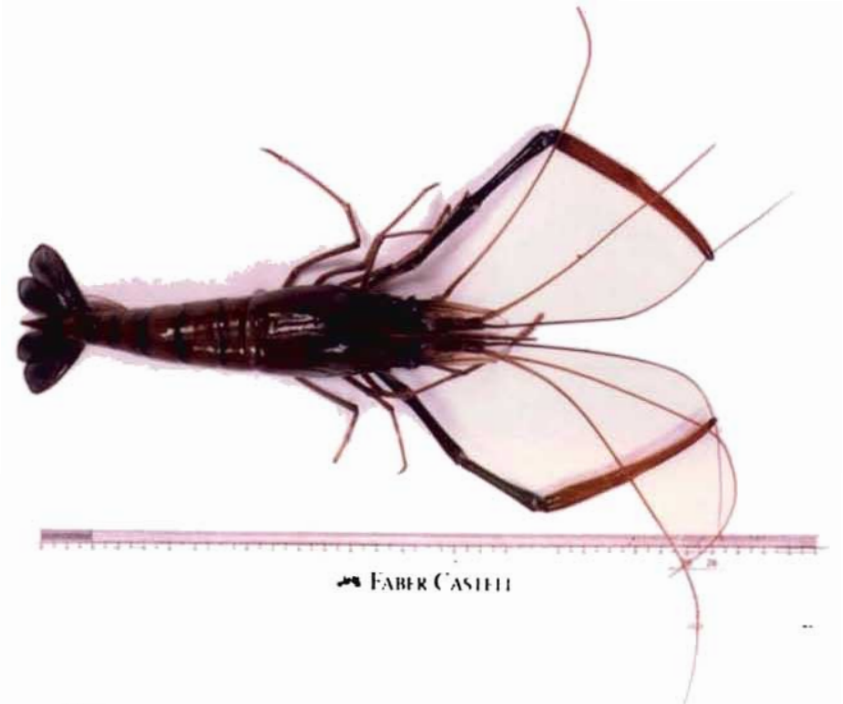


Fig 2.3 : *Macrobrachium rosenbergii* (de Man)
Strong Orange Clawed Male (SOC)

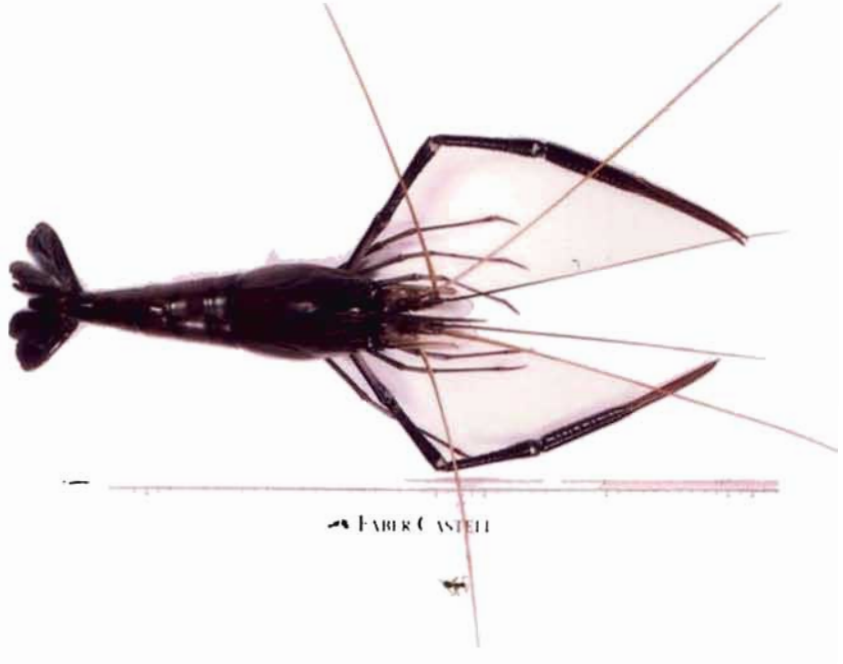


Fig 2.4 : *Macrobrachium rosenbergii* (de Man)
Pre-transforming Orange Clawed Male (t-SOC)

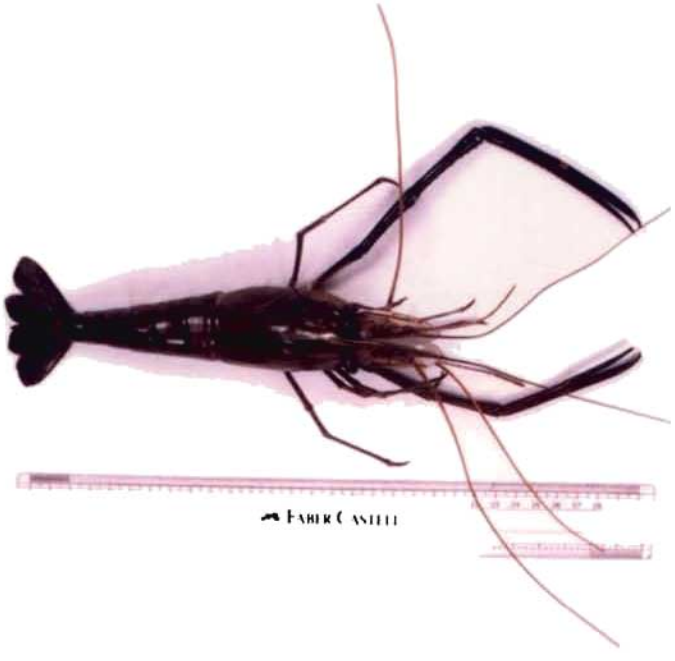


Fig 2.5 : *Macrobrachium rosenbergii* (de Man)
Weak Blue Clawed Male (WBC)

Fig 2.6 : *Macrobrachium rosenbergii* (de Man)
Strong Blue Clawed Male (SBC)

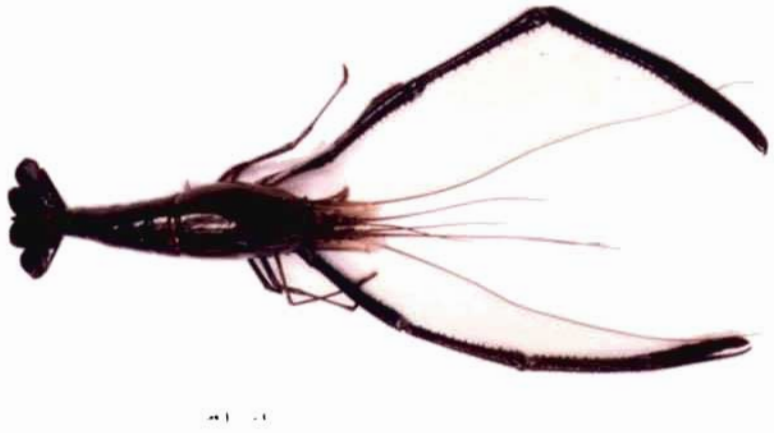


Fig2.7 : *Macrobrachium rosenbergii* (de Man)
Old Blue Clawed Male (OBC)

CHAPTER 3

REVIEW OF LITERATURE

The extensive literature on the reproductive biology of Palaemonid prawns are of male and female gross reproductive anatomy, artificial insemination, cryopreservation and functional evolutionary significance. In contrast, specific studies on reproductive anatomy of *M. rosenbergii* is quite sparse. Studies on the male reproductive system and fertilization of *M. rosenbergii* have been carried out by Chow *et al.* (1982). A morphological examination of sperm-egg interaction in the freshwater prawn *M. rosenbergii* was reported by Lynn and Clark (1983a). Lynn and Clark (1983b) also reported the fine structure of the mature sperm of the freshwater prawn, *M. rosenbergii.* Junctional relationship between germinal cells and sustentacular cells in the testes of *M. rosenbergii* was studied by Dougherty and Sandifer (1984). O'Donovan *et al.* (1984) studied the ovarian cycle during the intermoult in ovigerous *M.rosenbergii* while Chow *et al.* (1985) reported cryopreservation of spermatophore of *M. rosenbergii.* Dougherty and Harris (1986) reported about the spermatozoa in the spermatophore of *M. rosenbergii.* Sagi and Ra'anan (1988) conducted studies on the changes in midgut glands and the reproductive system and the morphotypic differentiation of males of *M. rosenbergii.* Sagi *et al.* (1988) worked on spermatogenesis and sperm storage in the testes of the behaviorally distinctive male morphotypes of *M. rosenbergii.* Some aspects of the morphology and histology of the Malasian fresh water prawn, *M. rosenbergii* was carried out by Ang *et al.* (1990). Varghese *et al.* (1992) conducted studies on the sex ratio in

broodstock rearing of *M. rosenbergii* (de Man). The reproductive anatomy of male fresh water prawns *M. australiense* (Holthius, 1890) of South East Queensland was reported by Butcher *et al.* (1994). Reproductive activity of male morphotypes of *M. rosenbergii* (de Man) and their performance in broodstock rearing and larval production were carried out by Sureshkumar and Kurup (1998).

Studies on the experimental hybridization of fresh water prawn, *Macrobrachium nipponence* and *M. formosense* were carried out by Uno and Fujita (1972). A method for artificial insemination of *Macrobrachium prawns* and its potential use in inheritance and hybridization studies was evolved by Sandifer and Smith (1979). Sreekumar *et al.* (1982) studied the aspects of semen production in *Macrobrachium spp.* The origin of spermatophores in six palaemonid prawns has been reported by Chow *et al.* (1989).

Some of the lesser known fresh water prawn species subjected to investigation include *Palaemon lamarrei* (Nath, 1937), *Palaemon elegans* (Pochon-Masson, 1969), *P. serratus* (Papathanasiou and King, 1984a, 1984b), *Palaemonetes vulgaris* (Burkenroad, 1947) and *P. paludosus* (Kochler, 1979).

The male reproductive system and spermatophore formation in other custaceans were also subjected to a series of studies. Cronin (1947) worked on the anatomy and histology of male reproductive system of *Callinectus sapidus.* The structure and function of the reproductive system of the crab *Portunus sanguinolentus* was studied by Ryan (1967). Greenwood (1972)

reported about the male reproductive system and spermatophore formation in *Pagurus novaezealandiae*. A comparative study of the origin, distribution and fine structure of extra cellular tubules in the male reproductive system of species of isopods, amphipods, copepods and cumacea was carried out by Reger and Fain-Maurel (1973). Histological observations on the vas deferens of the spider crab *Labinia emarginata* was done by Hinsch and Walker (1974). Hopkins (1978) reported about the male genital system and spermatophore production and function in *Euchaeta norvegica*. The male reproductive tract and accessory gland of a stomatopod *Squilla holoschista* was studied by Deecaraman and Subramoniam (1980). Blades and Youngbluth (1981) observed the ultra structure of the male reproductive system and spermatophore formation in *Labidocera aestiva*. Studies on the reproductive tract and accessory sex organs of the ghost crab Ocypoda platyarsis was carried out by Varadarajan and Subramoniam (1982). Haley (1984) studied the spermatogenesis and spermatophore production in the Hawaiian red lobster *Enoplometopus occidentalis*. Ryan (1984) worked on the spermatogenesis and male reproductive system in the Hawaiian crab *Ranina hariva*. Subramoniam (1982) studied the spermatophore formation in two intertidal anomuran crabs *Emerita asiatica* and *Albunea symnista*. Ultra structure and role of the lobster vas deferens in spermatophore formation was carried out by Kooda-Cisco and Talbot (1986). Champion (1987) studied the functonal anatomy of the male reproductive system in *Penaeus indicus*.. Diesel (1989) reported the structure and function of the male reproductive system of spider crab *Inachus phalangium*. The structure and function of the vas

deferens in the shrimp *Penaeus setiferus* was studied by Ro *et al.* (1990). Bauer and Cash (1991) reported about the spermatophore structure and anatomy of the ejaculatory duct in *penaeus setiferus*, *P. duorarum* and *P.azeticus*.

The androgenic gland was first described in the crab *Callinectes sapidus* (Cronin, 1947), while the first insight into its function was provided by Charniaux –Cotton (1954) in the amphipod *Orchestia* and in species representing nearly all the orders within the Malacostraca. The androgenic gland is usually located at the dorsomedian surface of the terminal ampoule at the distal end of the sperm duct (Thampy and John, 1972, 1973; Sagi and Ra'anan, 1988; Philip and Subramoniam, 1992). The cells may be arranged as thin, parallel and anastomosing cords (Carpenter and De Roos, 1970; Thampy and John, 1972, 1973) or in a compact lobed structure (Kleinhotz and Keller, 1979; Veith and Malecha, 1983). This gland secretes a hormone which determines the development of primary and secondary sexual characters (Thampy and John, 1972, 1973; Nagamine *et al.*, 1980a; Nagamine *et al.*, 1980b; Taketomi *et al.*, 1996) and also behavioral sexual characters (Charnaiux-Cotton, 1958, 1962; Nagamine *et al.*, 1980a, 1980b). A wide range of abnormalities in gonadal development was observed in andrectomised males, depending on the age at which the andrectomy was performed (Katakura, 1984; Malecha *et al.*, 1992; Sagi and Cohen, 1990).

The differential growth shown in *M. rosenbergii* ie. the males growing faster than females (Sagi *et al.*, 1986) adds to the significance of this gland in influencing the growth. The growth rate among males

vary greatly (Fujimura and Okamoto, 1972; Smith *et al.*, 1978; Malecha *et al.*, 1984) resulting in the existence of different morphotypes (Ra'anan and Cohen, 1985; Kuris *et al.*, 1987; Harikrishnan and Kurup, 1997). It was demonstrated (Sagi *et al.*, 1990) that the androgenic gland ablation affects growth rate and morphotypic differentiation in the three distinctive adult male morphotypes that coexist in *M. rosenbergii* population. Andrectomy of small males (SM) did not prevent transformation into the Orange claw (OC) morphotype but did prevent further transformation into the Blue claw (BC) morphotype. The androgenic gland undergoes hypertrophy after eye stalk ablation in several decapods (Adiyodi and Adiyodi, 1970). Hypertrophy and hyperactivity of gland tissue and precocious spermatogenesis in destalked crabs suggest that the androgenic gland is under the direct neuro hormonal inhibiting control of X-organ Sinus gland system of the eye stalk (Demeusy and Veillet, 1958). Histological and histochemical studies of androgenic gland of *M. rosenbergii* includes that of Veith and Malecha (1983), Kiran and Dubey (1999), Sun *et al.* (2000) and Joseph and Kurup (2001).

Hepatopancreas plays a significant role in the food assimilation and mobilisation of energy during moulting, pigmentation, gluconeogenesis and carbohydrate storage (Dhall and Moraity, 1983; Skinner, 1985; Ghidalia, 1985). Histophysiology and pattern of proteins in relation to reproduction and moult in the hepatopancreas of *Paratelphusa hydrodromous* was investigated by Adiyodi and Adiyodi, (1972). Al-Mohanna *et al.* (1985a) conducted a detailed study of the 'E' and secretory 'F' cells in the hepatopancreas

of the shrimp *Penaeus semisulcatus*. Al-Mohanna *et al.* (1985b) also gave a detailed report of M-Midget cells in the hepatopancreas of *Penaeus semisulcatus*. An ultra structural study of the hepatopancreas of *Procambarus clarkii* was conducted by Bunt (1968). Dall (1967b) reported the functional anatomy of the digestive tract of a shrimp *Metapenaeus bonnettae*. The growth and cell differentiation of the hepatopancreas of the Cray fish was studied by Davis and Burnett (1964). Hopkin and Nott (1979) made some observations on concentrically structured intracellular granules in the hepatopancreas of the shore crab *Carcinus meanas*. Histochemical studies on the hepatopancreas of the Cray fish *Procambarus clarkii* was carried out by Miyawaki *et al.*(1961). Stainer *et al.* (1968) studied the fine structure of the hepatopancreas of *Carcinus meanas*. Pre-ecdysial and post-ecdysial histological and histochemical changes in the hepatopancreas and integuments of the lobster *Panulirus homarus* was reported by Travis (1955, 1957). Interpretation of Cray fish hepatopancreatic function based on fine structural analysis of epithelial cell lines and muscle net work was studied by Loizzi (1971).

A careful survey of the available literature reveals that our knowledge on the neurosecretory system in natantia in general and in fresh water prawns in particular is very limited. Our present knowledge regarding the studies on neuroendocrine system in *Macrobrachium spp.* is scanty and is also restricted to neuroendocrine control on reproduction. Reproduction of crustacea is under the control of neuroendocrine factors (Adiyodi and Adiyodi, 1970). The

role of the neurosecretory material produced by the eye stalk, brain and thoracic ganglion in reproduction and several other physiological functions in crustacea, is sufficiently worked out but the exact source (cell types) of the production of particular hormone is not yet ascertained in any of the crustacean member. Otsu (1963) working on the fresh water crab, *Potamon dehaani* conformed the hypothesis that the sinus gland and/or the eye stalk contain an ovary inhibiting hormone. Otsu (1964) had also, for the first time, demonstrated the presence of gonad stimulating substance in the thoracic ganglion of the same crab. Gomez and Nayar (1965) demonstrated that like thoracic ganglion the brain also secrets a gonad stimulating hormone in the fresh water crab, *Paratelphusa hydrodromous.*

Few reports are available regarding the correlation between the reproductive cycle and neurosecretion in crustaceans. Passano (1951a) reported the X-organ sinus gland neurosecretory system in crabs. Studies on the shrimp neurosecretory system of *Caridina leavis* was carried out by Pillai (1960). Observations on the neurosecretory system of portunid crabs made by Potter (1958). Parameswaran (1956) studied the neurosecretory cells of the central nervous system of the crab *Paratelphusa hydrodromous.* Morphological studies on the neurosecretion in crabs was carried out by Matsumoto (1958). Ramadan and Matta (1976) conducted histological study of mapping the neurosecretory cells of the prawn. Smith and Naylar (1972) reported the neurosecretory system of eye stalk of *Carcinus meanas.* Van Herp *et al.* (1977) made a histophysiological study of the eye stalk of Palaemon serratus.

Studies on the reproductive biology and neurosecretion of fresh water prawn *Macrobrachium kistnensis* was conducted by Mirajkar (1980). Studies were also carried out by Mirajkar *et al.* (1983) on the neurosectory control of the annual reproduction cycle in the fresh water prawn *M. kistnensis*. Miyawaki (1960) made investigations on the neurosecretory cells of some decapod crustacea. Neuroendocrine control of reproduction of female crab *Barytelphusa cunicularis* was studied by Nagabhushanam and Diwan (1974). The neurosecretory system of portunid crab, *Scylla serrata* was reported by Nagabhushanam and Rao (1966).Studies on the neurosecretory cells of the thoracic ganglion of *Potamon magnum magnum* was carried out Baid *et al.*(1968). Durand (1956) reported the neurosecretory cell types and their secretory activity in the cray fish. The eye stalk neurosecretory cell type in the fresh water prawn *Palaemon paucidens* was described by Hisano (1974). Investigations on the neurosecretory cells of *Pachygraspus crassipus* was made by Inoue (1957). Johanson and Schreiner (1965) reported the neurosecretory cells in the ventral ganglia of the lobster *Homarus vulgaris*.

Available information on population profile development, morphotypic differentiation and their transformation, description of morphotypes based on morphology, morphometry and allometric characterisation are only based on harvested male population of *M.rosenbergii* from the grow outs, mostly from Israel (Brody et al., 1980; Cohen *et al.*, 1987) and from the natural waters from India. The pioneer work in this direction was carried out by Harikrishnan and Kurup, (1997) who studied population characteristics, fishery

and post larval distribution of *M.rosenbergii* in Vembanad lake. Population structure and morphotypic composition in the natural population of *M.rosenbergii* (de Man) was investigated by Harikrishnan and Kurup (1997a). Allometric relationships in M. rosenbergii (de Man) reared in a grow out pond under extensive system was carried out by Suresh kumar and Kurup, (1996). Reproductive activity of male morphotypes of *M. rosenbergii* (de Man) and their performance in broodstock rearing and larval production was reported by Suresh kumar and Kurup, (1998). Kurup and Harikrishnan (1999) reported the developmental pathways of male moprhotypes of *M. rosenbergii* (de Man) in natural habitat. Length weight relationships of male morphotypes of M. rosenbergii (de Man) as valid index for differentiating their developmental pathway and growth phases was established by Kurup *et al.* (1997a). Kurup *et al.* (1998) made observations on the population structure, weight distribution and yeild characteristics of M.rosenbergii reared in polders of Kuttanad, Kerala. Ranjeet and Kurup (2001) investigated on primary intrinsic factors governing the heterogeneous individual growth in male morphotypes of *M. rosenbergii.*

Though, morphotypic differentiation could be established in the grow out and natural population of *M. rosenbergii* (Cohen *et al.*, 1981; Ra'anan and Cohen, 1985; Sagi and Ra'anan, 1988; Harikrishnan and Kurup, 1997a) only very little studies have been made to characterise them biochemically. Sherief *et al.* (1992) studied the biochemical composition of pond reared *M. rosenbergii* giving due emphasis to fast growing bulls and stunted runts. A comparative

study on the nutrient composition of hepatopancreas of *M. rosenbergii* and *Penaeus indicus* was attempted by Sherief and Xavier (1994). Maugle *et al.* (1980) studied the variation in carotenoid composition in junvenile *M. rosenbergii* during eye stalk ablation. Rubbi *et al.* (1985) while examining quality changes during short term preservation observed that the proximate composition of *M. rosenbergii* vary with sex, maturity, different anatamical properties and also with different seasons. Biochemical characterisation of different male morphotypes of *M. rosenbergii* (de Man) from grow out has been studied by Sureshkumar and Kurup (1998). Significant difference could be seen in protein, DNA and RNA contents of muscle tissue, carbohydrate and RNA contents in hepatopancreas and DNA and RNA contents of the gonads of various male morphotypes. Sureshkumar and Kurup (1999) also reported that variations in hepatosomatic index and biochemical profiles among the male morphotypes of *M. rosenbergii*. A marked variation in hepatosomatic index of morphotypes indicated the possibility of difference in food assimilation and growth rates among the male morphotypes of *M. rosenbergii*. Tidwell *et al.* (1998) reported that the fatty acids and amino acid profiles in three tissues (eggs, muscle and midgut gland) from freshwater prawn *M. rosenbergii* raised unfed in ponds with no organic fertilization, unfed in ponds with organic fertilization or fed a formulated pelleted diet. Ranjeet and Kurup (2002) made investigations on the factors underlying heterogeneous individual growth associated with male morphotypes of M. rosenbergii and its implications on technoeconomic feasibility of farming.

CHAPTER 4

MATERIALS AND METHODS

SOURCE OF ANIMALS

Live specimens of three male morphotypes of *M. rosenbergii* such as SM, SOC and SBC and their four transitional stages such as WOC, t-SOC, WBC and OBC belonging to single age group were collected from a growout adjacent to Vembanad Lake, Kerala, South India, during 1998-2000 period.These morphotypes of unknown age were also collected from the Kumarakum –Muhamma regions of the Vembanad lake for catering the requirements of wild population in the present study. They were identified into three main morphotypes and their four transitional stages following Kuris *et al.* (1987), Sagi and Ra'anan (1988), Ang *et al.* (1990) and Harikrishnan and Kurup (1997a). The organ tissues selected for the study include the reproductive system viz. testes, vas deferens and androgenic gland, hepatopancreas and the neurosecretory system such as eye stalk, brain and thoracic ganglion. Sample tissues from five specimens displaying unequivocal morphotypic characteristics as described in chapter 2 were selected for the histological study.

HISTOLOGICAL METHODS (Humason, 1972)

Reproductive System

Prawns were dissected out and the testes, vas deferens including androgenic gland were removed and fixed in alcoholic Bouin's solution over night.

Composition of alcoholic Bouin's fixative

Picric acid	1 g
Formalin (40% HCHO)	60 c.c
Acetic acid (glacial)	15 c.c
80% alcohol	150 c.c

After fixation the tissues were processed in the following manner (Pantin, 1948).

1. Transferred to 70% alcohol.
2. Transferred to 90% alcohol for 2 hours.
3. Transferred to 95% alcohol for 1 hour.
4. Transferred to two changes of absolute alcohol for 1 hour each.
5. Placed the tissue in 1:1 mixture of absolute alcohol and methyl benzoate for 30 minutes.
6. Cleared in methyl benzoate until the tissue became transparent.
7. The tissues were transferred to xylene saturated with paraffin wax of melting point 58-60°C for 6 hours.
8. Infiltered the tissue in 2-3 changes of molten paraffin wax of melting point 58-60°C for 1 hour each.
9. Embedded in paraffin wax of melting point 60-62°C.

The blocks were sectioned at 6-7μ thickness and mounted on glass slides. The stain used for the reproductive system was Mayer's Haemalum (Humason, 1972).

Staining technique with Mayer's Haemalum (Humason, 1972)

Mayer's Haemalum

Hematoxyline	0.5 g
Distilled water	500.0 ml
Aluminium potassium sulphate	25.0 g
Sodium iodate	0.1 g
Acetic acid	20.0 ml

Scott's solution

Sodium bicarbonate	2 g
Magnesium sulphate	20 g
Distilled water	1000 ml

Eosin

Eosin Y	1 g
70% ethyl alcohol	1000 ml
Glacial acetic acid	5 ml

Dilute with equal volume of 70% alcohol for use and add 2-3 drops of acetic acid.

Procedure:

1. Deparaffinised and hydrated slides to water.
2. Stained in Mayer's haemalum : 11-15 minutes.
3. Washed in running water 3 minutes.
4. Blued in Scott's solution 3 minutes.
5. Washed in running water 3-5 minutes.

6. Counter stained in eosin 1-2 minutes.
7. Dehydrated quickly through 70 and 95% alcohols.
8. Dehydrated, cleared in xylene and mounted in DPX.

Sections were stained with Mayer's Haemalum stain (Humason, 1972). The slides were examined by light microscopy and photographed using a binocular microscope and Nippon camera combination at high power.

Hepatopancreas

Source of animals and tissue preparations for the histological studies were the same as mentioned above. Serial sections of 6 to 7μ thick were prepared and mounted on glass slides. Sections of hepatopancreas of various male morphotypes and their transitional stages were stained with Hiedenhein's azan stain (Humason, 1972).

Heidenhain's Azan staining technique (Pantin, 1948)

Solutions

Azocarmine

Azocarmine	0.1 g
Distilled water	100 ml
Glacial acetic acid	1 ml

Boiled azocarmine in distilled water for five minutes. Cooled and filtered through a soft filter paper. Added one ml of glacial acetic acid per 100 ml.

Aniline alcohol

Aniline	1 ml
90% ethyl alcohol	1000 ml

Acid alcohol

Glacial acetic acid	5 ml
96% ethyl alcohol	100 ml

Phosphotungstic acid

Phosphotungstic acid	5 g
Distilled water	100 ml

Aniline Blue stain

Aniline Blue	0.5 g
Orange G	2 g
Distilled water	100 ml

Added 8 ml glacial acetic acid. Boiled. Filtered when cold. Diluted with twice the volume of distilled water.

Procedure

1. Deparaffinised and hydrated slides down to water.
2. Stained in azocarmine at 56 – 60°C in soppered jar for 45-60 minutes.
3. Washed in distilled water.
4. Differentiated in aniline alcohol under the microscope. Only nuclei should remain pink, other parts greyish.

5. Stopped differentiation by washing off aniline in acetic acid: 1 minute
6. Mordanted connective tissue in 5% phosphotungstic acid: 1-3 hours.
7. Washed rapidly in distilled water.
8. Stained in aniline blue: 1-3 hours.
9. Washed briefly (seconds) in water.
10. Differentiated in 96% alcohol.
11. Dehydrated, cleared in xylene and mounted in DPX.

Neurosecretory system – eye stalk, brain and thoracic ganglion

Source of animals and tissue preparations for the histological studies were the same as mentioned above. The fixative used was aqueous Bouin's solution. Eye stalk, brain and thoracic ganglion from various male morphotypes and their transitional stages were dissected out and transferred to the fixative. The tissues were fixed overnight in this fixative. The cuticle of the eye stalk was gently removed to expose the optic peduncle. The tissues were dehydrated, infiltrated and embedded as described earlier. Serial sections of 6 to 7μ thick were prepared and mounted on glass slides. The stains used were Mallory's triple stain and Gomori's chrome haematoxyline phloxin stain.

Mallory's triple staining technique (Humason, 1972)

Solutions:

Mallory – I

Acid fuchsin	1.0 g
Distilled water	100 ml

Phosphomolybdic acid

Phosphomolybdic acid	1.0 g
Distilled water	100 ml

Mallory – II

Aniline blue	0.5 g
Orange G	2.0 g
Distilled water	100.0 ml

Procedure

1. The sections were deparaffinised and hydrated the slides in water.
2. Stained with Mallory I for 15 seconds.
3. Rinsed in distilled water for 10 seconds.
4. Treated with phosphomolybdic acid for 1-5 minutes.
5. Rinsed briefly in distilled water.
6. Stained with Mallory II for 2 minutes.
7. Rinsed in distilled water.
8. Differentiated with aniline blue in 90% ethyl alcohol.
9. Dehydrated in absolute alcohol.
10. Cleared in Xylene.
11. Mounted in DPX.

Gomori's chrome hematoxylin phloxin staining technique)

Solutions:

Bouin's fluid

Puric acid, saturated aqueous solution	75 ml
Formalin	25 ml

Glacial Acetic Acid	5 ml

Potassium permanganate solution

Potassium permanganate	0.3 g
Distilled water	100.0 ml
Sulphuric acid	0.3 ml

Sodium bisulphite solution:

Sodium bisulphite	5 g
Distilled water	100 ml

Chrome-Hematoxylin Solution

Hematoxylin, 1% aqueous solution	50 ml
Chromium, alum, 3% aqueous solution	50 ml

To 100ml of Chrome-Hematoxylin added 0.1 gm of potassium iodate and boiled until a deep blue colour developed. The mixture was filtered before use.

Phloxin B solution

Phloxin B	0.5 g
Distilled water	100.0 ml

Phosphotungstic acid solution

Phosphotungstic acid	5 g
Distilled water	100 ml

Procedure

1. Slides were deparaffinised and hydrated in water

2. Refixed in Bouin's fluid for 12 to 24 hours
3. Washed thoroughly in tap water to remove picric acid.
4. Treated the sections for about 1 minute with potassium solution.
5. Differentiated with a 5% solution of sodium bisulfite
6. Washed well in running tap water.
7. Stained in Chrome-Haematoxylin solution.
8. Differentiated in 1% Hydrochloric acid alcohol for 1 minute.
9. Wahed in tap water until the section becomes clear blue.
10. Counterstained with phloxin B for 5 minutes.
11. Rinsed in distilled water.
12. Immersed in 5% phosphotungstic acid solution for 1 minute.
13. Washed in tap water for 5 minutes.
14. Differentiated in 95% alcohol.
15. Dehydrated, cleared in xylene and mounted in DPX.

The sections were examined under research microscope for their histological details. The measurement of the cells and nuclear diameters were made with the help of calibrated eye piece.

HISTOCHEMICAL METHODS (Pearse, 1968)

The source of animals for the histochemical studies were the same as mentioned above. The organ tissues selected for the study include the reproductive system viz. testes, vas deferens including androgenic gland and hepatopancreas. Samples of these tissues, collected from five specimens, displaying unequivocal morphotypic characteristics were used for histochemical study.

Tissue samples were taken from live animal and directly put into fixatives. The fixatives employed were aqueous Bouin's fixative, alcoholic Bouin's fixative, Carnoy's fixative, Lillies alcoholic lead nitrate fixative and Baker's Formol-calcium fixative.

Composition of fixatives

1. Aqueous Bouin's fixative

Picric acid, saturated aqueous solution	75 c.c
Formalin (40% HCHO)	25 c.c
Acetic acid (glacial)	5 c.c

Solution keeps indefinitely. Fix 12 hours or more

2. Alcoholic Bouin's fixative

Picric acid	1 g
Acetic acid glacial	15 c.c
Formalin (40% HCHO)	60 c.c
80% alcohol	150 c.c

Fix for 2 hours or more

3. Carnoy's fixative

Ethyl alcohol	60 ml
Chloroform	30 ml
Glacial accetic acid	10 ml

4. Lillies alcoholic lead nitrate solution:-

Lead nitrate	8 g
40% formaldehyde	10 ml
Water	10 ml

Ethanol	80 ml

Fixation time 24 hours at room temperature.

5. Lipid fixative (Baker's formol calcium fixative)

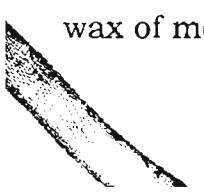
Cobalt nitrate	1 g
Formalin (40% HCHO)	10 c.c
10% Ca Cl_2 (an hydrous)	10 c.c
Distilled water	80 c.c

(Powdered Ca $C0_3$ in slight excess)

Fixation time 1-5 weeks.

After fixation in the different fixatives, the tissues were processed in the following manner.

1. Washed overnight in running water to remove the yellow colour of the picric acid (Tissues fixed in aqueous Boui'ns fixative, others were not)
2. Transferred to 70% alcohol.
3. Transferred to 90% alcohol for 2 hours.
4. Transferred to 95% alcohol for 1 hour. (Tissues fixed in alcoholic Bouin's fixatives were transferred directly to 95% alcohol).
5. Transferred to 2 changes of absolute alcohol for 1 hour each.
6. Placed the tissue in 1:1 mixture of absolute alcohol and methyl benzoate for 30 minutes.
7. Cleared in methyl benzoate until the tissue became transparent.
8. The tissues are then transferred to xylene saturated with paraffin wax of melting point of 58-68°C for 6 hours.



9. Infiltrated the tissues in 2-3 changes of molten paraffin wax of melting point 58-60°Cfor 1 hour each.
10. Embedded in paraffin wax of melting point of 60-62°C

For microscopical studies of these organ tissues paraffin blocks were sectioned at 6-7μ thickness. Histochemical localisation of glycogen, protein, lipid, RNA and DNA of various tissues were carried out (Pears, 1968). The slides were examined by the light microscopy and photographed using a binocular microscope and Nippon camera combination at high power.

For histochemical localisation of glycogen Periodic Acid Schiff (PAS) technique was employed (Humason, 1972).

Fixation :

Alcoholic Bouin's

Solutions :

Periodic acid

Periodic acid	1.0 g
90% Ethyl alcohol	100.0 ml.

Schiff's Reagent

Dissolved 1 g of basic fuchsin in 200ml of boiling distilled water. Shaked for five minutes and cooled to exactly 50°C Filtered and added to the filtrate 20 ml. Of N-Hcl. Cooled to 25°C and added 1 g of sodium metabisulphite. Kept this solution in the dark for 14-24 hours. Added 2 g of activated charcol and shaked for 1 minute. Filtered and kept the filtrate in the dark at 0-4°C. Allowed to reach room temperature before use.

Sodium bisulfite

Sodium metabisulphite $Na_2S_2O_5$	0.5	g
Distillted water	100.0	ml.

Hematoxylin

Haematoxylin	1.0	g
Alcohol, 95%	100.0	ml.
Distilled water	288.0	ml
Ferric chloride (29.1% Fe cl_3)	4.0	ml.
Hydrochloric acid, concentrated	8.0	ml.
Ferrous sulphate	4.44	g.

Procedure

1. Deparaffinized and run slides down to 90% alcohol.
2. Treated with alcoholic periodic acid : 2 hours
3. Washed with 90% alcohol 5 minutes.
4. Hydrated quickly to water.
5. Treated with Schiff's reagent 10 minutes.
6. Transferred through Sulphate solutions, 3 changes each. 2minutes
7. Washed in running water : 2 minutes.
8. Countered stain in Hematoxylin : 5 minutes.
9. Washed in running water 2 minutes
10. Dehydrated, cleared in Xylene and mount in DPX.

Control

The control slides after de-paraffinization and hydration up to 90% alcohol were treated with a 1% diastase solution in 0.2 M phosphate buffer (pH 6.2) at 37°C for one hour. After one hour the control slides were treated with alcoholic periodic acid and stained as described above.

For histochmical localization of protein, Mercury Bromphenol Blue Method was employed (Pearse, 1968).

Fixation : Alcoholic Bouin's

Solution :

Bromphenol Blue

Mercuric chloride	10.0 g
Bromphenol Blue	100.0 mg
95% ethyl alcohol	100.0 ml

Procedure

1.	Deparaffinised sections.	
2.	Transferred to absolute alocohol	1-2 minutes
3.	Stained in alcoholic Bromphenol Blue stain :	15 minutes
4.	washed in 0.5% acetic acid	20 minutes to remove excess dye.
5.	Immersed in tap water or buffer of pH 6.7	3 minutes to convert dye to blue alkaline form.

6. Transferred directly to tertiary butyl alcohol, cleared in xylene and mounted DPX.

For histochemical localization of lipids, Sudan Black B staining was followed (Pearse, 1968).

Fixation :

Tissues were fixed for 1-5 weeks in a solution of 1g cobalt nitrate in 80 ml distilled water with 10 ml 10% $CaCl_2$ and 10 ml 40% formalin.

After fixation the tissues were dehydrated in 3 changes of acetone, each of half an hour duration, and then infiltrated in molten paraffin wax of melting point 58-60°Cand embedded in paraffin wax of melting point 60-62°C.

Solution:

Saturated solution of Sudan Black B in 70% alcohol.

Procedure

1. Deparaffinised and hydrate sections to 70% alcohol.
2. Stained in Sudan Black B : 30 minutes – 1 hour
3. Removed excess dye by rinsing quickly in 70% alcohol.
4. Washed in running water
5. Counterstained in 1% aqueous neutral red 1 minute
6. Wash in water, and mount in glycerine jelly.

For histochemical localization of RNA, Methyl Green-Pyronin Y method was followed (Pearse, 1968).

Fixation: Carnoy's fixative

Solution : Methyl Green – Pyronin Solution

Solution A

5% aqueous Pyronin	17.5 ml
2% aqueous Methylgreen (chloroform washed)	10.0 ml
Distilled water	250.0 ml

Solution B

M/5 acetate buffer pH 4.8

For use mix equal volumes of A and B in a coplin jar.

Procedure

1. Deparaffinised and bring sections to water.
2. Stained in Methyl green-Pyronin solution for 10 minutes to 24 hours
3. Rinsed in distilled water for a few seconds.
4. Blotted dry
5. Dehydrated rapidly in absolute acetone.
6. Rinsed briefly in equal parts of acetone and xylene.
7. Rinsed briefly in 10% acetone in xylene.
8. Cleared in xylene and mount in DPX.

BIOCHEMICAL METHODS

Biochemical Composition

Source of animals for the biochemical analysis were the same as mentioned above. The prawns were transported to the laboratory in live conditions and segregated into different morphotypes and their transitional stages (Kuris *et al.*, 1987; Harikrishnan and Kurup, 1997a). Special care was taken to include only hard shelled prawns of the intermoult stage in all the biochemical estimations. The muscle tissue and hepatopancreas of various male morphotypes and their transitional stages were dissected out from live animal and used for the various biochemical analysis viz. protein, lipid, carbohydrate, moisture and ash content. When the tissues of a single individual was insufficient for all the determinations, identical samples were pooled for the various estimations.

Ash content:- The ash content of muscle tissue and hepatopancreas was obtained by igniting the oven-dried samples in a porcelain crucible in a muffle furnace at 550°C-600°C for about 5 hours. The ignited residue thus obtained has been taken as the ash content, and expressed as percentage in terms of dry weight of the body.

Moisture:- A weighed portion of the sample (10g) was kept in a hot air oven at 100-105°C for 12 hours until a constant weight was obtained. The moisture content was calculated as the difference between the wet weight and dry weight of the tissue. The percent moisture in the sample calculated as follows:

$$\% \text{ moisture} = \frac{\text{Difference in wet weight and Dry weight of samples}}{\text{Wet weight of samples}} \times 100$$

Protein:- The total protein was estimated by the method of Microkjeldahl's as outlined by Hawk (1954). Protein value was calculated by multiplying the protein nitrogen value with a factor of 6.25 as performed by Giese *et al.* (1958).

Lipid:- Total lipids were quantified by the AOAC (1990) method after extraction with chloroform methanol (2:1,V/V) by the procedure of Bligh and Dyer (1959)

Carbohydrate:- The carbohydrate content (NFE) was calculated using difference method on dry weight basis (Hasting, 1976).

100-Protien + lipid + moisture + ash = NFE

These estimations were performed in triplicate, the values presented represent the average of these determinations. The results of the biochemical analysis of various male morphotypes and their transitional stages were analyzed statistically using simple statistics and one way ANOVA (Snedecor and Cochran, 1962)

Aminoacid Analysis:

The source of animals and their segregation into various morphotypes were the same as mentioned above. The tail muscle tissue from five individuals from each morphotype was removed, chopped into small pieces, pooled and immediately stored in one screw cap vial at -40°c until amino acid analysis was carried

out.Aminoacid composition was determined using Shimadzu LC-10As (Japan) HPLC system. Muscle sample after homogenising, was weighed accurately in triplicate in test tubes (nearly 100 mg). Acid hydrolysis was done in an atmosphere devoid of oxygen (by filling nitrogen gas), in an oven at 110°C for 24 hours. Hcl was removed by flash evaporation and the membrane filtered samples were injected into the HPLC at an oven temperature of 60°C. Column consists of a strongly acidic cation exchange resin made up of styrene divinyl benzene copolymer with sulfinic group. Post column derivatisation was done with ophthalaldehyde and hypochlorite reagent and the fluorescence developed was detected by a spectrophotometer.

Lipid Fractionisation

Source of animals and their segregation into various morphotypes for the lipid analysis were the same as mentioned above. Special care was taken to include only hard shelled prawns of the inter moult stage for lipid analysis. The muscle tissue and hepatopancreas of various male morphotypes were dissected out from live animal and immediately used for the lipid extraction. Whenever sufficient quantity of tissues were not available from single animal, the sample of the tissues were pooled from males belonging to the same morphotype. Lipid content was determined by extraction with chloroform methanol mixture (Bligh and Dyer, 1959) and cholesterol by the ferric chloride method as described by Rudel and Morris (1973). Cholesterol standard was procured from Sigma chemicals. Other chemicals used were of analytical grade from BDH.

cholesterol: 30 g of sample tissue was extracted with 15 volumes of chloroform methanol (2 : 1) in cold condition. To the combined extract

20% water was added and allowed to stand overnight. The lower chloroform layer was filtered over sodium sulphate and flash evaporated. It was made to a definite volume. Weight of fat was determined by drying a small portion of it (Bligh and Dyer, 1959). Saponification was done using methanol and KOH and the unsaponifiable matter was separated as ether extract. Cholesterol in the nonsaponifiable extract was determined by Feric chloride colorimetric method.

Phospholipid: The organic phospholipid phosphorous in the sapmple was converted to inorganic phosphorous which reacted with amonium molybdate to form phosphomolybdic acid which on reduction and reacting with ANSA formed a stable blue colour which was read at 660 nm.

Triglycerides: After extracting fat using isopropanol, the impurities were removed using alumina. Suphonification was done using KOH. Metaperiodate and acetyl acetone reagents were used for the colour development and it was measured at 405nm in a spectrophotometer.

These estimations were carried out in triplicate and the average of these are presented. The results were analysed statistically using simple statistics and one-way ANOVA (Snedecor and Cochran, 1967).

SECTION 2

HISTOLOGICAL CHARACTERISATION OF MALE MORPHOTYPES AND THEIR TRANSITIONAL STAGES OF *Macrobrachium rosenbergii* (de Man)

CHAPTER 5

TESTES

Introduction

Histological study of male reproductive system and spermatophore formation of freshwater prawns have been subjected to detailed investigation by Nath (1937), Chow *et al.* (1982), Sreekumar *et al.* (1982), Lynn and Clark (1983a), Dougherty and Sandifer (1984), Papathanassiou and King (1984b), Dougherty and Harris (1986), Sagi and Ra'anan (1988), Sagi *et al.* (1988), Chow (1989), Butcher and Fielder (1994) and Verdi (1995). Though the male morphotypes are characterized morphologically, allometrically and biochemically (Cohen *et al.*, 1987; Harikrishnan and Kurup, 1997a; Ra'anan and Sagi, 1988; Suresh Kumar and Kurup, 1998) however, no attempt has so far been made to bring out the structural variations, if any, in the testes of various male morphotypes and their transitional stages of *M.rosenbergii* by resorting to histological examination and to examine how far it will conform with the reproductive potential of various male morphotypes(Telecky, 1984; Ra'anan and Sagi, 1985; Sagi and Ra'anan, 1988; Sagi *et al.*, 1988; Suresh Kumar and Kurup, 1998).

Materials and Methods

Details regarding the collection, transportation and segregation of samples and histological techniques used are described in chapter 4.

Results

Testes of *M. rosenbergii* are paired elongated structure lying above the posterior half of the dorsal surface of the hepatopancreas and beneath the pericardial sinus and heart. At the anterior ends, the two testes meet together and fuse to form a common lobe, while posteriorly they remain separate, although lying close together. Testicular lobes are composed of long cylinders or lobules compactly held together by the intervening connective tissue. The thin wall or cortex surrounding the testes consists of two layers, an outer epithelium and an inner layer of connective tissue. The cylinders or lobules are of varying size and highly convoluted structures, in which the reproductive cells are produced. The membrane like wall of each tubule is also of two layers, the outer tunica and the inner germinal epithelium. Light microscopic studies revealed differences in the spermatogenic elements of the cylinders among the different male morphotypes and their transitional stages, as described below.

1. Small Males (SM) **:** The testes of small males possessed cylinders, most of them are covered by a single layer of epithelium. Part of the epithelium is multi layered and included cells of variable size, forming a spermatogenic zone containing germinal cells and sustentacular or nurse cells. The nurse cells appear to be confined to the peripheral region of the tubule. The nurse cells have irregularly shaped nuclei embedded in syncitial mass of protoplasm with no visible cell boundaries (Fig 5.1). Germinal cells include mainly primary spermatogonial cells and secondary spermatogonial cells. Primary spermatogonia are the largest spherical cell types among the

spermatogenic cells in the testes of small males especially in a state of higher concentration in the peripheral lobules as well as in the vicinity of lobular wall. The cytoplasm of these cells exhibits less affinity towards basic dyes such as haematoxylin- eosin. Primary spematogonia divide mitotically and give rise to small round secondary spermatogonia with a centrally placed nucleus, structurally they are similar to primary spermatogonia but for their size. Mature spermatozoans were seen in the lumen of a few of the testicular lobules.

2. Weak Orange Clawed Male (WOC) **:** In weak orange clawed male, the transitional stage between small male and orange clawed male, the spermatogenic zone becomes thinner and occupying less space than in the testes of SM (Fig 5.2). The spermatogenic zone contains primary spermatogonial cells as well as secondary spermtogonial cells. Mature spermatozoans were seen in the lumen of all testicular cylinders.

3. Strong Orange Clawed Males (SOC) **:** The testicular cylinder of strong orange clawed male (Fig 5.3) is filled with spermatocytes of both primary spermatocytes as well as secondary spermatocytes. The primary spermatocytes are formed by the active multiplication of secondary spermatogonia, which are in various stages of their divisions and contain large vesicular eccentrically placed nucleus. At first, the chromatin of the nucleus of each spermatocytes remain diffused, which subsequently transform into a fine reticular mesh. The reduction division of primary spermatocytes resulted in the formation of secondary spermatocytes. The chromatin materials of

secondary spermatocytes are in the form of dark thick clumps with traces of cytoplasm.

4.Pre-transforming Orange Clawed Male (t-SOC) **:** In the pre-transforming orange clawed male, spermatogenic zone becomes thinner when compared to small males and in weak orange clawed male (Fig 5.4). The lumen of all testicular lobules are filled with spermatozoans.

5.Weak Blue Clawed Male (WBC) **:** The testes of weak blue clawed males contain spermatocytes and spermatids in their lobules (Fig 5.5). The spermatids are much smaller in size than the secondary spermatocytes and are deeply stained with basic dyes such as haematoxylin-eoxin.

6.Strong Blue Clawed Male (SBC) **:** Among the various male morphotypes examined, all the testicular lobules of strong blue clawed male contains spermatozoans only (Fig 5.6). Spermatozoan of *M. rosenbergii* are non-flagellated and non-motile. The main body base of the sperm is slightly cupped and a single appendage (spike) projects from the convex surface of the base. The spermatogenic zone almost disappears and spermatogenesis reached to standstill stage.

7.Old Blue Clawed Male (OBC) **:** In old blue clawed male, testicular cylinders also contain mature sperm though its intensity seems to be reduced. However, some empty lobules are present in the testicular lobe and the spermatogenic zone is totally absent (Fig 5.7).

Discussion

Histological examination of the testes of various male morphotypes of *M. rosenbergii* revealed that the testes show difference in structure and function among each other. Based on the histology of the testes of three male morphotypes and their four transitional stages studied, it appeared that the testes of SM, WOC, and t-SOC were characterized by a spermatogenic zone and also possessed spermatozoans in their testicular lobules (Figs 5.1, 5.2 and 5.4). It could also be seen that, the spermatogenic zone becomes thinner in WOC and t-SOC when compared to SM, whereas the spermatogenic zone of t-SOC is much thinner than to WOC. Similarly, an increase in the abundance of spermatozoan could also be seen in the testicular lobules as it transforms from SM → WOC → t-SOC, the intermediary stages. On the contrary, in the testes of strong orange clawed and weak blue clawed males, the other two intermediary stages, the testicular lobules which is characterized by a total absence of spermatozoans (Figs 5.3 and 5.5). It would thus appear that the testes of these morphotypes are modified into organ containing mainly spermatocytes. On the other hand, the testicular lobules of SBC and OBC morphotypes, the penultimate and terminal stages respectively in their morphotypic developmental pathway, were characterized with the presence of only spermatozoans (Figs 5.6 and 5.7). The complete absence of the spermatogenic zone in their testicular lobules of the above morphotypes is another unique feature observed in the present study.

The available reports suggest that the male morphotypes of *M. rosenbergii* such as SM, WOC, t-SOC, WBC, SBC and OBC show perceptible difference in reproductive activity and somatic growth among them (Sagi and Ra'anan, 1988; Sagi *et al.,* 1988; Suresh Kumar and Kurup, 1998). SM and SBC are sexually active, participating in mating and fertilization thereby utilizing little energy in somatic growth. The small males employ a 'sneak copulation' strategy (Telecky, 1984) which performs numerous mating attempts, however, it could achieve only relatively little reproductive success. The SBC is reported to be dominant, territorial and sexually active and always found in association with 8-10 females (Ra'anan, 1987). On the contrary, orange clawed males are characterized by a fast growth rate and reduced reproductive activity (Sagi and Ra'anan, 1988; Sagi *et al.*, 1988; Suresh Kumar and Kurup, 1998).

Among male morphotypes of *M. rosenbergii*, the reproductive system of SM is relatively large and the testes is very prominent (Sagi and Ra'anan, 1988) when compared to WOC and t-SOC, however, the failure in its reproductive success can be attributed to the low occurrence of spermatozoans in testicular lobules of SM. The results of the present study also showed that spermatozoans were present only in a few testicular lobules of SM and this finding would lend to support its weak mating strategy and poor reproductive success.

SOC, the intermediary stage of WOC and t-SOC, is characterized by faster growth rate (Ra'anan, 1982), low relative weight of the reproductive system (Sagi and Ra'anan, 1988) and

reduced reproductive activity (Ra'anan and Sagi, 1985; Kuris *et al.*, 1987; Sagi and Ra'anan, 1988; Suresh Kumar and Kurup, 1998). OC males are sub-dominant, non-territorial and were never observed to be courting and protecting a receptive female, although occasional fertilization by OC males have been recorded (Ra'anan and Cohen, 1985). Studies on the reproductive activity of OC morphotypes revealed that it is reproductively submissive and showed a lesser performance of reproductive activity only in the absence of BC (Ra'anan and Sagi, 1985; Kuris *et al.*, 1987; Sagi and Ra'anan, 1988; Suresh Kumar and Kurup, 1998). From the histological structure of the testes of SOC, it can reasonably be inferred that the seminiferous cords contain only spermatocytes, in contrast, devoid of spermatozoans. The reproductive submissiveness of SOC might be attributed by the complete absence of spermatozoans in their testicular lobules. This is further supported by the observation of Sagi *et al.* (1988) who found out that lack of spermatozoans and presence of spermatocytes are characteristics of the testicular lobules of SOC. It can reasonably be inferred that when the small males become an orange claw male, the balance between reproductive activity and somatic growth may shift which in turn would change the SOC male into an almost sexually inactive morphotype, and reciprocally improving its growth rate so as to attain the status of the dominant male in terms of size, appearance and behaviour.

In SBC, testicular cylinders are used exclusively for the storage of spermatozoans, a characteristics feature that complements the dominant reproductive status of the SBC as reported by Ra'anan

and Sagi (1985). It would thus appear that, SBC, which occupies the penultimate stage in the morphotypic developmental pathway, are reproductively very active, however, characterized by a reduced growth rate, uses the stored sperm, rather than producing sperm. This is further supported by the complete disappearance of spermatogenic zone in the testicular lobules as observed by Ra'anan and Sagi (1988). Whereas the terminal stage, OBC, is characterized by a reduced reproductive activity as manifested by the presence of empty lobules and lesser abundance of spermatozoans in their testicular lobules. Spermatogenic zone is also found to be completely disappeared in this morphotype.

In WOC, the intermediary stage of SM and SOC, small amounts of mature sperm present in the testes of SM reported a slight increase in its quantity in the WOC males, however, it was found disappeared at the SOC stage. Similarly, in t-SOC, the intermediary stage of SOC and WBC, showed greater abundance of spermatozoans in their testicular lobules, than that of WOC, in contrast disappears in WBC stage. But Sagi *et al.* (1988) have opined that there is a possibility of reduction in the abundance of spermatozoans in the WOC and its complete disappearance in t-SOC and this is at variance with the present observation.

In WBC, the transitional stage between t-SOC and SBC is also characterized by the total absence of spermatozoans, in which the presence of spermatocytes and spermatids in their testicular lobules is worth mentioning. The result of present study confirms the complete absence of spermatozoans in their testicular lobules as

observed in the case of SOC (Sagi *et al.*, 1988). This is further supported by the observation of Dougherty and sandifer (1984) who observed two apparently exceptional specimens which appeared to be in synchrony in respect to spermatogenesis. According to them, in one specimen, the entire testes seemed to be synchronous with most of the spermatogenic cells which are in the state of pachytene of meosis-1 while some cells are in metaphase and late anaphase stage. In another specimen, the entire testes again observed to be synchronous with most of the spermatogenic cells appearing as spermatids. While making comparison with the present findings, it may be inferred that the two specimens as described above might be the SOC and WBC morpbotypes as the characters of them show very strong agreement with that of SOC and WBC. However, a lower performance of reproductive activity of these morphotyes have been reported by several authors (Ra'anan and Sagi, 1988; Suresh Kumar and Kurup, 1998). It can, therefore, be inferred that there is a possibility of retention of spermatozoans in the seminal vesicle and that might be act as a source of sperms in these morphotypes, for oviposition of female during breeding. An antgonism between second vitellogenesis and somatic growth has been found in the crab, *Carcinus*, by Demeusy (1965). According to her, second vitellogenesis is strongly opposed to somatic growth. Similarly, an increased somatic growth coincided with an inactive reproductive state of SOC and WBC might be due to such type of antagonistic nature of these two activities as reported in crab(Demeusy, 1965).

The result of the present study revealed that there exist a very strong correlation between the structural properties of the testes

with the reproductive activity of these morphotypes. The testes of SM, WOC and t-SOC are characterized by the presence of a spermatogenic zone and spermatozoans in their seminiferous cords, on the contrary, SOC and WBC are characterized by the absence of spermatozoans and presence of spermatocytes only in their testicular lobules. On the other hand, the testicular loblules of SBC and OBC were exclusively filled by mature sperm. It can be concluded that SM, WOC and t-SOC produce and store sperms while SOC and WBC primarily produce spermatocytes whereas SBC and OBC appears to be using the spermatozoan stored in the testicular lobules. Thus, *M. rosenbergii* male morphotypes comprised of reproductively active intermediary stages (WOC and t-SOC) alternate with a reproductively inactive stages (SOC and WBC) till it reaches the terminal SBC stage.

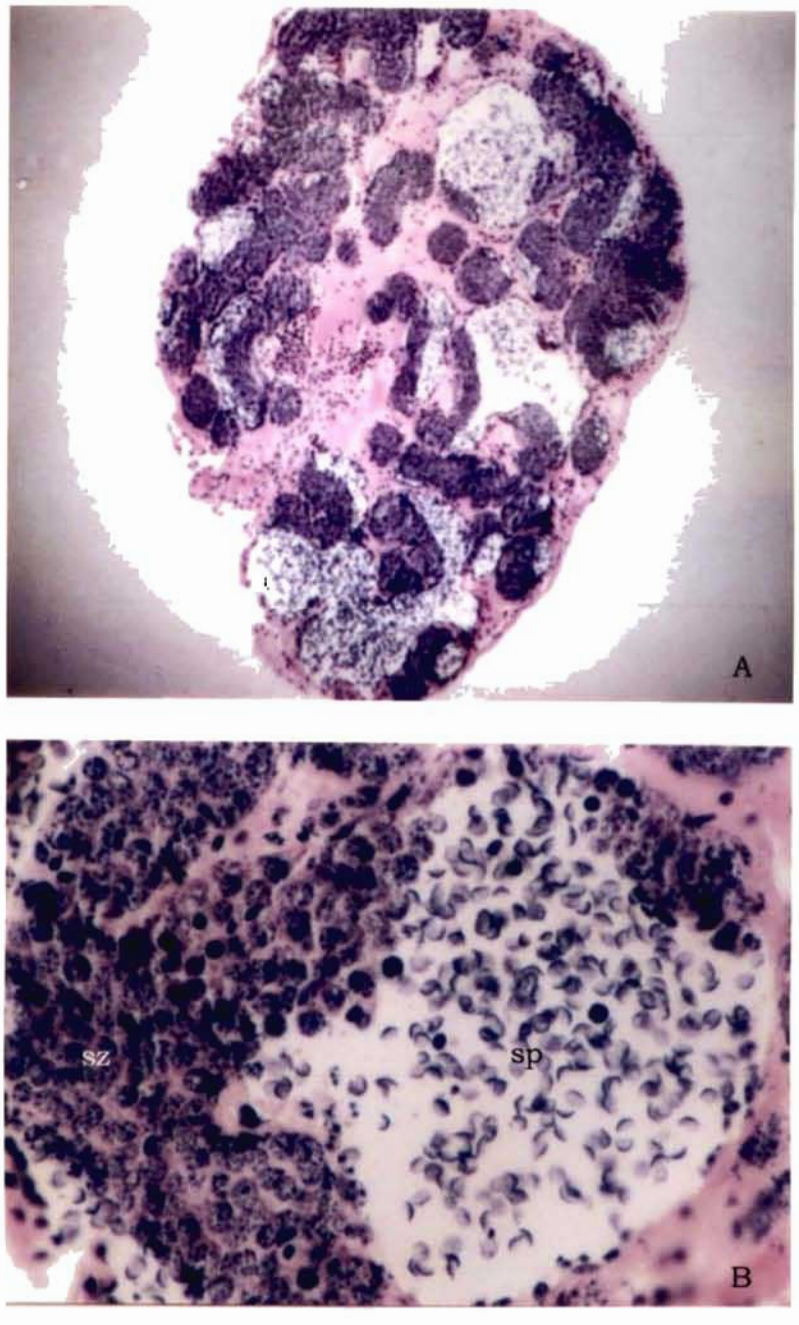
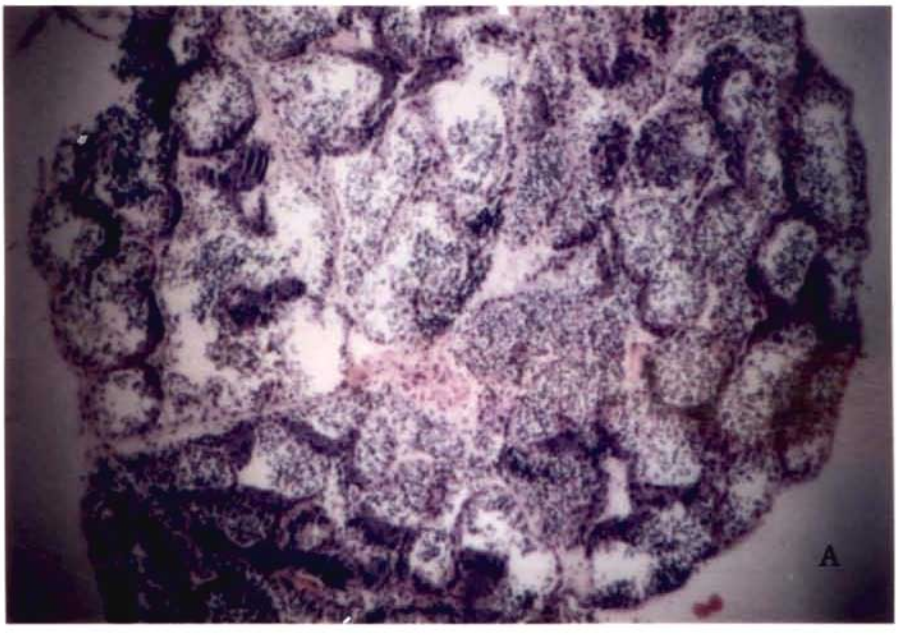


Fig 5.1 : Light photomicrography of a cross section through one testicular lobe removed from a small male. (A) general cross section (x40), (B) A typical testicular cylinder (x200), sz-spermatogenic Zone, sp-mature spermatozoa



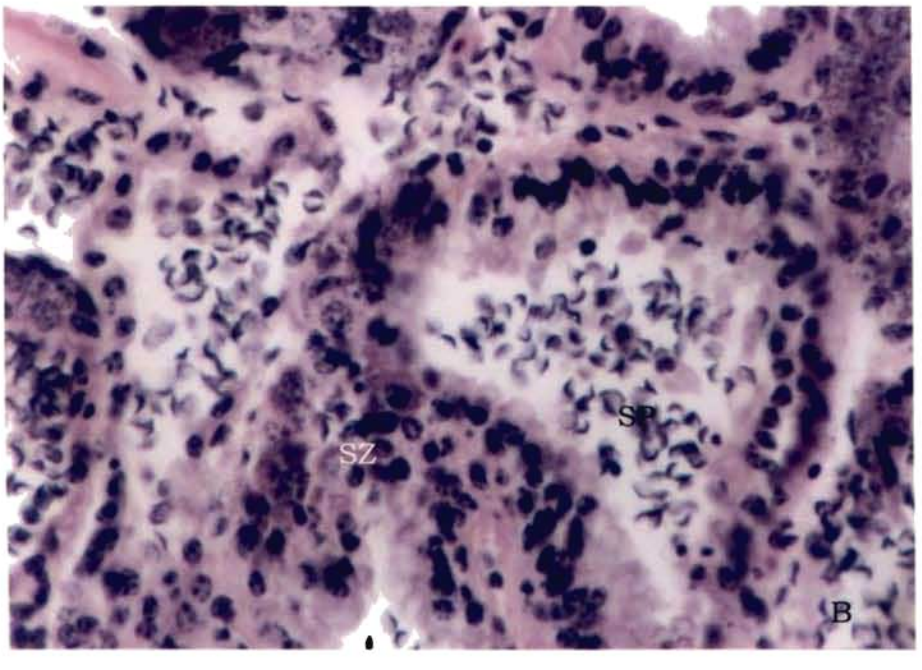


Fig 5.2 : Light photomicrography of a cross section through one testicular lobe removed from a Weak Orange Clawed male. A. General cross section (x40), B. A typical testicular cylinder (x200), sz-spermatogenic Zone, sp-mature spermatozoa

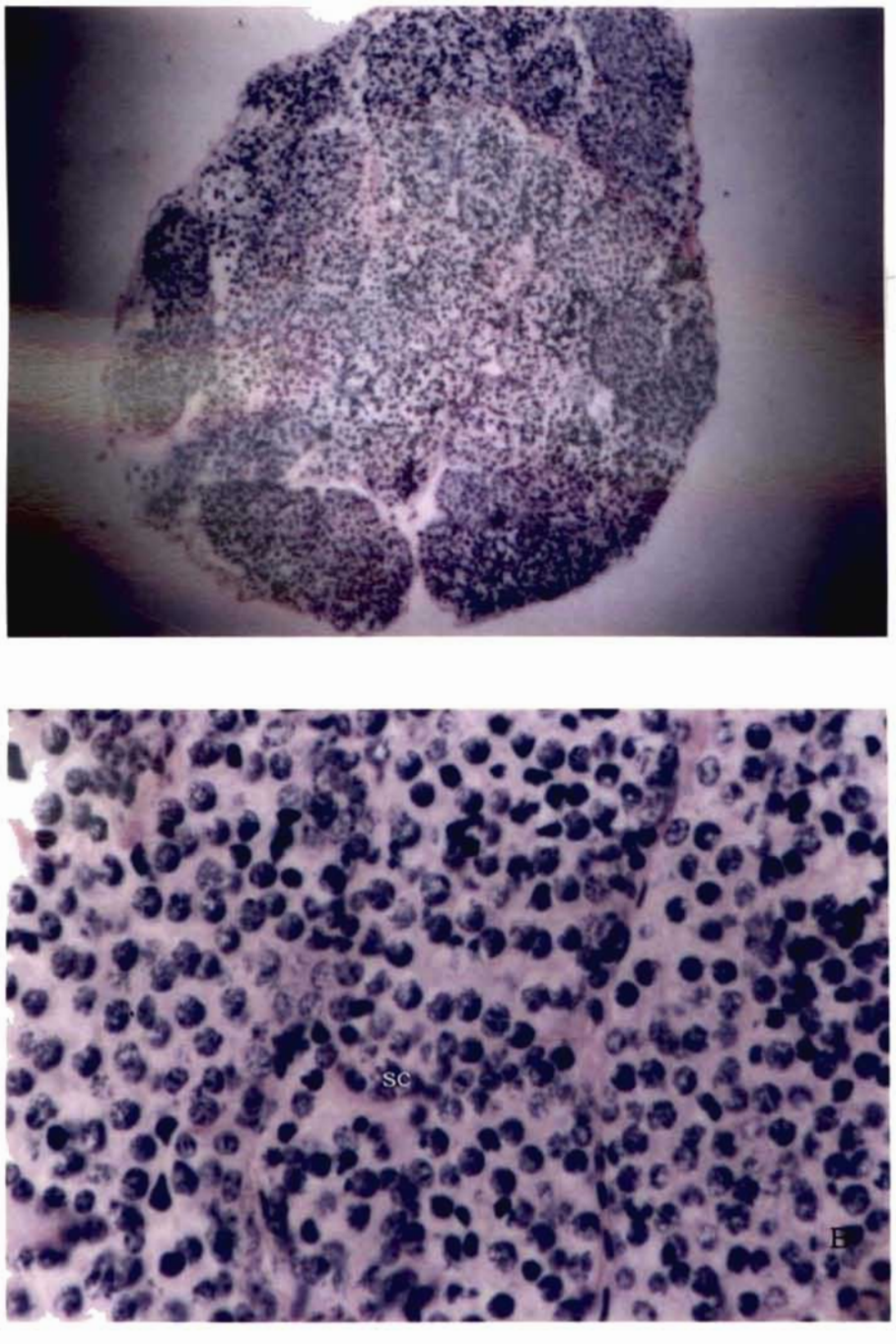
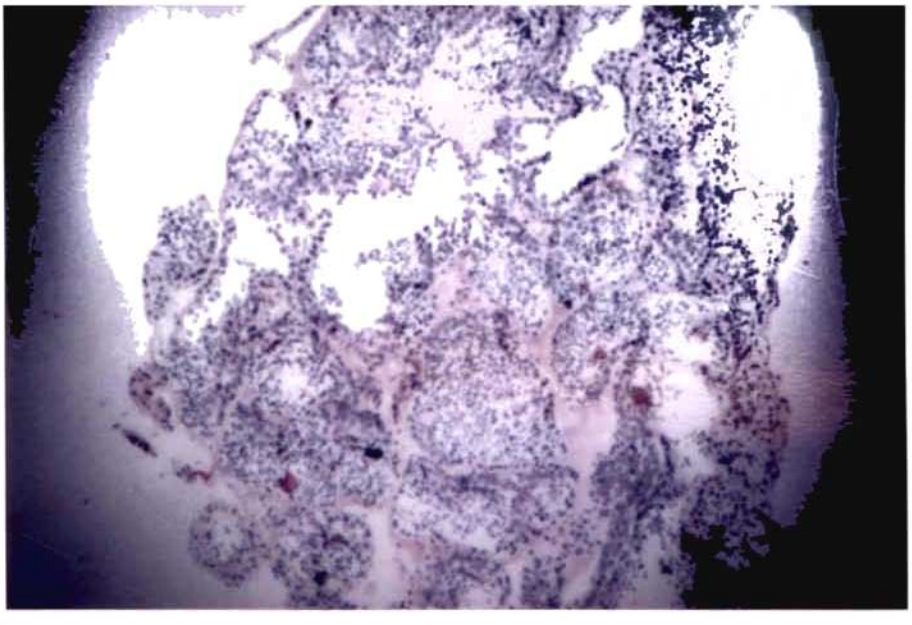


Fig 5.3 : Light photomicrography of a cross section through one testicular lobe removed from a Strong Orange Clawed male. (A) General cross section (x40), (B) A typical testicular cylinder (x200), sc-spermatocytes



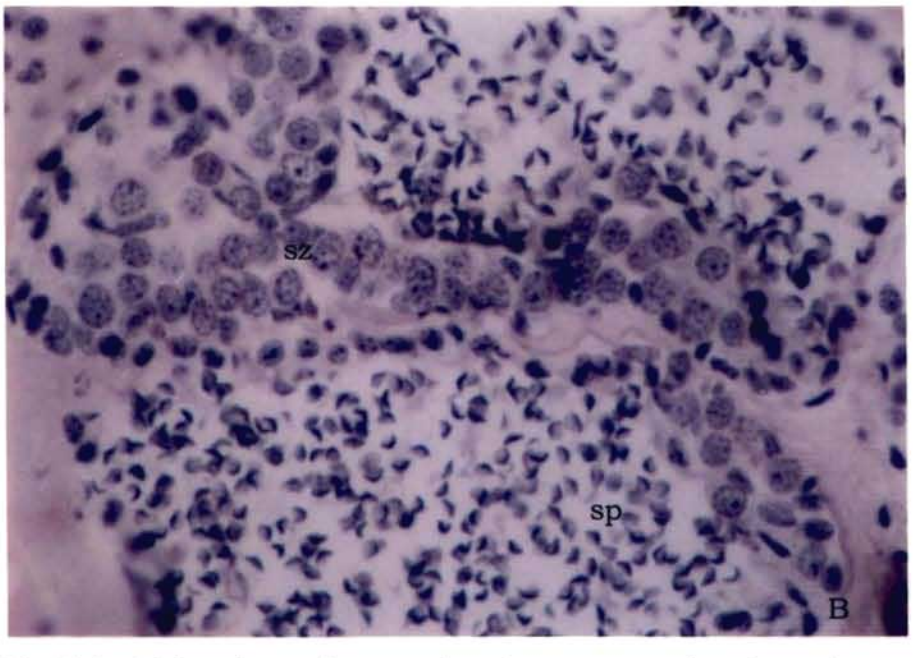


Fig 5.4 : Light photomicrography of a cross section through one testicular lobe removed from a Pre-transforming Orange Clawed Male. (A) General cross section (x40), (B) A typical testicular cylinder (x200), sz-spermatogenic zone,sp-mature spermatozoa

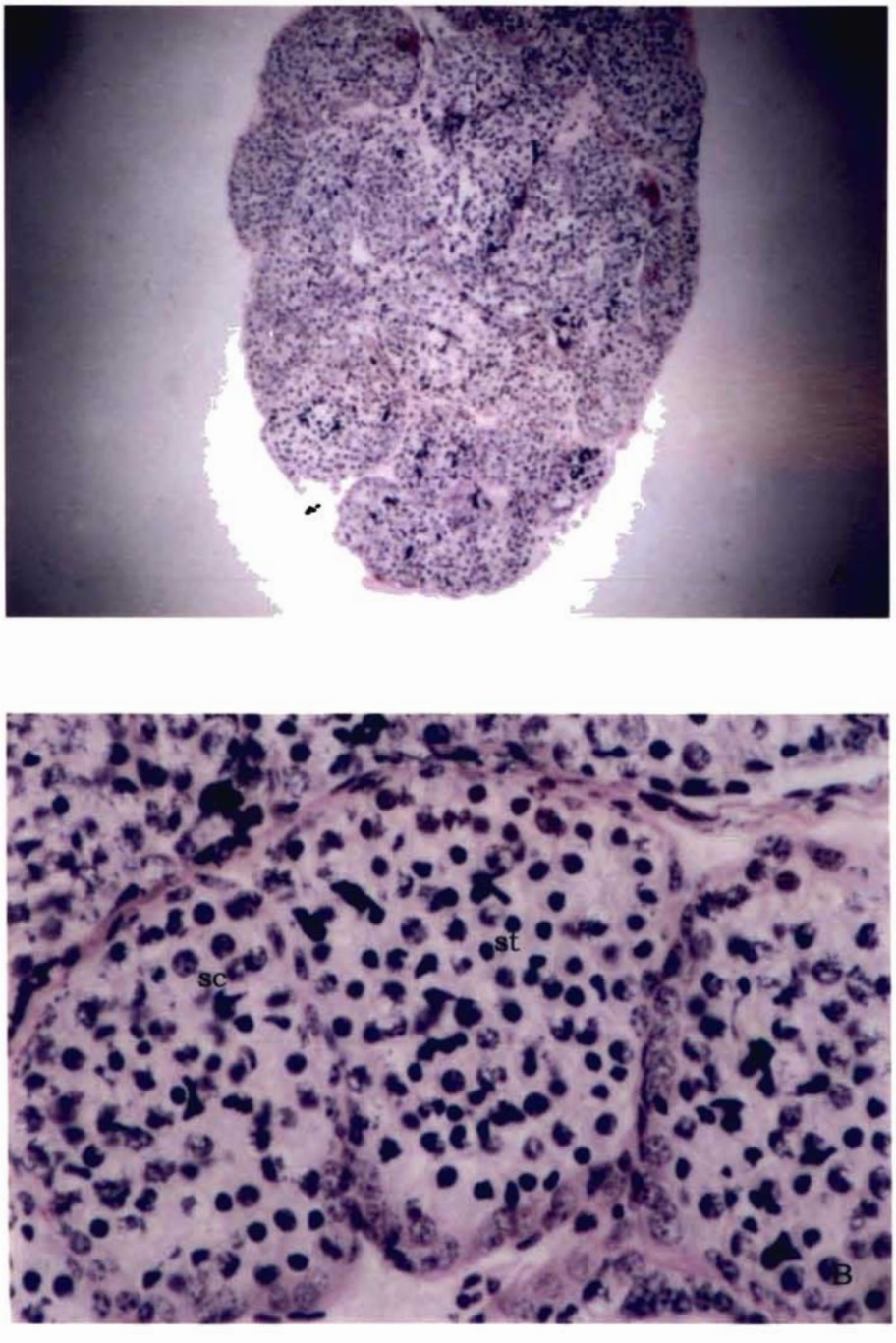


Fig 5.5 : Light photomicrography of a cross s ttection through one testicular lobe removed from a Weak Blue Clawed Male. (A.) General cross section (x40), (B) A typical testicular cylinder (x200), sc-spermatocytes, st-spermatids

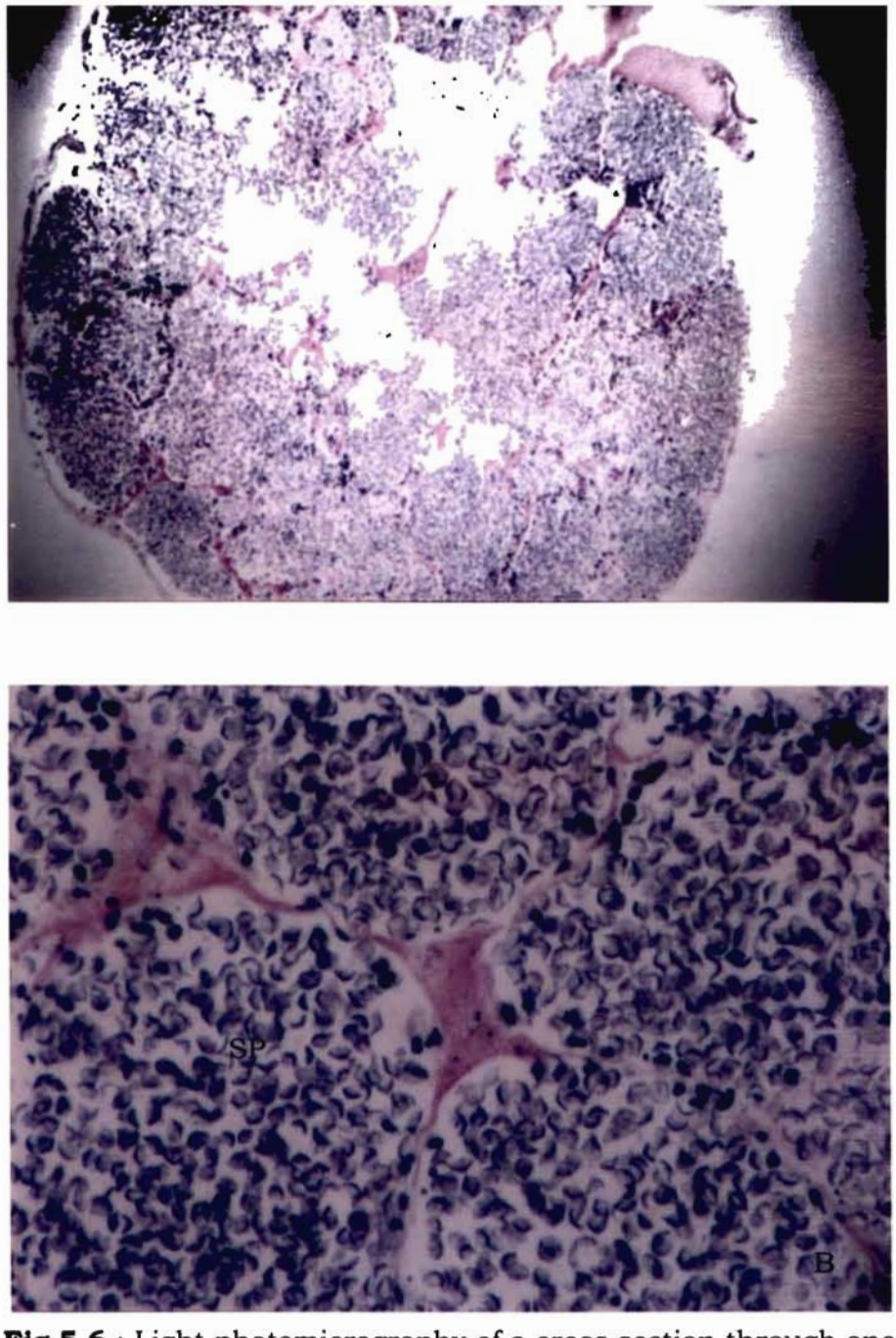


Fig 5.6 : Light photomicrography of a cross section through one testicular lobe removed from a Strong Blue Clawed Male. (A) General cross section (x50), (B) A typical testicular cylinder (x200), sp-mature spermatozoa

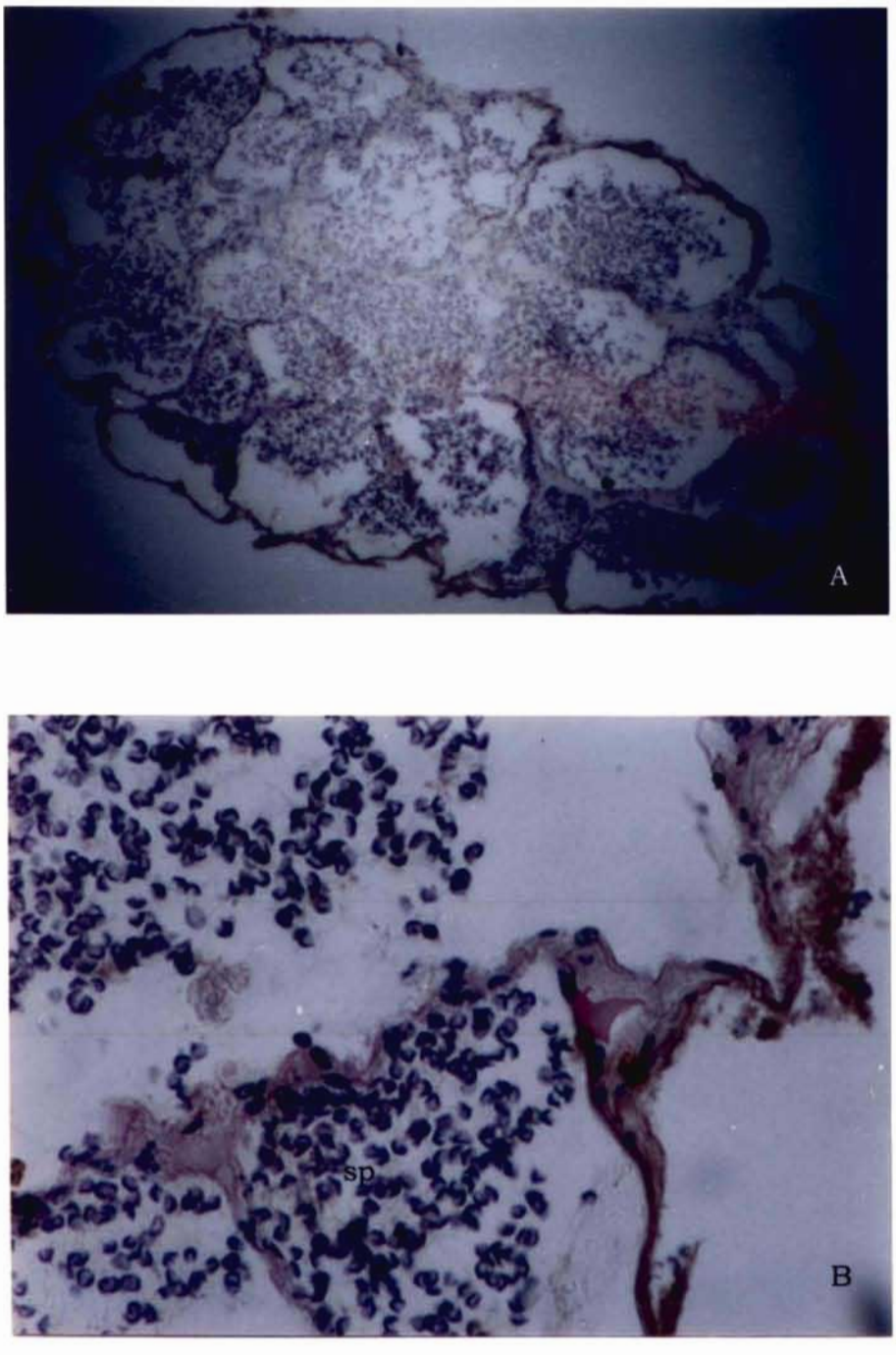


Fig 5.7 : Light photomicrography of a cross section through one testicular lobe removed from a old Blue Clawed Male. (A.) General cross section (x50), (B) A typical testicular cylinder (x200), sp-mature spermatozoa

CHAPTER 6

VAS DEFERENS

Introduction

Adult male morphotypes of *Macrobrachium rosenbergii* can be distinctly differentiated into three distinct morphotypes such as small males, strong orange clawed males and strong blue clawed males and their four transistional stages such weak orange clawed males, pre-transforming orange clawed males, weak blue clawed males and old blue clawed males. (Brody *et al.*, 1980; Harikrishnan and Kurup, 1997). The available reports suggest there is marked variation in somatic growth as well as reproductive activity among these morphotypes. (Ra'anan and Sagi, 1985; Sureshkumar and Kurup, 1998). Further, histological study of the testes of these morphotypes revealed that in SBC and OBC, the penultimate and terminal stages respectively contain only mature spermatozoan while the SM, WOC and t-Soc, the intermediary stages, are characterized by the presence of a spermatogenic zone as well as mature sperms in their testicular lobules (Sagi *et al.*, 1988; Joseph and Kurup, 2000, communicated; Joseph and Kurup, 2001). The SOC and WBC, the intermediary morphotypes are characterized by the absence of spermatozoans in their testicular lobules, however, they showed reproductive capability at a low rate (Telecky, 1984; Ra'anan and Sagi, 1985; Sagi and Ra'anan, 1988; Sagi *et al.*, 1988; Suresh kumar and Kurup, 1998). Against this background, an attempt is made to examine whether there is any difference in the histological structure of vas deferens of male morphotypes and their transistional stages

and also to characterize them on the basis of variations if any, in consonance with the difference established in reproductive activity of male morphotypes of *Machrobrachium rosenbergii.*

Studies on the vas deferens of various species of decapod crustaceans are of those of Matthews (1951, 1954a) in lobsters; Spalding (1942), Hinsch and Walker (1974) and Mathad and Adiyodi (1979) in crabs; King (1948), Subramaniam (1965), Malek and Bawab (1974) and Ro *et al.* (1990) in penaeids and Chow *et al.* (1982), Jayachandran and Joseph (1986) and Butcher and Fielder (1994) in palaemonids. However, no information is available on the histological structure of vas deferens of various male morphotypes and their transitional stages of *M.rosenbergii.*

Materials and methods

Details regarding the collection, transportation and segregation of samples and histological techniques used are described in chapter 4.

Results

The vas deferentia of three male morphotypes and their four transitional stages studied are white in colour and each originates from the mid lateral region of testes posterio-ventrally to the gonopore. Four separate regions could be distinguished within each vas deferens ie. a short and narrow proximal region (Pvd), a thicker strongly convoluted region (Mvd), an elongate distal region (Dvd) and an enlarged terminal ampoule (seminal vesicle, SV) that terminates at the

gonopore in the coxa of the fifth pereiopod (Fig 6). The terminal ampoule or seminal vesicle is the most dilated part of the vas deferens. It has characteristic pear shaped body which can be subdivided into a conical apex, a large trunk and a short cylindrical base. Histological details of the vas deferens of various male morphotypes and their transitional stages of *M. rosenbergii* are illustrated in Figs 6.1 to 6.7-A, B, C. Histological study of the vas deferens reveals no marked variation in the structural details of different regions of vas deferens in these morphotypes. Hence, a description about the vas deferens, in general is given in this chapter.

The vas deferens is dorsoventrally flattened so that in transverse sections it has more or less an oval contour. The lumen of the vas deferens increases in diameter along its length. The wall of the proximal region of the vas deferens consists almost entirely of cuboidal epithelium surrounded by a thin basal membrane. The lumen contains loosely packed mature spermatozoa. A small aggregation of columnar epithelium could be observed at one side of the proximal duct (Figs 6.1 to 6.7-A. anterior/proximal). The wall of the convoluted region of the vas deference is composed almost entirely of cuboidal epithelium except for a localised aggregation of higher columnar epithelium, both of which overlay a basal membrane. The lumen contains a more concentrated sperm mass and a small amount of basophilic matter adjacent to the high columnar epithelium (Figs 6.1 to 6.7 - B). The elongated distal region of the vas deferens has a thickened wall with a layer of muscle fibres overlaying the basal membrane. The lumen is primarily consists of

columnar epithelium with an increasing concentration of high columnar epithelium on one side. The sperm mass within the distal region is concentrated to a smaller volume than can be observed within the other regions of vas deferens (Figs 6.1 to 6.7 – C). A major concentration of basophilic matter lies adjacent to the aggregation of high columnar epithelium and projects into the lumen. The remaining part of the lumen adjacent to the high columnar epithelium is filled with a secondary eosinophilic element. In the terminal portion of the vas deferens, longitudinal muscle fibres are seen interspersed between the outer circular muscles and the epithelial cells, so that in this portion, the wall appeared more thick and muscular. The sperm mass appear to be tightly packed and concentrated into a small region within the lumen. It is surrounded by a basophilic matrix as well as an eosinophilic matrix adjacent to the epithelium. The sperm cord is stored in the enlarged terminal region until it is ejaculated simultaneously with the product of the other vas deferens which jointly form the spermatophore.

The epithelial layer is not of uniform structure and thickness for the entire length of the vas deferens. Commencing from the second limb of vas deferens the epithelial layer at the narrow side of the vas deferens is two to three cells thick, and these cells project into the lumen of the duct over a fold of the wall forming a structure like 'typhlosole' as in the case of penaeids. The cells in this region carry cilia at their distal ends which project into the lumen of the duct. These cells contain cytoplasm which is highly vacuolated. The nuclei are centrally placed, large and prominent with a spherical or oval shape. They have

each nucleolus which is also central in position. Nuclear bits can be seen attached to the inner side of the nuclear membrane, in addition to the chromatin granules present in the nucleus. The 'typhlosole' extends into the third limb also and on reaching the posterior one-third of the vas deferens merges imperceptibly into the general epithelium. On the side opposite the typhlosole, the epithelium is only one cell thick and the nuclei are also characteristically large and prominent with oval or spherical shape as in the typhlosole. They show signs of activity by the migration of nucleolar bits to the periphery of the nucleus. In the wall of the seminal vesicles, the cells of the general epithelium are low and compressed and pseudo stratified in appearance. Nuclear bits or chromatin granules as visible in the nuclei of the tall cells, are hardly visible in the nuclei of these cells. Thus, it can reasonably be inferred that at least in the 'typhlosole' like portion and part of the epithelium in the middle region of the vas deferens, it can be of secretory in function.

Within the lumen, the sperms appear to be loaded in a trellis formed of secretory matter which takes a deep blue colour stain under Mayer's Haemalum. The trellis is, however, situated away from the 'typhlosole', throughout the length of the vas deferens. Near to the 'typhlosole' there is a coagulum, which probably also is a secretion. The secretion, however, takes more stain and appearing pinkish under Mayer's Haemalum and is totally devoid of any sperm. Thus, it would appear that the two secretions, one which forms the trellis and the other which forms the coagulum, may be of different origin, because of their difference in staining activity.

Discussion

In decapods, the sperm ducts or vas deferens can be divided into several regions usually on the basis of structural and functional differences. Three regions were recognized in *penaeus indicus* (Subramanyam, 1965), *Nephrops norvegicus* (Farmer, 1974), tanner crabs (Sapelkin and Fedoseev, 1981), four regions in several brachiuran species (Spalding, 1942; Cronin, 1947; Ryan, 1967; Hinsch and Walker, 1974), *Penaeus setiferus* (King 1948; Ro *et al.*, 1990), *Penaeus kerathurus* (Malek and Bawab, 1974a), *M. rosenbergii* (Chow *et al.*, 1982), *Macrobrachium idella* (Jayachandran and Joseph, 1986), *Macrobrachium australienses* (Butcher and Fielder, 1994), five regions in *Hamarus americanus* (kooda-cisco and Talbot, 1986), three regions externally and seven regions internally in the hermit crab *Pagurus novaezealandiae* (Greenwood, 1972) and also as far as nine regions in *Diogenes pugilator* (Mouchet, 1931). The vas deferens of various male morphotypes and their transitional stages of *M.rosenbergii* contains four distinct regions viz. proximal vas deferens, medial vas deferens, distal vas deferens and seminal vesicle and this is comparable with that of *P. setiferus* (King, 1948; Ro *et al.*, 1990).

Well organized collateral glands responsible for secreting seminal plasma are not found in the vas deferens and the secretory function appears to be completely taken over by gland cells that border the lumen of the vas deferens, as in the male genital duct of *Paratelphusa hydrodromous* (Mathad and Adiyodi, 1979), *P. novaezealandiae* (Greenwood, 1972), *Libinia emarginata* (Hinsch and walker, 1974) and *P. Kerathurus* (Maleak and Bawab, 1974). Non

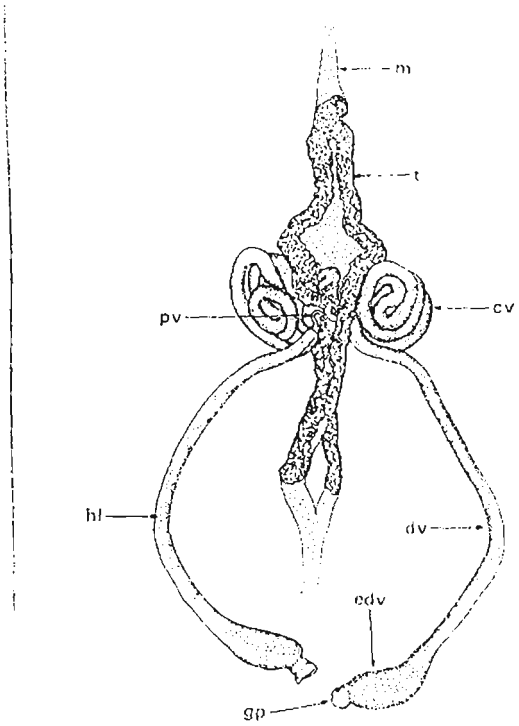
uniformity in the distribution of ductal gland cells along the entire luminal surface of the vas deferens in *Macrobrachium spp.* has already been reported by Anilkumar and Adiyodi (1984) and the present observation shows strong agreement with that of the above authors. Chow *et al.* (1982) are of the view that the eosinophilic element is secreted by the columnar epithelium and acts as an adhesive agent for the spermatophore, while the basophilic element is secreted by the high columnar epithelium and acts as protective matrix around the spermatophore. On the other hand, Dougherty and Sandifer (1984) identified the eosinophilic element as a non cellular protective capsule surrounding the extruded spermatophore and the basophilic element as a medial mucus mass in the extruded spermatophore of *M. rosenbergii.*

The thick band of circular and longitudinal muscle surrounding the enlarged terminal region of the vas deferens of male morphotypes of *M. rosenbergii* is probably utilised during ejaculation. This is in agreement with the reports of Chow *et al.* (1982) and Butcher and Fielder (1985) in *M. rosenbergii* and *M. australiensis* respectively.

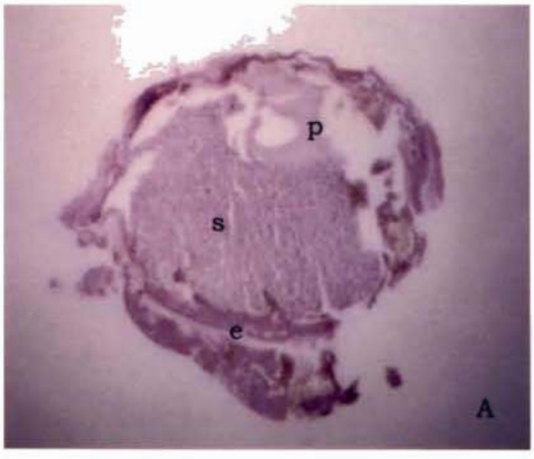
Sexual maturity is determined by the presence of mature spermatozoan in the vas deferens. Burkenroad (1947) reported that the spermatophore of *Palaemonetes vulgaris* was formed from a continuous column of sperm mass and associated matrices in the vas deferens. The funtioning of vas deferens of the three male morphotypes, SM, SOC and SBC and the four transitional stages, WOC, t-SOC, WBC, and OBC of *M. rosenbergii* are comparable to that of *P. setiferus* (Ro *et al.*, 1990) and *Macrobrachium australiense*

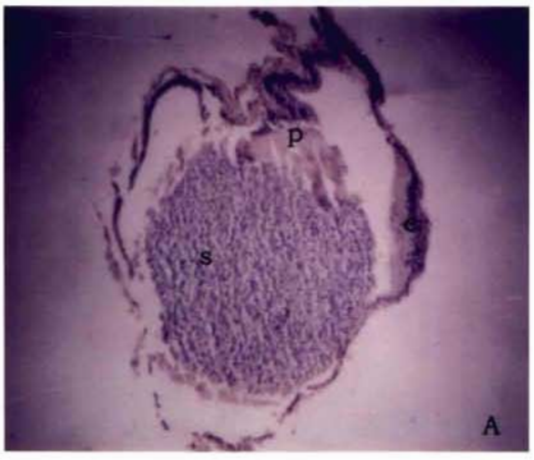
(Butcher and Fielder, 1994) where in, it concentrates and conducts the sperm mass, adds supporting matrix and stores the sperm cord prior to ejaculation.

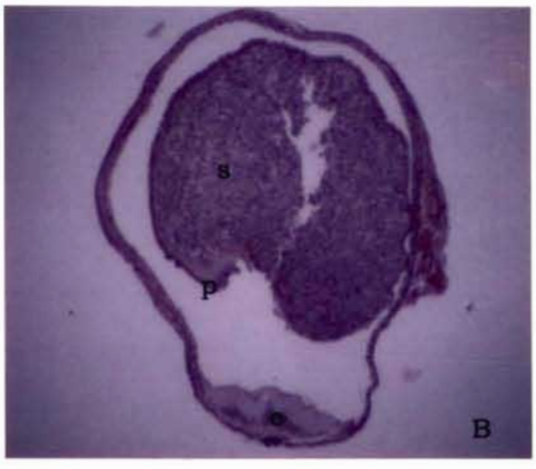
Histological study of the testes of SOC and WBC male morphotypes revealed that, these transitional stages are devoid of spermatozoans in their teaticular lobules (Sagi et al., 1988; Joseph and Kurup, 2001). At the same time, presence of spermatozoa could be observed in the four regions of the vas deferens of these morphotypes as that of SM, WOC, t-SOC, SBC and OBC male morphotypes, which are reproductively active. Telecky (1984) and Sureshkumar and Kurup (1998) have conducted some breeding experiments and also found that SOC male morphotype has a low reproductive activity during breeding processes. Therefore, it can be concluded that, the strong orange clawed and weak blue clawed male morphotypes, irrespective of differences in their gonadal activity, could perform low rate of reproductive activity during breeding processes, like other sexually active male morphotypes. This is because of the retention of spermatozoa in the seminiferous tubules (ie. vas deferens and seminal vesicle), produced by the WOC and t-SOC morphotypes and might be used by the SOC and WBC morphotypes, for oviposition of females (refer Chapter 5).

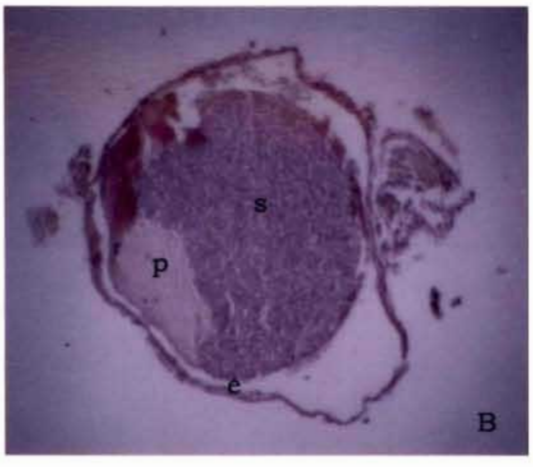


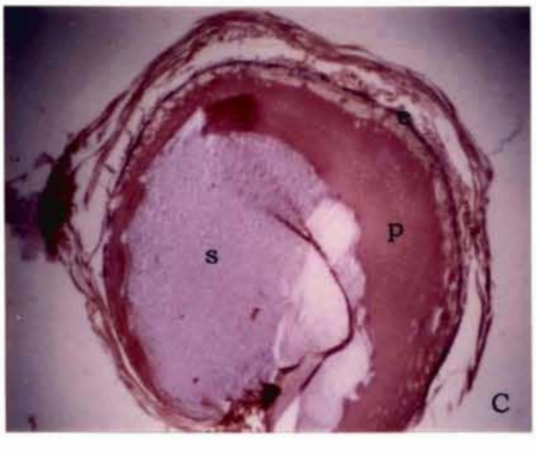
Schematic diagram of the Male reproductive system of *Macrobrachium rosenbergii* (de Man)











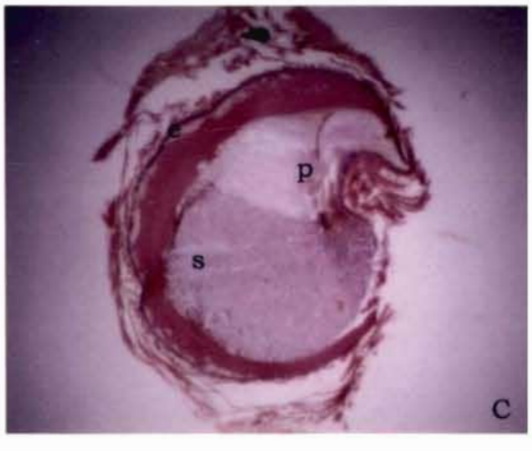
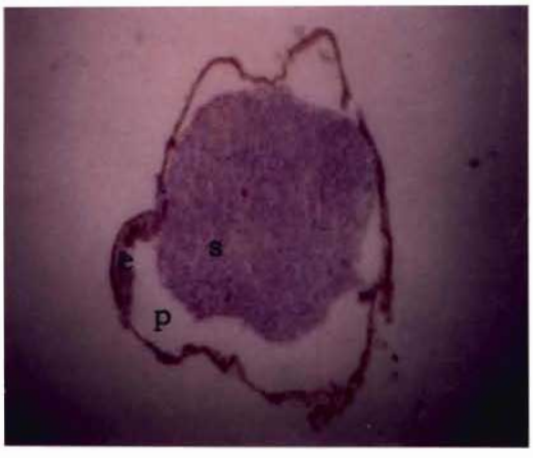
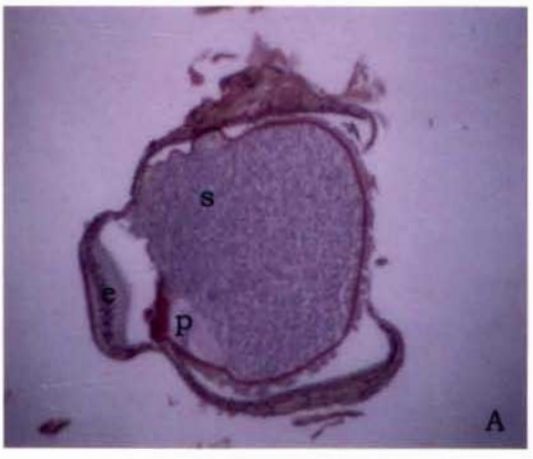
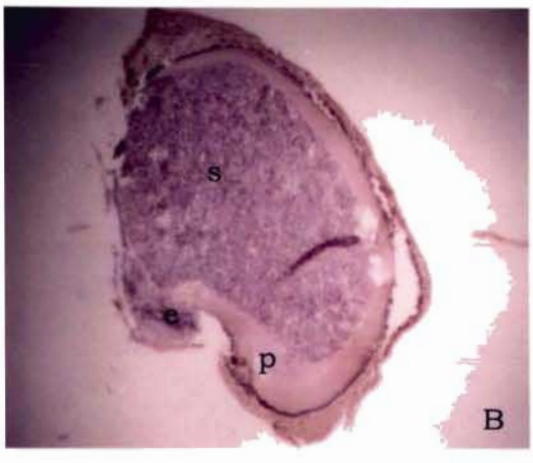


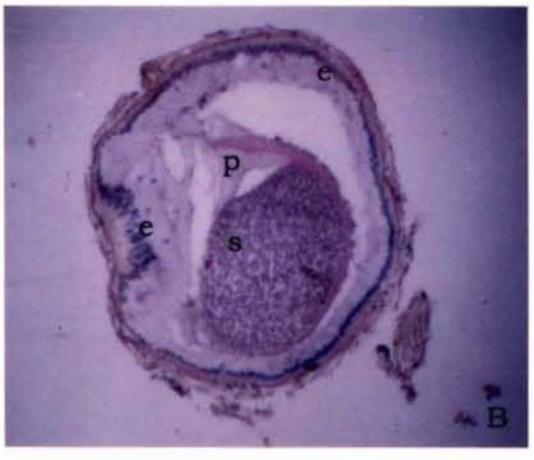
Fig 6.1 : Transverse sections of the vas deferens of SM (A) anterior vas deferens B) mid vas deferens (C) distal vas deferens p-seminal plasma, s-sperm mass, e-secretory epithelium

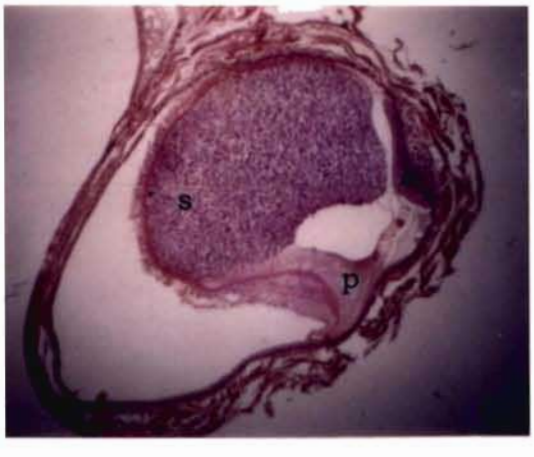
Fig 6.2 : Transverse sections of the vas deferens of WOC (A) anterior vas deferens B) mid vas deferens (C) distal vas deferens p-seminal plasma, s-sperm mass, e-secretory epithelium











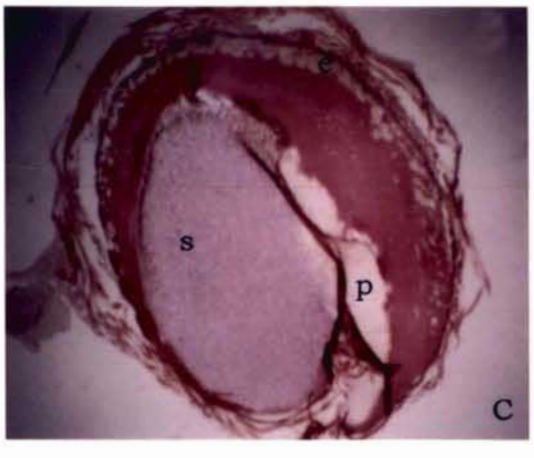
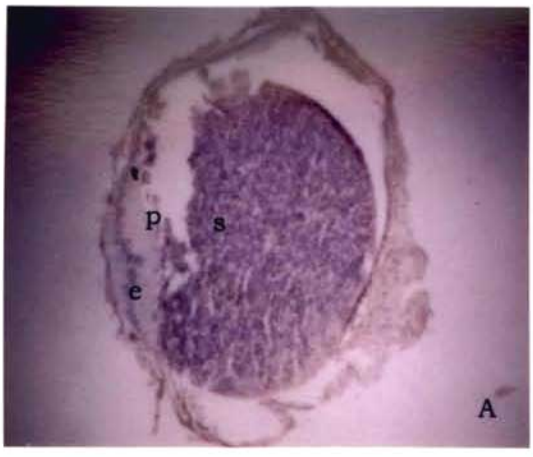
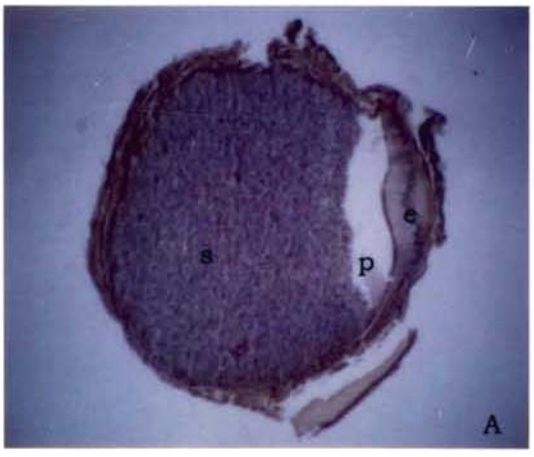
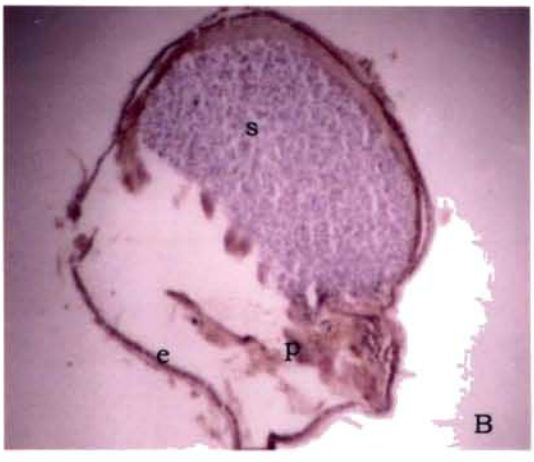


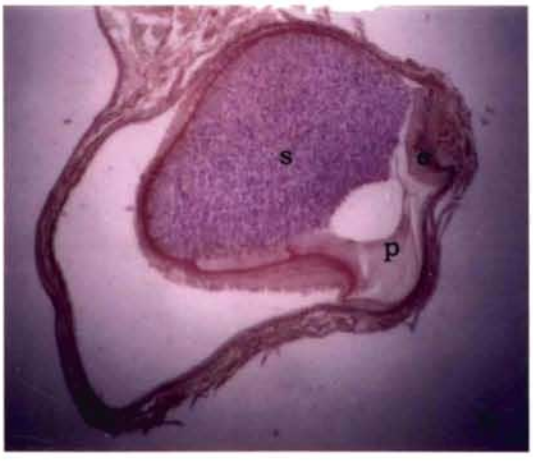
Fig 6.3 : Transverse sections of the vas deferens of SOC (A) anterior vas deferens (B) mid vas deferens (C) distal vas deferens p-seminal plasma, s-sperm mass, e-secretory epithelium

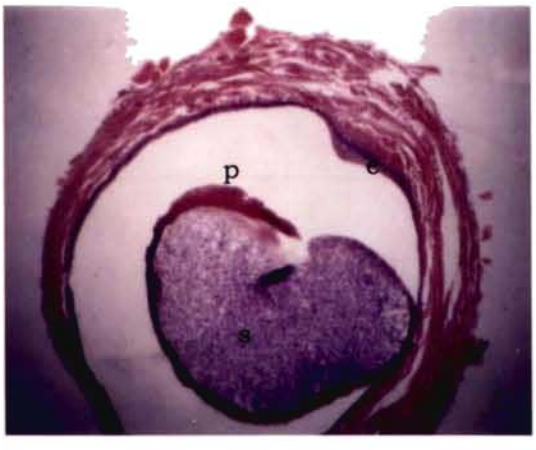
Fig 6.4 : Transverse sections of the vas deferens of t-SOC (A) anterior vas deferens (B) mid vas deferens (C) distal vas deferens p-seminal plasma, s-sperm mass, e-secretory epithelium











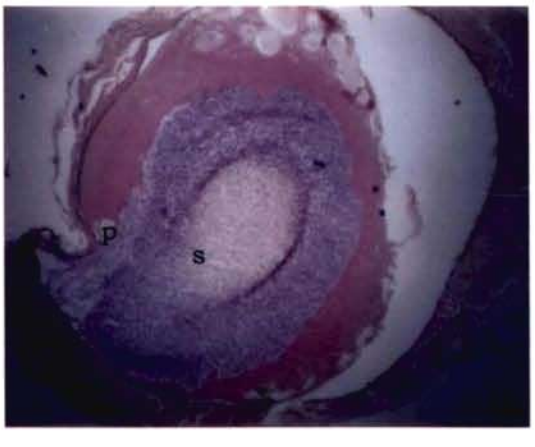


Fig 6.5 : Transverse sections of the vas deferens of WBC (A) anterior vas deferens (B) mid vas deferens (C) distal vas deferens p-seminal plasma, s-sperm mass, e-secretory epithelium

Fig 6.6 : Transverse sections of the vas deferens of SBC (A) anterior vas deferens (B) mid vas deferens (C) distal vas deferens p-seminal plasma, s-sperm mass, e-secretory epithelium

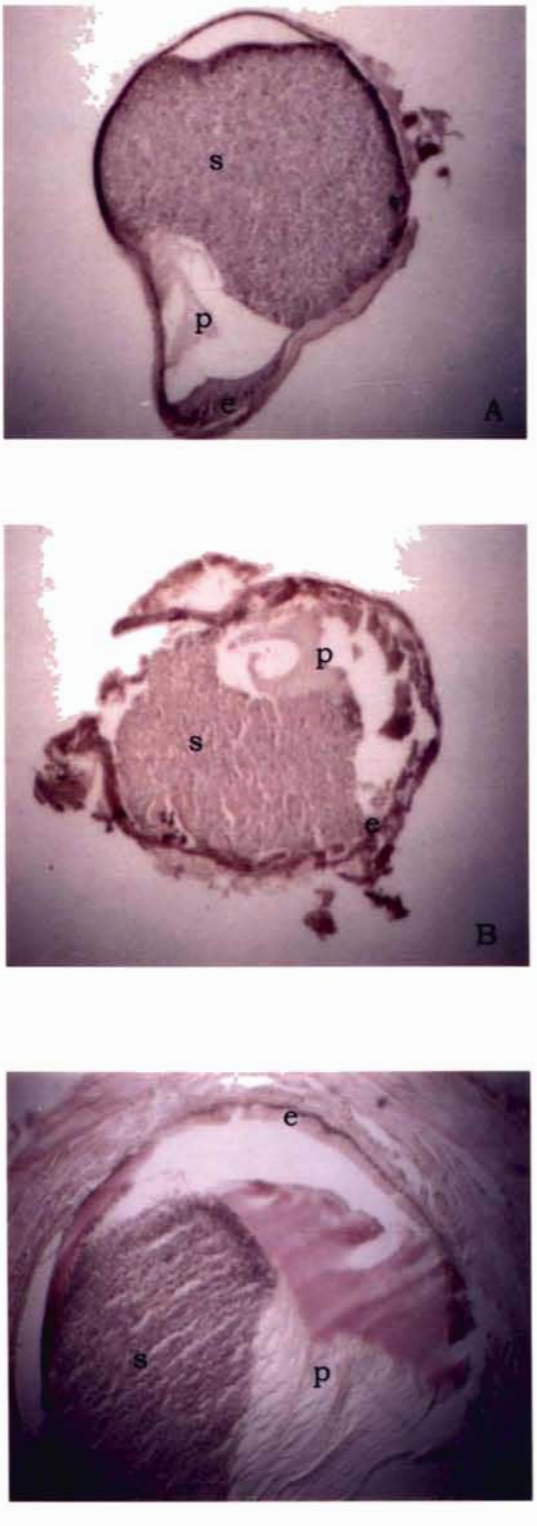


Fig 6.7 : Transverse sections of the vas deferens of OBC (A) anterior vas deferens (B) mid vas deferens (C) distal vas deferens p-seminal plasma, s-sperm mass, e-secretory epithelium

CHAPTER 7

ANDROGENIC GLAND

Introduction

The androgenic glands, the epithelial endocrine glands of crustacean, are known to control the functioning of the male sexual characteristics [Taketomi *et al.* 1996]. This gland [AG] was first described in the crab *Callinectes sapidus* [Cronin, 1947], however, its role was first established by Charniaux- Cotton [1954] in amphipod *Orchestia.* A detailed review of the androgenic gland in malacostracans [apart from syncarids] was first published by Charniaux–Cotton *et al.* [1962], whereas Zerbib [1967] subsequently made description of this gland in syncarids. This gland in amphipods and isopods is known to be control differentiation of the male reproductive system [Balesdent-Marquet, 1958; Katakura, 1960, 1961, 1984; Charniaux-Cotton, 1962; Juchault and Legrand, 1964]. The anatomy, histology and development of the androgenic gland was observed in *Penaeus chinensis* by Li, Xia and Li, Jiayong (1993). Fowler and Leonard (1999) first described the structure and function of the androgenic gland in the freshwater crayfish *Cherax destructor.* Studies on the development of the testes and androgenic gland commensurate with the development of the external sexual characteristic of the Cray fish, *Procambarus clarkii* [Girard] was carried out by Yokotaketom *et al.* [1996]. The role of the androgenic gland in the fresh water prawn *Macrobrachium rosenbergii* was demonstrated by resorting to its removal [Nagamine *et al.,* 1980 a, b]. Maintenance and functioning of the androgenic gland is controlled by

eyestalks, brain and the thoracic ganglion [Payen *et al.*, 1971]. This gland plays an important role in androgenesis and expressing external male characteristics [Touir, 1977; Juchault and Legrand, 1978]. Several experiments have been conducted to demonstrate the endocrine role of the androgenic gland in crustacea while studies on the histological structure of androgenic gland of *Macrobrachium spp. was* found to be limited to that of Thampy &John [1972]; Sreekumar *et al.* [1982]; Mirjakar *et al.* [1984]; Awari &Kiran [1999] and Sun *et al.* [2000]; Joseph and Kurup [2001]

In Macrobrachium *rosenbergii*, the differential growth in male is remarkably higher than that of the female [Fujimura and Okamota, 1972; Smith *et al.*, 1978; Sagi *et al.*, 1986] and this is due to the existence of different male morphotypes within the single aged population [Ra`anan and Cohen, 1985; Kuris *et al.*, 1987; Harikrishnan and Kurup, 1996]. These male morphotypes are characterized by distinct territorial behavior, complex courtship, mating and male guarding behavior [Peeble, 1979; Rao, 1965] varied reproductive capabilities [Ra`anan and Sagi, 1985; Suresh kumar and Kurup, 1998] and growth rates [Ra`anan and Cohen, 1985; Kurup and Harikrishnan, 1996]. These male morphotypes also differ from each other morphologically, anatomically and physiologically and the hierarchy among these morphotypes are closely associated with social roles and reproductive activities [Ra`anan and Sagi, 1985; Kuris *et al.*, 1987; Sagi and Ra`anan, 1988; Sagi *et al.*, 1988 b; Sureshkumar and Kurup, 1998; Joseph and Kurup, 2000 (in press)]. In the present study, an attempt is made to bring out the structural

difference, if any, in the androgenic gland of various male morphotypes of M. *rosenbergii* such as small male [SM], strong orange clawed male [SOC] and strong blue clawed male [SBC] and their transitional stages such as weak orange clawed male [WOC], pre-transforming orange clawed male [t-SOC], weak blue clawed male [WBC] and old blue clawed male [OBC]. An attempt is also made to critically examine the structural variations of androgenic gland of male morphotypes in the light of the difference already reported in respect of reproductive capability and growth associated with these morphotypes.

Materials and methods

Live specimens of male morphotypes of M. *rosenbergii* viz: SM, SOC and SBC and their transitional stages such as WOC, t-SOC, WBC and OBC belonging to single age group were collected from a growout located adjacent to Vembanad Lake, Kerala, South India during 1999-2000. They were identified into seven morphotypes following Kuris *et al.* [1987], Sagi and Ra`anan [1988], Ang *et al.* [1990] and Harikrishnan and Kurup [1997 a]. Androgenic gland samples from five specimens displaying unequivocal morphotypic characteristics as mentioned above were selected for histological study. The androgenic gland cell types are categorized followed by the classification of Thampy and John [1974]. Prawns were dissected out and the androgenic gland along with the seminal vesicles were removed and fixed in alcoholic Bouin`s solution overnight. Following fixation the specimens were dehydrated through an ethyl alcohol series, cleared in methyl benzoate and benzene [Pantin, 1946] and

embedded in paraffin wax [M.P 58°-62°C]. Serial sections of 7μ thickness were cut and mounted on glass slides and sections were stained with Mayer`s Haemalum stain [Humason, 1972]. The slides were examined by light microscopy and photographed using a binocular microscope and Nippon camera combination at high power.

Results

Location and Morphology

As in other malacostracans the androgenic gland of male morphotypes of *M.rosenbergii* appeared as a very thin elongated plate like structure, whose proximal end is broad and firmly attached to the wall of the vas deferens. At the distal part there is a lateral projection in the gland, which is long characterized by its pointed tip inserted among the coxal muscles. Structurally it is compact with several longitudinal strands ensheathed by a membrane, with non–uniform number while disposition of the strands are not uniform through out the length of the gland, the number being maximum towards the distal end.

Cytological characteristic of androgenic gland

Each of the strand of androgenic gland is formed of a basement membrane and an inner layer of glandular epithelium. The glandular epithelium is one cell thick in the proximal tip; in contrast distally it is many cells thick. Though the boundaries of the individual cords are distinct, the boundaries of cells forming the individual cords are not clearly visible. The cells are polygonal in shape and possess in active states, a centrally placed nucleus which

are of two types – small and large. The small size of 3.33 μ in diameter is having a spherical shape. These nuclei have a clear nuclear membrane and the chromatin is dense and granular. The nucleoplasm appears to be denser and absorbs more stain. The other types of nuclei are larger in size measuring 6.7 μ along the long axis. These nuclei have an irregular in appearance and their nuclear membrane is hazy. The nucleoplasm is less dense and absorbs less stain. In some cases a nucleolus is present towards the center. In addition to these two types of nuclei, which can be identified, based on their morphological features, a third type of nuclei which are pycnotic showing no signs of chromatin. They are of polygonal or spindle shaped and stains brightly under Haematoxylin eosin.

The cytoplasm of the individual cells also show variations. In certain cells the cytoplasm is dense and homogenous and uniformly present around the nuclei, usually lightly basophilic in reaction, the presence of small, spherical type of nuclei are characteristics of such cells. In some other cells, the cytoplasm is reduced to fragments and prone to intense vacuolization. Where in few others, the cytoplasm is completely disintegrated, leaving only traces of the outer wall of the nucleus.

Based on the cytological characteristics, five types of glandular tissue were identified such as type I, II, III, IV, and V. Type 1 cells are apparently appeared as young tissue wherein the cells are small, filled with dense, homogeneous, deeply staining cytoplasm. In this the cell boundaries are more or less clear. The nuclei are small in size, centrally placed so also the nucleolus. Type II cells are

comparatively older tissues in which the cells are large characterized by low staining and vesicular cytoplasm. The cytoplasm is also less dense when compared to young tissue. The nuclei are large in size, spherical or oval in shape and contain each a centrally placed nucleolus. Type III cells appear as old and degenerating tissue where the boundaries of individual cells are found totally lacking and even individual cords are hardly distinguishable. The cytoplasm was disintegrated and only remnants are left within the cells showing no affinity towards usual stains. The nuclei are reduced in size and many of them have shriveled up and lost their characteristic spherical shape. In type IV cells, only strands of protoplasmic remnants are present. Nuclei show signs of degeneration. In type V cells there is no cellular demarcation. The entire cells mass looks aberrant.

A well defined localization of a particular type of cells to a specific region of the gland could be observed which appeared as specific to various morphotypes and their transitional stages of *M. rosenbergii*.

Morphotypic variation in the histological structure of androgenic gland

***SM*:** - The androgenic gland of small male morphotype comprised of type I cells. The cells of the glandular epithelium are almost spherical in shape and measuring on an average of 10 μ in diameter. The cells are filled with dense, homogenous, deeply staining cytoplasm in which the cell boundaries are more or less clearly discernable. The nuclei are small, spherical in shape, prominent and centrally placed and contained each a centrally positioned nucleolus [Figs 7.1-A,B,C].

***WOC*:** - The cytological characteristics show some regional variations in relation to the entire gland. Around the periphery and at the base of the gland, signs of degeneration could be discernable which is mainly constituted by type III cells. The cytoplasm is very sparse and absorbs very little stain. The size of the type III cells varies from 6.67 μ to 10 μ. Towards the center core of the gland, the cells are visible as smaller in size containing a dense homogenous cytoplasm without any vacuole. The active cells of androgenic gland are constituted mainly by type I cells as in the case of SM and are measuring around 10 μ [Figs 7.2-A,B,C].

***SOC*:** - In SOC morphotype, the entire androgenic gland shows clear signs of degeneration except stray cords of active cells. The gland comprises mainly of type IV and V cells. The cytoplasm is very sparse and takes up very little stain. The nuclei also show signs of degeneration. The nuclei are mostly pycnotic, staining very intensely and without any signs of the presence of nucleotic and chromatin material. Along the base facing the vasdeferens, degeneration is complete and the cells are only represented by filamentous protoplasmic strands, without any signs of nuclei [Figs 7.3- A,B,C].

***t- SOC*:** - In t-SOC, along the sides of the gland and also at the base by which it is attached to the vasdeferens, show signs of degeneration, is the type III cells which are characterized by the presence of pycnotic nuclei. Towards the center, active cells are seen mainly constituted by type II cells which measure an average size of 16.65 μ and are vacuolated and also less denser than the young tissue (Figs 7.4-A,B,C).

WBC: - In WBC, the gland consists of degenerated cells at the basal region as well as active cells at the anterior region and along the lateral elongation. The active cells mainly constituted by Type II cells and the size ranges between 13.32 to 14.49μ(Figs 7.5-A,B,C).

SBC.- In SBC, the entire gland is filled with Type II cells. The size of the cells varies from 16.65 to 23.31μ. The nuclei are centrally placed and measuring 6.67μ with a centrally placed nucleolus (Figs 7. 6-A,B,C).

OBC: - In OBC, the proximal and outer regions of the gland show stray evidences of degeneration that are characterised by the presence of empty cells and pycnotic nuclei. Towards the center stray cords of active type II cells are present with vacuolated cytoplasm and chromatin rich nuclei. The size of type II cells ranged from 10 μ to 12. 94 μ. (Figs 7.7-A,B,C).

Discussion

Cronin (1947) in an anatomical study provided the first description of androgenic gland, which in most species of Malacostracans is attached to the sperm duct. In shape, the androgenic gland of *M. rosenbergii* resembles other Natantians like *Lysmata seticaudata, Palaemon serratus* (Charniaux-Cotton, 1958), *Pandalus boreales* (Carlisle, 1959a), *Atyaephyra desmaresti* (Huget, 1968), *Pandalus platyceros* (Hoffman, 1969), *M. dayanus* (Thampy & John, 1972), *M. idae* (Thampy & John, 1973) *M.kistnensis* (Mirajker, 1984).

Charniaux -Cotton (1954) provided a pioneer impetus to this field through discovery of the role of the androgenic gland. It has been suggested that the androgenic gland is the exclusive source of hormone responsible for sex differentiation in crustaceans (Charniaux-Cotton, 1954., Charniaux-Cotton and Payen, 1985) and the AG hormone production in turn is dependant mostly on the type of cell, whether active or degenerative, that are present in the androgenic gland.

The results of the present study revealed that there exist variations in the histological structure of the androgenic gland among the male morphotypes of *M. rosenbergii*. Based on the cytological characters of cells, it could be inferred that the androgenic gland of small males is active, since it is mainly constituted by type I cell, which are characterized by the presence of active nuclei, surrounded by rich homogenous cytoplasm. Similarly, in strong blue clawed male morphotypes, the androgenic gland shows very high level of activity as evidenced by the presence of more active type II cell, which are larger in size with a vacuolated cytoplasm and a centrally placed large nucleus. A similar observation was made by Awari and Dube (1999) while studying the histology of the androgenic gland of mature male of *M.rosenbergii* and found that type II cells are responsible for the high activity of androgenic gland. Light and electron microscopic study of the androgenic gland in the crayfish, *Procambarus clarkii* by Taketomi (1986) revealed that the gland consists of two types of cells called type A and type B. Type A cells have a cell matrix of high electron density, whereas the type B cells have a low electron density

and he opined that the two types of cells actually represent different secretary phases of a single type. Developmental changes in structure of the androgenic gland of *M.rosenbergii* was studied by Sun *et al.* (2000) and found that the dominant Blue Clawed (BC) morphotypes has the highest cell density, a 'transitional' morphotype named Orange Blue Clawed (OBC) form, which is intermediary between the Orange Clawed (OC) morphotype and Blue Clawed (BC) morphotype, medium cell density and the subdominant Orange Clawed (OC) morphotypes, the least cell density. Although, the mechanism of sex differentiation in crustaceans has yet to be defined, the androgenic gland (AG) is thought to be the exclusive organ that produces the androgenic hormone (AH), which induces male sexual development (Hasegawa *et al.*, 1993). The available reports, suggest that the male morphotypes of *M.rosenbergii* viz. SM, SOC and SBC and their intermediary stages viz. WOC, t-SOC, WBC and OBC show perceptible difference in reproductive activity and somatic growth among them [Sagi and Ra'anan, 1988; Sagi *et al.*, 1988; Sureshkumar and Kurup,1998; Joseph and Kurup, 2000 (in prsess); Joseph and Kurup, 2001] SM and SBC are sexually active, participating in mating and fertilization, on the contrary, SOC are reproductively submissive and showed a lesser performance of reproductive activity (Sagi and Ra'anan,1988; Sagi *et al.*, 1988; Sureshkumar and Kurup,1998;). Taketome *et al.* (1996) observed the histological changes of the androgenic gland of the Cray fish, *`Procambarus Clarkii* and the testes during development and found that the activity of the androgenic gland is different in each developmental stage. In the earlier stages, the gland had an extremely

low level of activity, because it was composed of only a few cells that showed little activity, in which the nuclei had condensed chromatin and there was only a small quantity of cytoplasm. The androgenic gland in mature males, with stage E, was endowed with the presence of large lump of tissue containing numerous cells and are characterized by a large nucleus, with chromatin and large quantity of cytoplasm. Studies conducted by Thampy and John (1972, 1973) revealed that there is a positive correlation between the reproductive activity and the hypertrophy of androgenic gland in *Palaemon dayanus* and *M.idea*. Histology of the androgenic gland of *M. kistensis* (Mirjakar *et al.*, 1984) showed variations in the size of the androgenic gland with reproductive cycle being maximum during the breeding season. These studies are in agreement with the present observation that the high activity of androgenic gland in the SBC morphotype as this exhibit high reproductive activity among the various morphotypes and their intermediary stages [Ra'anan and Sagi, 1985; Kuris *et al.*, 1987; Sagi and Ra'anan, 1988; Sureshkumar and Kurup, 1998; Joseph and Kurup,2000 (in press); Joseph and Kurup, 2001]

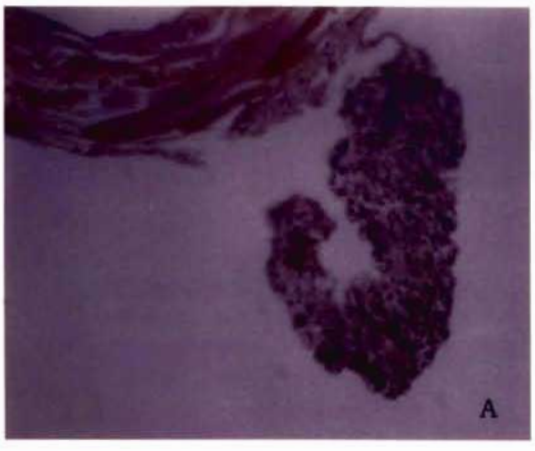
Where as in SOC morphotype extensive disintegration of the glandular tissue could be discernible. In the distal sheet like portion, there are only remnants of disintegrated protoplasm of the posterior half and the cells in this region belong to the type III, IV and V. Disintegration is found advancing forward, and the pycnotic nuclei are visible at the posterior end of the remaining gland. In a limited area at the center it is formed of type II cells as evidenced by clear boundary of individual cords and cells. Sreekumar & Adiyodi (1982) made a similar

observation in Macrobrachium species during winter season. They found that the activity of the androgenic gland of macrobrachium species is low during winter season as evidenced by the high incidence of nuclear pycnosis and cytoplasmic vacuolation of the androgenic gland cells along with the presence of areas showing late phases of degeneration. The sexual inactiveness of the SOC morphotype is apparently appears to be due to the low activity of the androgenic gland, as type III, IV and V cells are predominantly seen in the androgenic gland. Initiation of spermatogenesis in crustacean is due to circulating androgenic gland hormone (Payen, 1973). Removal of the androgenic glands from the adult generally results in the cessation of spermatogenesis (Charniaux- Cotton, 1964; Puckett, 1964; Nagamine *et al.*, 1980 b). The role of the androgenic glands in spermatogenesis is in the form of regulating its intensity. Lack of the androgenic glands results in a reduction of spermatogenesis (Berreur-Bonnenfant, 1970; Touir, 1977 a). The common occurrence of spermatogenesis and primary spermatocytes but not of later spermatogenic stages in andrectomised *M. rosenbergii* suggests that many primary spermatocytes may not complete meiosis. The tendency of spermatogenic development to be inhibited at meiosis in the absence of the androgenic glands has been noted in Cray fish and other Natantia. (Puckett, 1964; Kracht, 1975; Touir, 1977a,b; Payen and Amato, 1978). Payen and Amato (1978) hypothesized that in Crayfish, the inability of the spermatocytes to complete meiosis in the absence of the androgenic glands may be due to insufficient syntesis of RNA.

Crustacean reproductive system is under hormonal control and this is known for more than fifty years. The general stimulating hormone is present in the brain and thoracic ganglion (Otsu, 1963;Gomez, 1965; Eastman Reks and Fingerman, 1984; Kulkarni *et al.*, 1991; Yano, 1993). Gonad stimulating hormone (GSH) and gonad inhibiting hormone (GIH) act directly on the ovary (Adiyodi, 1985), where as in the male GSH and GIH act only indirectly on the testes by directly affecting the androgenic glands, thus androgenic gland hormone being directly responsible for stimulating testicular maturation (Adiyodi & Adiyodi, 1970, Ginsburger-Vogel and Charniaux-Cotton, 1982; Charniaux-Cotton & Payen, 1988). Gupta *et al.*, (1989) reported in crab, *Paratelphusa hydrodromous*, that the inactive phase of the testes is due to an increase in the haemolymph titre of GIH with concomitant decrease in the titres of GSH & AGH. It has been found that adult male Cray fishes viz. *Orconetes nais* and *O. sanborni*, undergo seasonal changes in morphology, ie. from sexually inactive form II to sexually active form I, showed a positive correlation between androgenic gland growth and attainment of form I (Carpenter and de Roos, 1970).

In WOC, t-SOC and WBC, the intermediary stages and OBC the terminal stages of male morphotypic transformation, the androgenic gland is characterised by the presence of active (Type I&IIcells) as well as degenerated areas (Type III cells). The presence of such degenerated area along with tissues which can be considered as young by virtue of the presence of active nuclei surrounded by rich homogenous cytoplasm indicate that a medium activity of androgenic

gland in these transitional stages of male morphotypes of *M. rosenbergii* (Sun *et al.*, 2000). The histological picture of the androgenic gland of these intermediary stages appears to be in agreement with their respective reproductive activity [Sagi *et al.*, 1988; Sureshkumar & Kurup, 1998; Joseph & Kurup (in press, 2000); Joseph and Kurup, 2001]. Hence, the results of the present work suggest that in male morphotypes of *M.rosenbergii* and their transitional stages, the androgenic gland exhibit distinct variations which can be correlated with the reproductive activity of these morphotypes. It may be possible that androgenic gland which controls the primary and secondary sex characters of male Crustacean has a role in the development of male morphotypes of *M.rosenbergii* and growth variation among them as growth rate of *M.rosenbergii* is closely associated with morphotypic status which is based on social roles and reproductive activities.



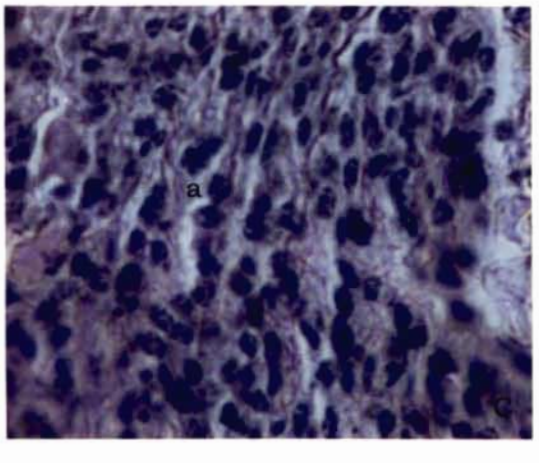
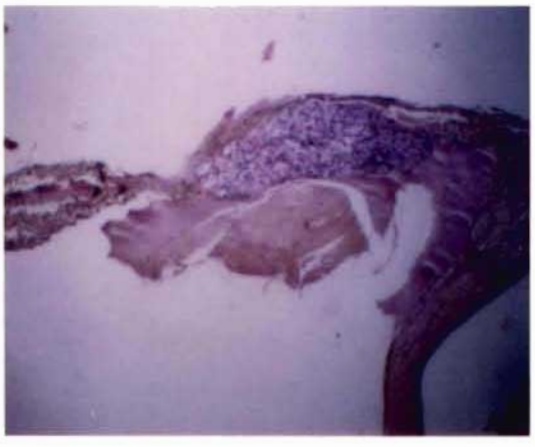
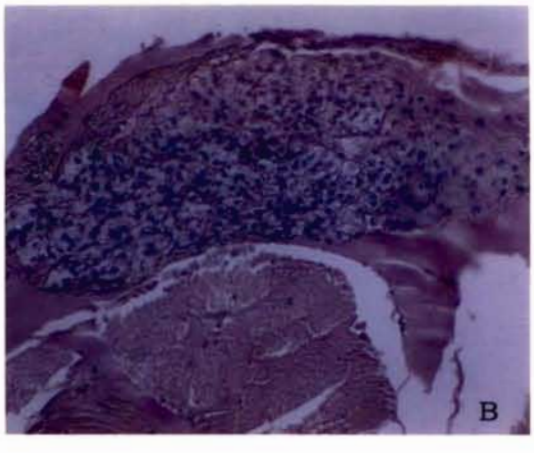


Fig 7.1 : Cross section of the androgenic gland of SM (A) General cross section (x40), (B) Enlarged portion (x100) (C) Enlarged portion (x200) a. Type I cell





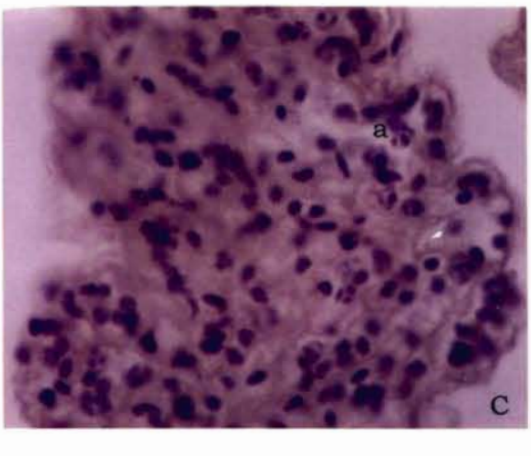
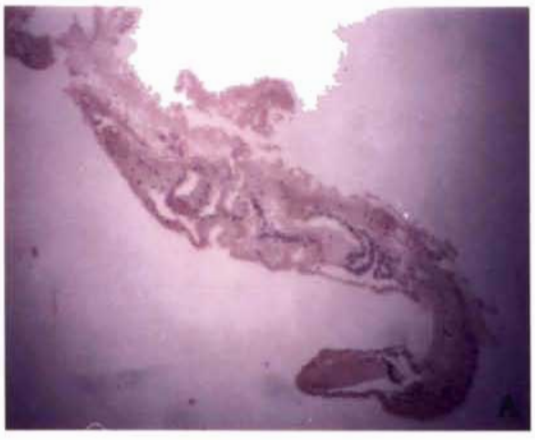
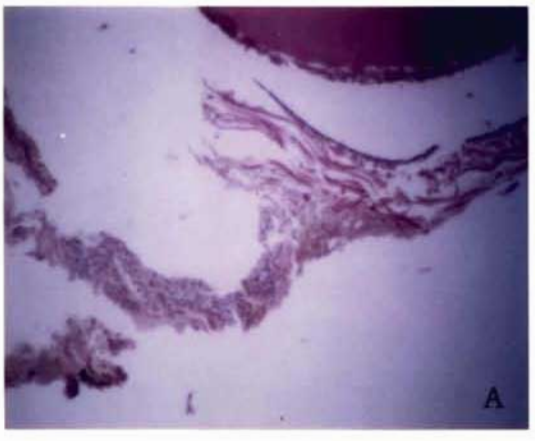
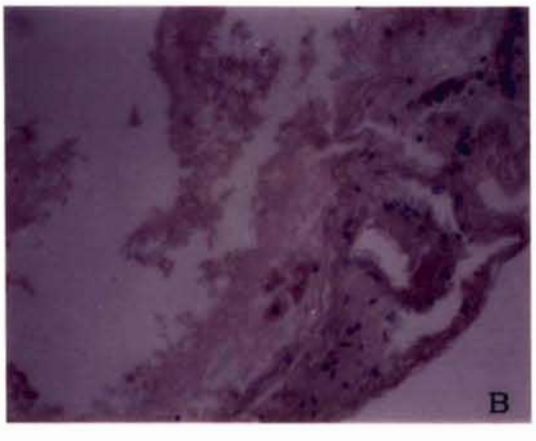
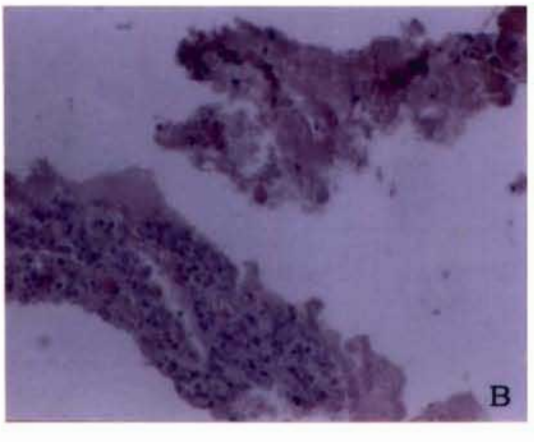


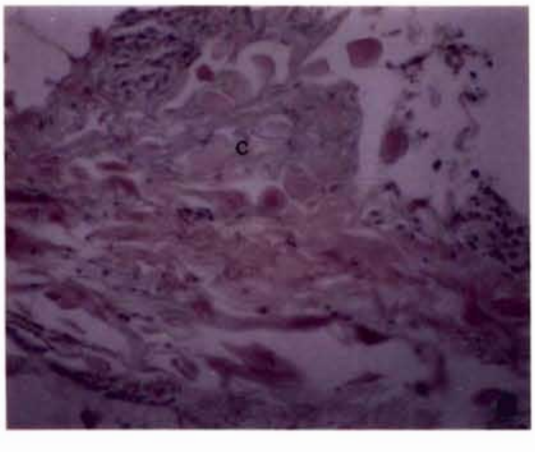
Fig 7.2 : Cross section of the androgenic gland of WOC (A) General cross section (x40), (B) Enlarged portion (x100) (C) Enlarged portion (x200) a. Type I cell











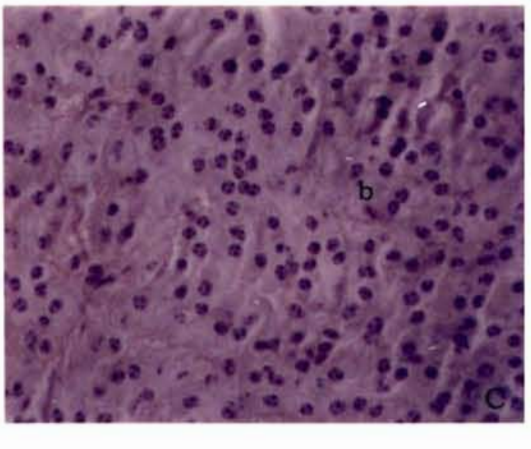
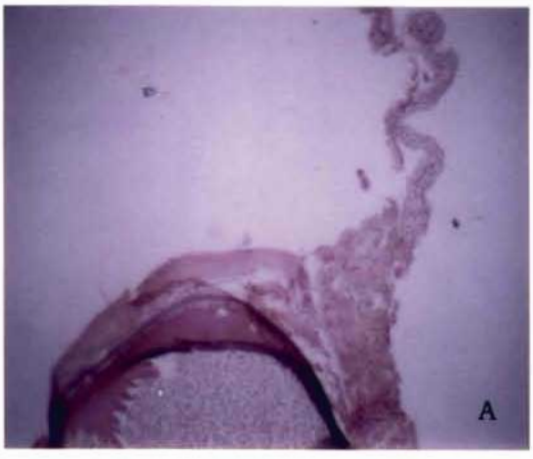
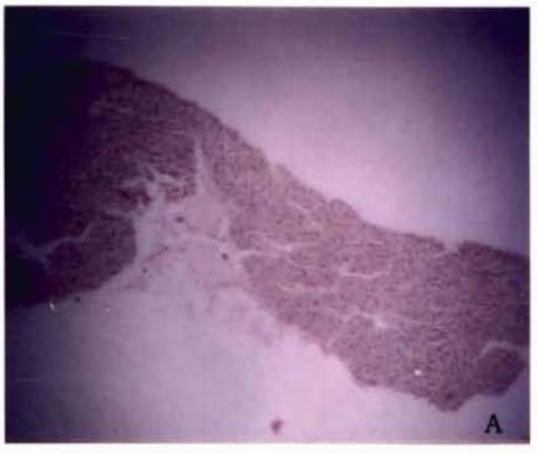
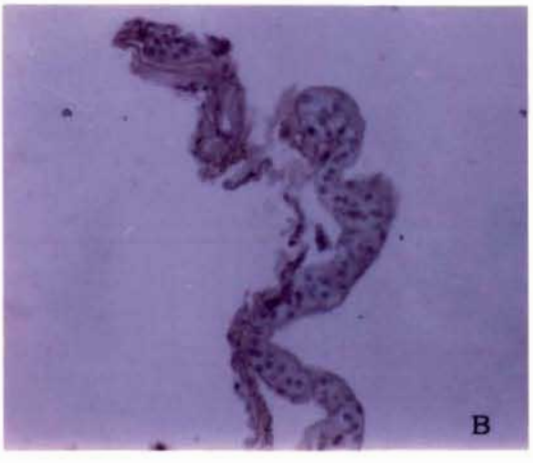


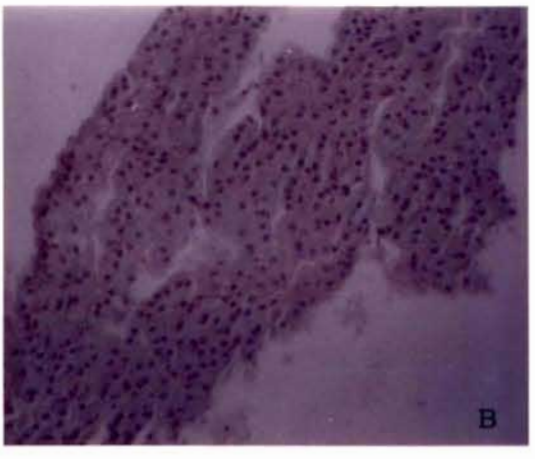
Fig 7.3 : Cross section of the androgenic gland of SOC (A) General cross section (x40), (B) Enlarged portion (x100) (C) Enlarged portion (x200) c. Type III cell

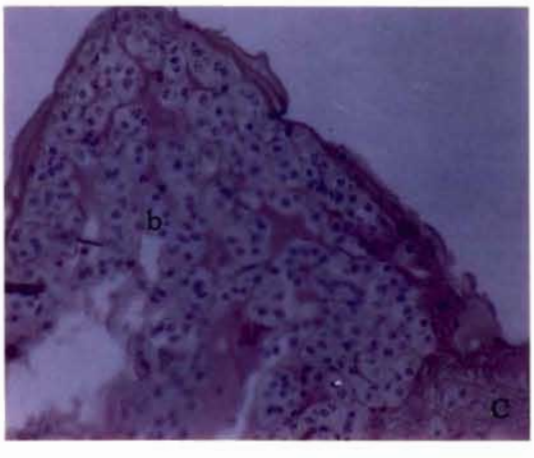
Fig 7.4 : Cross section of the androgen gland of t-SOC (A) General cross section ((B) Enlarged portion (x100) (C) Enlarge portion (x200) b. Type II cell











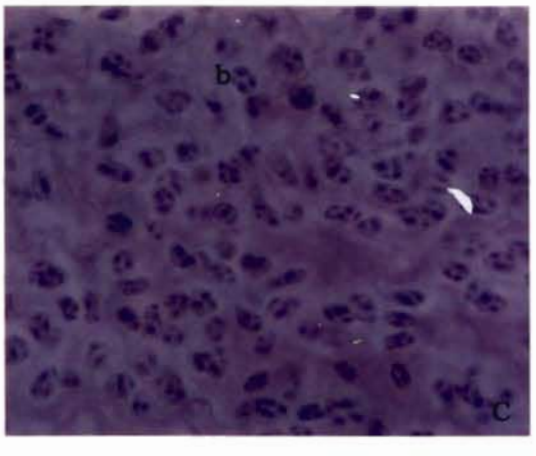


Fig 7.5 : Cross section of the androgenic gland of WBC (A) General cross section (x40), (B) Enlarged portion (x100) (C) Enlarged portion (x100) b. Type II cell

Fig 7.6 : Cross section of the androgenic gland of SBC (A) General cross section (x40), (B) Enlarged portion (x100) (C) Enlarged portion (x200) b. Type II cell

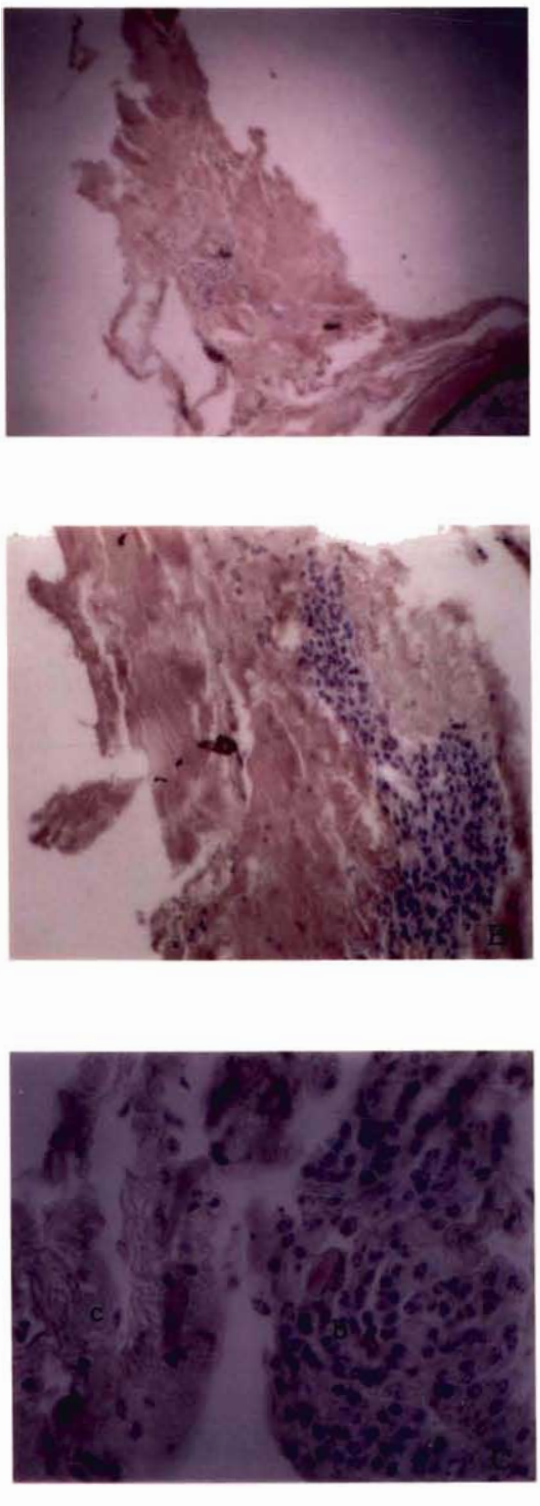


Fig 7.7 : Cross section of the androgenic gland of OBC (A) General cross section (x40), (B) Enlarged portion (x100) (C) Enlarged portion (x200) b. Type II cell c. Type III cell

CHAPTER 8

HEPATOPANCREAS

Introduction

Detailed description about the male morphotypes and their transitional stages of *M. rosenbergii* is given in chapter 2. Each morphotype develops in sequence in the adult male population from Small males through Orange Clawed males to dominant Blue Clawed males. The male morphotypes differ from each other in their claw colour, relative claw length and spination (Kuris *et al.*, 1987), reproductive behaviour (Ra'anan and Sagi, 1985; Suresh Kumar and Kurup, 1998) and growth rates (Ra'anan and Cohen 1985). Blue Clawed males have very long second pereopods (claws) that are deep blue in colour. These males are dominant and territorial, sequestered post moult adult female prawns prior to mating and grow slowly (Ra'anan and sagi, 1985). Orange Clawed males are so large and have long claws and are usually orange in colour (Ra'anan, 1982; Ra'anan and Cohen, 1985; Kuris *et al.*, 1987). These subordinate animals are not territorial, characterized by poor mating success and very high growth rates (Ra'anan, 1982; Sagi and Ra'anan, 1988; Sureshkumar and Kurup 1997). Small males are small and claws are short which are often relatively unpigmented and translucent. These animals are subordinate, not territorial and mating with females using "sneak" reproductive behaviour in the presence of blue clawed males. Small males grow slowly (Teleckey, 1984; Ra'anan and Sagi, 1985; Sagi and Ra'anan 1988). Variations in the hepatosomatic index of male morphotypes are well documented (Sureshkumar and Kurup,1999),

however, no attempt has so far been made to bring out the structural differences, if any, among the male morphotypes and therefore, the present study is aimed towards this. Knowledge on the histological details and functions of the hepatopancreas of various male morphotypes of *M. rosenbergii* is meagre. Studies on decapod hepatopancreas are those of in *Astacus leptodactylus* (Jacobs, 1928; Hirsch and Jacobs, 1928; 1930); *Atya spinipes* (Van Weel, 1955); *Procambarus clarkii* (Ogura, 1959); Miyawaki *et al.*, 1961; Bunt,1968; Loizzi, 1971); *Orconectes virilis* (Loizzi, 1966); *Carcinus meanas* (Stainer *et al.*, 1968; Hopkin and Nott, 1979; 1980); *Austropotamobius pallipes* (Lyon and Simkiss,1984) and *Penaeus semisulcatus* (Al-Mohanna *et al.*, 1985 a,b).

Materials and Methods

Details regarding the collection, transportation and segregation of samples and histological techniques used are described in chapter 4.

Results

General Morphology of hepatopancreas

The hepatopancreas, the main digestive gland in male morphotypes of *M. rosenbergii*, a bilateral evagination of the mesenteron, is a large compact organ which occupies the greater part of the cephalothoracic cavity, posterior to the cardiac portion of the stomach. They are composed of compact arrays of blind ending tubules which are held ventrally with the gut at the junction of the pyloric foregut and anterior end of the mid gut. The bulk of the tissue

comprises of simple tubules, whose wall consists of a single layer of secretory epithelium. In between the individual tubules are found interstitial cells (IC) and small blood spaces. The tubules are connected by connective tissue. The whole organ is enclosed in a greenish- yellow membrane.

Histology

The tubules of the hepatopancreas of three male morphotypes viz., SM, SOC and SBC and their four transitional stages such as WOC, t-SOC, WBC and OBC of *M. rosenbergii* are lined with an epithelium in which four cells can be recognized viz. Embryonic (E) cells, Fibrillar (F) cells, Secretory (B) cells and Absorptive (R) cells.

1. ***Embryonic (E) cells*** : These are small undifferentiated columnar cells seen at the distal end of the tubules, the only part where the epithelium is more than one cell thick. The size of the cell ranged between 10 to13.32μ and contain an ovoid nucleus. These cells undergo differentiation to become other cells viz. F,B and R cells of the tubules.
2. ***Fibrillar (F) Cells*** : The size of the cells ranged from 26.42 to 56.1μ. Their nuclei are located at the basal region of the cell. A single vacuole of variable dimensions positioned distal to the nucleus.
3. ***Secretory (B) Cells***:- The size of the cells ranged from 29.65 to 66μ. These cells have a large vacuole occupying 80-90% of the total cell volume. An aggregation of small vacuoles may sometimes

be present between the large vacuoles and the distal cell border forming the apical complex often found coalescing with the larger vacuole, apparently causing it to swell. The nucleus of the B cell lie proximal to the large vacuole and appears to be compressed as the latter enlarges.

4. ***Absorptive (R) Cells***:- The absorptive (R) cells are found lining the lumen of the hepatopancreatic tubules. The size of the cell varies from 33.3 to 99μ. These cells have a dense granular cytoplasm with a large round nucleus. Their nuclei lie medio proximally within the cells, and have a prominent nucleolus.

Variations in the histology among male morphotypes and their tansitional stages

The histological preparations of hepatopancreas which are viewed under light microscope showed variations among the morphotypes in respect of the type of cells. The salient differences are given below :

Small males (SM) : In SM, the hepatopancreatic tubule epithelium is mainly constituted by embryonic cells followed by fibrillar cells while secretary cells and absorptive cells were found to be very less. The size of the embryonic cells ranged between 6.67.to 10μ, in contrast, the size of the fibrillar cell was in the range 26.64 to 29.98μ (Fig 8.1).

Weak Orange Clawed Male (WOC) : In WOC morphotype, the hepatopancreatic tubular epithelium comprised mostly of fibrillar cells followed by embryonic cells. Secretory cells and absorptive cells are also present in very few number. The size of the fibrillar cell

ranged from 26.64 to 33.3μ where embryonic cell ranged between 6.67 to 10μ (Fig. 2)

Strong Orange Clawed Male (SOC) : Large size secretory cells characterized by large vacuoules are the major cell type seen in the hepatopancreatic tubular epithelium of SOC male mophotype with a few absorptive cells and fibrillar cells. The size of the secretory cells varied from 43.29 to 56.61μ where the size of the vacuoules ranged from 10 to 26.64μ (Fig 8.3).

Pre – transforming Orange Clawed male (t-SOC) : In t-SOC, the intermediate morphotypic stage, the secretory cells form a major portion of the hepatopancreatic tubular epithelium followed by absorptive cells. The average size of secretory cell is 66.6μ and absorptive cell is 56.61μ. The size of the vacuole ranged from 26.64 to 33.33μ (Fig 8.4).

Weak Blue Clawed Male (WBC) : In WBC morphotype also the secretory cells showed its dominance followed by absorptive cells in the hepatopnacreatic tubular epithelium. The average size of secretory cell and absorptive cells are 56.61 and 49.95μ respectively. (Fig 8.5).

Strong Blue Clawed Male (SBC) : In SBC male morphotype, absorptive cells are occupying a major protion of the hepatopancreatic tubular epithelium. The size of absorptive cells ranged from 53.28 to 79.9μ (Fig 8.6).

Old Blue Clawed Male (OBC) : In OBC male morphotype also absorptive cells are contributing to the major cells type of the hepatopancreatic tubular epithelium. The average size of absorptive cell is 88.245μ (Fig 8.7).

Discussion

Four types of cells in the hepatopancreatic tubular epithelium observed in male morphotype of M. rosenbergii have also been reported in *Astacus leptodactylus* (Hirsch and Jacobs, 1928), *Panulirus argus* (Travis, 1955, 1957), *Procambarks clarkii* (Ogura, 1959; Miyawaki *et al.*, 1961), *Caridina leavis* (Pillai, 1960), *Homarus gammarus* and *Scylla serrata* (Barker and Gibson, 1977,1978), *Carcinus meanas* (Hopkin and nott, 1979,1980), *Menippe rumphii* (Erribabu *et al.*, 1982) and in *Palaemon serratus* (Papathanassiou and King, 1984c). On the contrary *Metapeuneus bennettae*, Dall (1967b) reported only three types cells; however, in *penaeus semisulcatus* a fifth type of cell, the " Mikjet " (M) cell was also reported by Al-Mohanna *et al.* (1985b).

Oizzi (1971) presented a model for the sequential differentiation of the various cell types in the tubular epithelium of hepatopancreas of cray fish. Accordingly, the apical embryonic (E) cells give rise to two basic types, the absorptive (R) cells which store nutients and the Fibrillar (F) cells which are secretory in nature. On the basis of electron microscopical studies on *Orconectes virilis* and *Procambarus clarkii*, Loizzi (1971) established that (E) cells are associated with mitosis, (F) cells with synthesis of digestive enzymes and their storage in supra nuclear vacuole which enlarges by

pinocytic intake of luminal nutrients and fluids. The vacuoules of Fibrillar (F) cells continue to enlarge and coalesce until only the nucleus and a pinocytolically active apical complex remain thereby completing the transformation into secretory (B) cells. According to Loizzi (1971) (R) cells absorb luminal nutrients mainly via contact digestion and molecular transport. They store and metabolize glycogen and lipid. The (R) cells seem to perform the function of storage, transport and metabolism of organic and inorganic matter. With regard to their funtions Loizzi (1971) compares these cells to the hepatic cells for strorage and with intestinal absorbing cells for the transport through cell membrane. Thus, a functional analogy between the (B) cells of the hepatopancreas and the hepatic and intestinal absorbing cells of vertebrates is quite apparent. Since this cell possesses a striated apical border strikingly similar to that of vertebrate cells known to function input absorption (Yamada, 1955; Ito, 1965). Hopkin and Nott (1980) also supported the developmental sequence of E →, R → and E →, F →, B → cells. In *P.semisulcatus* the fibrillar cells (F) synthesis and secrete zumogen granules and gradually differentiated into secretoy (B) cells (Al-Mohanna *et al.*, 1985a). In M.rosenbergii, the vacuolar contents in the (B) cells (secretory) are liberated by pinching off the apical complex in an apocrine fashion as reported in *P. argus* (Travis, 1955). The histological details of cell types in the hepatopancreatic tubular epithelium of various male morphotypes of *M. rosenbergii* also supports Loizzis model. ie. the developmental sequence E → S and E → F→ R cells. M. rosenbergii is characterised by sexually dimorphic growth, with a relative slow and uniform growth rates in females and

differential growth rates in males. (Smith *et al.*, 1978; Brody *et al.*, 1980; Cohen *et al.*, 1981; Ra'anan and Cohen, 1985; Karplus *et al.*, 1986a). Males can be distinctly divided into three main morphotypes and four transitional stages, each exhibiting a different growth rate (Cohen *et al.*, 1981; Sagi and Ra'anan, 1988; Harikrishnan and Kurup, 1997a). Each morphotype develops in sequence in the adult male population from small males through Orange clawed males to blue clawed males (Cohen *et al.*, 1981; Ra'anan and sagi, 1985; Sagi *et al.*, 1988). Small and blue claw males reproduce but grow slowly (Ra'anan and Sagi, 1985), whereas the Orange clawed males represent the intermediate fast growing phase (Kuris *et al.*, 1985; Sagi and Ra'anan, 1988; Kurup *et al.*, unpublished) which divert more energy towards somatic growth (Sagi, 1984; Ra'anan and Cohen, 1985; Ra'anan and Sagi, 1985). SM which occupies the initial stage where WOC is the first transitional stage of SM and SOC. Growth of SM is also reported to be very slow as they convert a large part of their energy for mating attempts using a sneak mating behavior (Ra'anan and Sagi, 1985). Similarly, the lowest hepatosomatic index observed in SM by Cohen *et al.* (1981); Sagi and Ra'anan (1988) and Sureshkumar and Kurup (1999) may manifest its retarded growth rate. Relatively slow growth rate seen in small male and Weak orange clawed male could be attributed to the presence of large proportion of embryonic cells and fibrillar cells in the epithelial layer of hepatopancreatic tubules as these morphotypes occupy the initial stage of their developmental pathway. This is in agreement with the findings of Travis (1955), van Weel (1955), Davis and Burnett (1964), Loizzi (1971) and Al-Mohanna *et al.* (1985a) that the

embryonic cells give rise to the remaining cell types, in the epithelial layer of the hepatopancreas; the organism grows secretory (B) cells form the most dominating cell type in the hepatopancreatic tubular epithelium of SOC morphotypes and in its intermediary stages viz., t-Soc and WBC. Relative weight of hepatopancreas in SOC is larger when compared to other morphotypes (Sureshkumar and Kurup, 1999). This morphotype may have distinct advantage of better food assimilation and carbohydrate storage which in turn can explain the higher somatic growth seen in this morphotype (Dhall and Moriaty, 1984., Sagi and Ra'anan, 1988). Moreover, in an intermoult animal of late stage C, a period during when active feeding occurs, the most conspicous cell observed in the epithelial tissue of the hepatopancreas are large, mature secretory cells (Hirsch and Jacobs, 1928, 1930). These are swollen and enclosed in large vacuoules and their contents plus adjacent cytoplasm are released into the lumen, leaving only the basal region and nucleus of the cell intact, ie. apocrine secretion, and it is probable that these cells are reconstructed from the remaining basal end (Hirsch and Jacobs, 1928). The secretory (B) cells are suggested to function in the synthesis and release of digestive enzymes (Van Weel, 1955; Travis, 1955; Davis Burnett, 1964). In its fine structure this cell resembles the vertebrate pancreatic exocrine cells which are known to function in secretion of digestive enzymes (Van Weel, 1955 ; Travis, 1955; Davis and Burnett, 1964). The exocrine cell of the vertebrate pancreas, specialised for secretion of digestive enzymes, is characterised by a large nucleus containing a prominent nucleolus, numerous profiles of rough endoplasmic reticulum and a prominent

golgi complex (Fawcett, 1966). These fine structural characteristics within the hepatopancreas are observable only in the fibrillar cells. This suggests that the fibrillar cell is in fact responsible for secretion of the digestive enzymes known to derive from the hepatopancreas. The fibrillar (F) cells in the present study can be considered to be immature secretory (B) cells as suggested by Loizzi (1971). The secretory (B) cells are characterized by single large vacuoule, in which small secretory vacuoules have coalesced to form a single large vacuoule, occupying a large proportion of the cell volume, leaving a rim of cytoplasm and a compressed basal nucleus (Loizzi, 1971). The secretory (B) cells are a source of enzymes for extra cellular digestion (Loizzi, 1971; Gibson and Barker, 1979). The dominance of secretory (B) cells in the hepatopancreatic epithilium would manifest the possibility of better food assimilation and growth in the SOC morphotype and the t-SOC and WBC, the intermediary stages, due to the presence of high amount of digestive enzymes. This observation strongly corroborates with the earlier reports (Kuris *et al.* 1987; Kurup *et al.*, unpublished) that SOC and its transitional stages have relatively high somatic growth rate.

In SBC and OBC morphotypes the penultimate and terminal stages respectively, the absorptive (R) cells are the most conspicuous cell type present in the hepatopancreatic tubular epithelium. Mature absorptive cells are tall, columnar cells, with numerous vacuoules and with either a basal or central nucleus, and an opical striated border depending upon the stage of development (Travis, 1955). Essentially, the function of the absorptive (R) cells are food asborption, ie. to absorb diffusible and small particles from the

haemelymph (Travis, 1955; Davis and Burnett, 1964). This cell also functions like a storage site as manifested from the high content of tipid droplets, glycogen and calcium phosphate crystals (Travis, 1955; Davis and Burnett, 1964). Sureshkumar and Kurup (1999) reported a gradual decrease in Hepatosomatic index (HIS) from SOC to OBC commensurating with the developmental pathway of these morphotypes and this observation is in full agreement with Ra'anan and Sagi (1985) who reported that growth rate is reduced and moulting is in frequent in the latter stages of male morphogenesis. The absorptive (R) cells in SBC and OBC are functioning as food absorptive cells. From the high content of lipid droplets and glycogen observed in these cells, it can reasonably be inferred that they function also as storage site as reported by Travis, 1955; Davis and Burnett (1964). In the latter stages of male morphogenesis, the morphotypes are characterised with a reduced growth rate and infrequent moulting as observed in SBC and OBC by Ra'anan and Sagi (1985) and the main food reserve is fat and glycogen, which forms the energy store in these morphotypes. The storage of glycogen and fat in the absorptive cells during the intermoult cycle has been reported in majority of crustaceans (Travis, 1955; Miyawaki and Sasahi, 1961; Davis and Burnett, 1964; Stainer, 1968; Loizzi, 1971). The dominance of absorptive (R) cells in the hepatopancreatic epithelium of SBC and OBC morphotypes as observed in the present study showed strong agreement with the earlier reports that it acts as a storage site for glycogen and fat (Yonge, 1924; Travis, 1955; Davis and Burnett, 1964).

Hepatopancreas plays a significant role in the food assimilation and mobilization of energy during moulting, pigmentation, gluconeogenesis and carbohydrate storage (Dall and Moriaty, 1984; Gidalia, 1985; Skinner, 1985). A major seat of bulk storage, synthesis and tranformations of a variety of organic and inorganic substances, the hepatopnacreas of crustaceans, which is analogous to the liver of vertebrates and the fat body of insects, can aptly be ascribed as a metabolic factory par excellence (Lockwood, 1968; Huggins and Munday, 1968; Adiyodi and Adiyodi, 1970). Sagi and Ra'anan (1988) opined that the relative size of the hepatopancreas can be highly corelated with the morphotypic stage of development and its relative energy expenditure in growth and sexual maturity. Hepatosomatic index (HSI) showed an increase from SM to SOC, in contrast a gradual decrease from SOC toOBC was quite discernible (Sureshkumar and Kurup, 1999). Relative weight of hepatopancreas, which plays a key role in food assimilation (Dhall and Morairty, 1984) as well as the functional roles of different cell types comprising the hepatopancreatic epithelium of various male morphotypes of *M. rosenbergii* observed in the present study will lend to support the difference in growth among various male morphotypes as reported by Cohen *et al.* (1981), Kuris *et al.* 1987) and sagi and Ra'anan (1988). In *M. rosenbergii*, size of hepatopancreas of SOC is larger when compared to other morphotypes and this coupled with dominance of secretory (B) cells in the hepatopancreatic epithelium as noticed in the present study, would suggest the possibility of better food assimilation (Dhall and Morairty, 1984) which in turn might have contributed to the higher somatic growth of this

morphotype. (sagi and Ra'anan, 1988). Type of cells in hepatopancreatic epithelium of SOC, t-SOC and WBC also showed distinct variations with that of SBC and OBC, which are dominated with absorptive (R) cells, and this observation would also support the structural and functional variations of hepatopancreas between OC and BC males commensurate with the respective growth and reproductive stages represented by these morphotypes (Sagi and Ra'anan, 1984). Similarly, the dominance of embryonic (E) and Fibrillar (F) cells in SM and WOC as observed in the present study together with the lowest hepatosomatic index in SM as observed by Sureshkumar and Kurup (1999) would also serve as strong evidences for its retarded growth as reported by Cohen *et al.* (1981) and Sagi and Ra'anan (1988). In conclusion, there exists glaring differences in the histological structure of the hepatopancreas among various male morphotypes of *M.rosenbergii* and this finding would be immensely useful in explaining the differential growth manifested by various male morphotypes and their transitional stages.

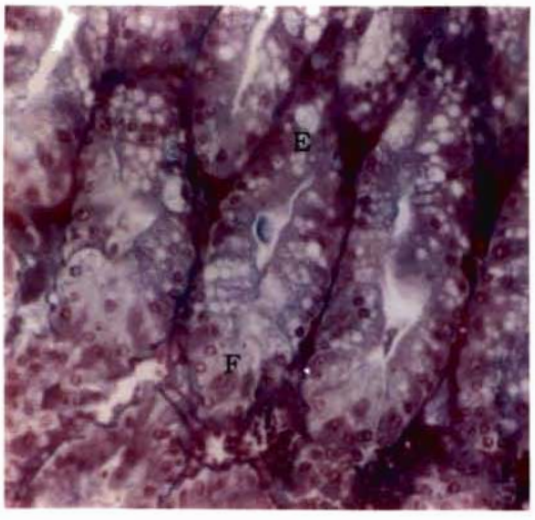


Fig 8.1 : Cross Section of hepatopancreas of SM with embrionic cells (E) & fibrillar cells (F) (x100)

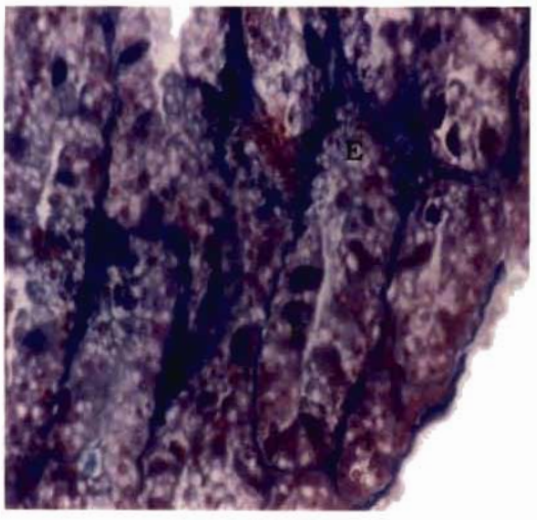


Fig 8.2 : Cross Section of hepatopancreas of WOC with embrionic cells (E) & fibrillar cells (F) (x100)

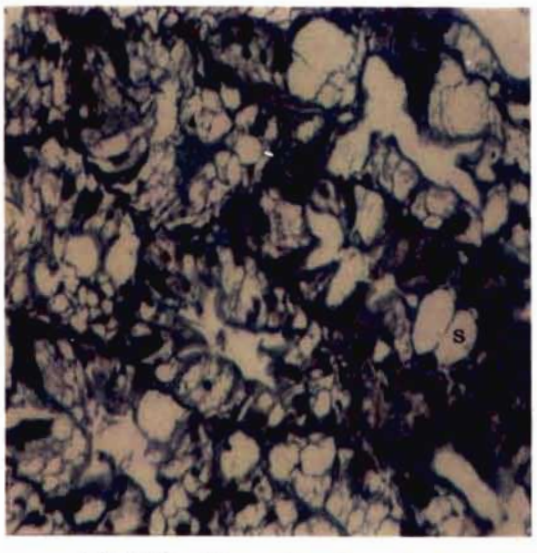


Fig 8.3 : Cross Section of hepatopancreas of SOC with secretory cells (B) (x100)

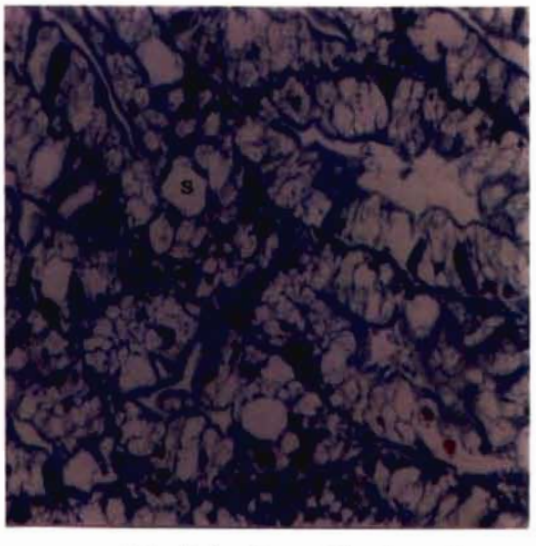


Fig 8.4 : Cross Section of hepatopancreas of t-SOC with secretory cells (B) (x100)

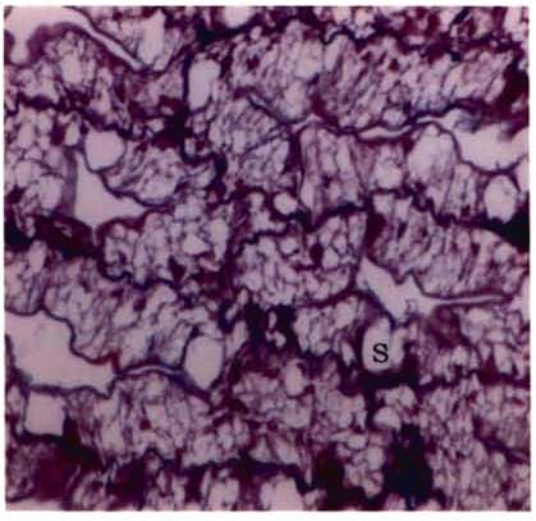


Fig 8.5 : Cross Section of hepatopancreas of WBC with secretory cells (B) (x100)

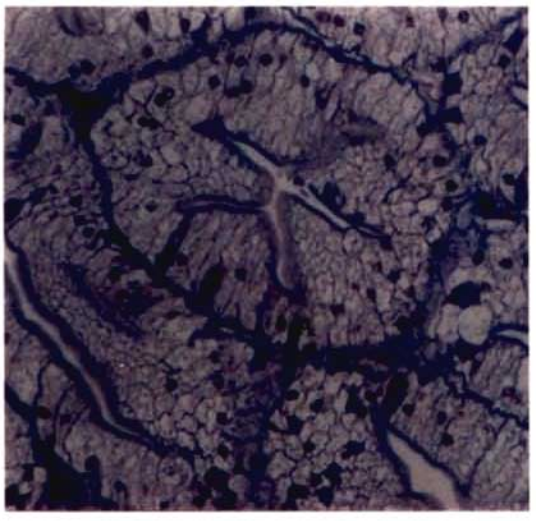


Fig 8.6 : Cross Section of hepatopancreas of SBC with absorptive cells (B) (x100)

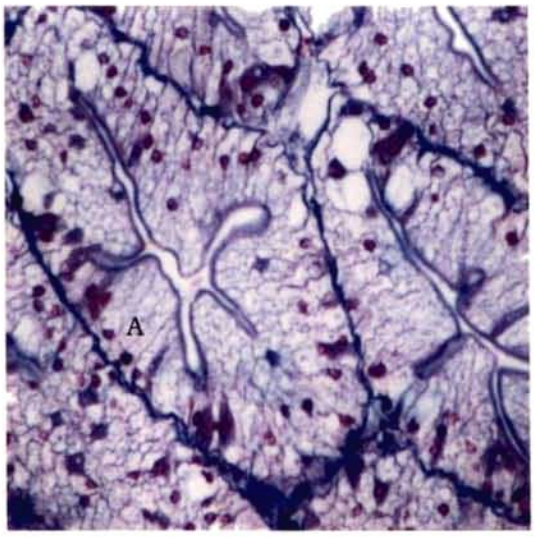


Fig 8.7 : Cross Section of hepatopancreas of OBC with absorptive cells (B) (x100)

CHAPTER 9

NEUROSECRETORY CELLS IN THE EYE STALK, BRAIN AND THORACIC GANGLION

Introduction

Neurosecretory cells are defined as neurons with axonal terminals that show specialization and localization for release of substances to the haemolymph (Cook and Sullivan, 1982). Since ordinary neurons may release neurohumors that function in the transmission process at synapses and neuroeffector junctions, a neurosecretory system is distinguished by the grouping and specialization of axon terminals for storage and release of its products, usually into the circulation through blood. The neurosecretory cell can, therefore, be recognized by its cytological characteristics, and their axons terminate in close proximity to a blood vessel or sinus. These axons contain appreciable quantities of chemical substances which can be shown by extraction and injection experiments. Similar type of chemical substances can also be found in the blood stream and they have high biological activity on certain tissues (Carlisle and Knowles, 1959). Neurons showing clear glandular properties have been described as neurosecretory cells (Scharrer and Scharrer, 1954). The reviews of Brown (1944, 1948) and Panuouse (1947) reveal that internal secretions in crustaceans originate in the nervous system and that most known endocrine organs of crustaceans are in fact neurosecretory systems analogous to the hypothalamus–hypophysial pathways seen in chordates (Bargmann, 1949) and the pars intercerebrales-corpora cardiaca

system described in insects (Scharrer, 1952). Brown (1944) suggested that many hormones of crustaceans originated in the central nervous system. But a morphological basis of the theory was established only when injection and extraction experiments could be correlated with the presence of secretory elements within the nervous tissue (Knowles, 1951; Enami, 1951; Bliss and Welsh, 1952; Carlisle, 1953a,c; Bliss *et al.*, 1954). It is now well known that physiologically active substances are produced in neurosecretory cells located throughout the central nervous system of crustaceans (Bliss, 1951, 1952, 1953; Bliss, Durand and Welsh, 1954; Bliss and Welsh 1952; Carlisle, 1953; Enami, 1951; Passano, 1951a, 1952, 1953). Furthermore, it has been observed that the neurosecretory cells are distributed as distinct groups in the optic lobe peduncle within the eye stalk, brain and the thoracic ganglion. The presence of neurosecretory cells within the eye stalk of crustaceans has been reported by Adiyodi and Adiyodi (1970). Studies on the neurosecretory cells in brain include that of Enami (1951); Bliss and Welsh (1952); Carlisle (1959c); Bliss *et al.* (1954) and Knowles and Carlisle (1956). The neurosecretory cells located in the thoracic ganglion have been investigated by Smith (1948); Brown *et al.* (1949); Sandeen (1950); Enami (1951); Carlisle (1953a); Matsumoto (1954); Parameswaran (1955) and Nayar and Parameswaran (1955). The neurohormones are usually transported along the definite neuronal pathways and stored in their swollen axon terminals till their release into the blood until upon receiving necessary appropriate stimulus. Some neurosecretory cells may discharge their products peripherally (Matsumoto, 1958).

Histoarchitecture studies of the neurosecretory organs in crustaceans have been attempted in *Sesarma dehaani* (Enami, 1951), *Lysmata seticaudata* (Carlisle, 1953), *Penaeus braziliensis, Leander affinis, L. serratus* and Orconectes sp. (Knowles, 1953; Bliss *et al.*, 1954), *Callinectes sapidus, Carcinidas, Ocypode* and *Calappa*(Potter, 1954, 1956, 1958), *Orconectes virilis* (Durand, 1956), *Paratelphusa hydrodromous* (Parameswaran, 1956), *Chionectes, Eriocheir, Potamon* and *Neptunus* (Matsumoto, 1958), *Pandalus borealis* (Carlisle, 1959a), *L. serratus* (Carlisle and Knowles, 1959), *C. laevis* (Pillai, 1961b), *Penaeus durarum* (Gabe, 1966),*Paratelphusa hydrodromous* (Adiyodi, 1967)and *Carcinus maenas* (Smith and Naylor, 1972). Neurosecretory cells of Natantians were described by Rangnekar and Madhystha (1971), Nagabhushanam and Vasantha, (1972), Hisano (1974), Sarojini and Gyananath (1984), Sarojini and Victor (1985), Sarojini *et al.* (1987) and Rao (1969). Hitherto no attempt has been made to study the neurosecretory system of freshwater prawns, barring the preliminary studies made in this direction by Nagabhushanam (1983), Sarojini and Victor (1985), Sarojini and Gyananath (1984), Mirajkar *et al.* (1982), Rao (1969) and Joseph and Kurup (2001).

A Review of the literature reveals that knowledge on the neurosecretion in Natantia in general and in freshwater prawns in particular is very limited. Relatively little is known about the specific localization of the sources of the neurohormones affecting particular physiological processes. Furthermore, there is no cytological information available to indicate which of the different neurosecretory cell types are involved or often go hand in hand with differences in

function. In view of the meager knowledge on neurosecretory control on morphotypic transformation in male *M. rosenbergii*, the present investigation was undertaken to find out whether there exists any structural and functional interrelationship between the neurosecretory system of various male morphotypes of *M. rosenbergii* such as small male, strong orange clawed male and strong blue clawed male and their transitional stages viz. weak orange clawed male, pre-transforming orange clawed male, weak blue clawed male, and old blue clawed male.

Materials and methods

Details regarding the collection, transportation and segregation of sample and histological technique followed are described in chapter 4.

Results

Neurosecretory system : Much effort was made to locate the neurosecretory centres in the eye stalk, brain and thoracic ganglion in order to assess their functional efficacy in terms of secretory behavior. These studies were found indispensable for the assessment of physiological implication of cell types in the formation of various male morphotypes of *M. rosenbergii.*

Eye stalk and its secretory centres : The eye is attached to the head region by the proximal stalk. The eye stalk of *M. rosenbergii*, like that of other crustaceans, possesses a ganglion known as the optic ganglion. The gross internal anatomy of the eye stalk does not differ

from the general plan of the Malacostraca. Anatomically each optic ganglion has been demarcated into four distinct zones, namely, the lamina ganglionaris (LG), medulla externa (ME), medulla interna (MI) and medulla terminalis (MT) (Fig 9). The medulla terminalis, is in turn, connected to the protocerebrum with the pedunculus lobus opticus. All the optic centres are formed of nerve fibres which arise from the neurons, the latter cover these neuropiles. The pedunculus lobus opticus is made of bundles of nerve fibres intermingled with neuroglia. The lamina gnaglionaris and the medulla externa are connected together by intercrossing of fibres forming the external chiasma. The medulla externa and the medulla interna are connected together by the internal chiasma. But there is no such intercrossing of fibres between the medulla interna and medulla terminalis. Fibres which start from the medulla interna traverse through the terminalis probably to end in the brain or even beyond in the ventral nerve cord.

Lamina ganglionaris : Lamina ganglionaris is situated at the distal most tip of the eye stalk, just beneath the basement membrane of the compound eye. It is bow shaped, with its convex side facing outwards and separated from the ommatidia of the compound eye by the basement membrane alone.

Medulla externa : The lamina ganglionaris is followed by the medulla externa which is sickle shaped with a loose network of fibres separating it from the lamina ganglionaris and medulla interna.

Medulla interna : The medulla interna has more or less oval contour and is situated behind the medulla externa. It is also enveloped by an outer sheath of connective tissue.

Medulla terminalis : The most complex area of the optic ganglion is the medulla terminalis which is more or less conical in outline. It is situated in the proximal region of the ocular peduncle. Towards the proximal end, it tapers into the pedunculus lobi optici. As already stated the medulla terminalis is actually a part of the protocerebrum which has moved into the eye stalk in most of the stalked-eyed crustaceans. In many of the decapods it has resumed its position within the brain. But in *M. rosenbergii*, as in the 'Carcinus type' brain described by Hanstrom (1947), this portion is lodged in the eye stalk and connected with the brain through the pedunculus lobus opticus.

Distribution of neurosecretory cell groups in the eye stalk of *M. rosenbergii*.

In the eye stalk of *M. rosenbergii*, neurosecretory centres are present in three proximal neuropiles namely the medulla terminalis, medulla interna and medulla externa. Except the lamina ganglionaris, each of the other three zones possesses distinct ganglionic X-organs which are comprised of distinct groups of neurosecretory cells (NSC) and neuroglial elements. The neurosecretory apparatus, in the optic ganglion, exhibits the presence of scattered neurosecretory cells (NSC) in the lamina ganglionaris (Fig 9-E) while the groups of neurosecretory cells forming the X-organ sinus gland complex, are present in the medulla

externa, medulla interna and medulla terminalis. Two groups of neurosecretory cells are present in the medulla externa, each of which is called as medulla externa ganglionic X-organ (MEGX). These two groups are designated as $MEGX_1$ and $MEGX_2$ for the purpose of identification (Fig 9-E). A single group of the neurosecretory cell called the medulla interna ganglionic X-organ (MIGX) is present in the medulla interna (Fig 9-E). The medulla terminalis consists of two groups of neurosecretory cells, each of which is called the medulla terminalis ganglionic X-organ. These two groups are designated as $MTGX_1$ and $MTGX_2$ (Fig 9-E).

Neurosecretory cell types

The neurosecretory cells in the central nervous system of crustaceans can be grouped into various types based on differences in size, shape, locality, cytological and cytophysiological details and tinctorial behavior (Parameswaran, 1955, 1956; Matsumoto, 1958, 1962; Szudarski, 1963; Adiyodi, 1967).

Eye stalk

Detailed examination of the serial sections of the eye stalk revealed that they contain certain specialized cells. Depending on their cytological characteristics such as the shape, size, with or without axons, condition of cytoplasm, the shape and size of their nuclei and the staining properties; the neurosecretory cells of the eye stalk can be classified and designated as type 'C,'D' and 'E'.

'C' type neurosecretory cell : 'C' type cells are the largest of the neurosecretory cells in the eye stalk and polygonal in shape, ranging between 18.65 and 29.30μ in diameter. The nucleus is comparatively large, spherical or slightly elliptical in shape measuring from 6.67 to 15.32μ in diameter. The nucleolus is single and is prominent. These cells exhibit neurosecretory cycle. They stain pinkish with Mallory's triple stain and blue with Gomori's chromealum haematoxylin phloxin stain.

'D' type neurosecretory cells : These are smaller than 'C' type cells, ranging between 11.32 and 17.32μ and are round to oval in shape. The nucleus is fairly large and centrally placed, with a prominent central nucleolus. The cytoplasm of these cells are comparatively lesser in volume containing small secretory granules. They also exhibit neurosecretory cycle. The staining properties of these cells are similar to those of 'C' type cells.

'E' type neurosecretory cells : 'E' type cells are very small having an average size of 6.67μ and round in shape. The quantity of the cytoplasm in these cells is very less and are devoid of vacuoles. The nucleus occupy the greater part of the cell bodies. Each nucleus contains a single nucleolus. These cells do not exhibit cyclic neurosecretory activity. They appear pinkish with Mallory's triple stain and also positive to the Gomori's chromealum phloxin stain.

Distribution of different types of neurosecretory cells in the eyestalk neurosecretory centers.

In lamina ganglionaris, the most common neurosecretory cells are those of 'E' type with a few 'D' type. The most common cell

found in the medulla externa ganglionic X-organ$_1$ and medulla externa ganglionic X-organ$_2$ are of type'D' and 'E' with very few cells of type 'C'. In the medulla interna ganglionic x-organ, mostly type 'C'cells are found along with a few of the 'D' and 'E' types. The most abundant cells in the medualla terminalis ganglionic X-organ$_1$ and medulla terminalis ganglionic X-organ$_2$ are type 'D'cells. Very few cells of the type 'C' and 'E' cells are also seen in these organs.

Neurosecretory cell types in the eye stalk of various male morphotypes and their transitional stages of *M.rosenbergii*

Small male : The average cell diameter of the 'C', 'D', and 'E' type cells recorded were 21.32, 13.32 and 6.67μ respectively. The nuclear diameter measured on an average size of 9.99, 6.67 and 3.33μ in the 'C', 'D' and 'E' type cells respectively (Fig 9.1).

Weak Orange Clawed Male : For 'C' and 'D' type cells the cell diameters were 22.64 and 14.69 respectively. The respective nuclear diameters of 'C' and 'D' type cells were 10 and 6.67 μ. 'E' type cells do not exhibit any variation (Fig 9.2).

Strong Orange Clawed Male : While in SOC morphotype the cell diameters were 29.3 and 17.32μ respectively for 'C' and 'D' type cells, their nuclear diameters were 15.32 and 10μ respectively. The 'E' cells do not exhibit any variation (Fig 9.3).

Pre-transforming Orange Clawed Male : The average cell diameter of 'C' and 'D' type cells were recorded 27.97 and 16.65μ respectively. The nuclear diameter measured on an average size of 13.32μ and

8.66μ in the 'C' and 'D' type cells respectively. The 'E' type cells do not exhibit any variation (Fig 9.4).

Weak Blue Clawed Male : For 'C' and 'D' type cells the cell diameters were 24.64 and 15.98μ respective. The respective cell diameters of 'C' and 'D' type cells were 10 and 7.99μ. 'E' type cells do not exhibit any variation (Fig 9.5).

Strong Blue Clawed male : The cell diameters of 18.65 and 11.32μ were recorded for 'C' and 'D' types of cells. The nuclear diameters of 6.67μ and 3.33μ were recorded for 'C' and 'D' types of cells. 'E' type cells do not exhibit any variation (Fig 9.6).

Old Blue Clawed Male : The average cell diameter of the 'C' and 'D' type cell recorded were 17.33 and 9.99μ respectively. The nuclear diameter measured on an average size of 6.67 and 3.33μ in the 'C' and 'D' type cell respectively. 'E' type cells do not exhibit any variation (Fig 9.7).

Brain and its neurosecretory centres

The terminologies used for describing the different components of the brain of *M. rosenbergii* are that of Hanstrom (1947). The brain is roughly rectangular in shape and is situated on the anterio-ventral side of the cephalothorax just in front of the esophagus. It is enveloped by a blood space which is anteriorly bordered by the optic sternum. Eventhough rectangular in shape, it has a convex dorsal and a flat ventral surface and the sides are slightly bulged at the region of the olfactory lobes. The brain is

slightly deflected dorsoventrally with the anterior part lying in a more dorsal plane than the posterior part.

The brain of *M.rosenbergii* can be subdivided into three main regions i.e. the protocerebrum, the deutocerebrum and the tritocerebrum, with a principal portion of the protocerebrum ie. the medulla terminalis having moved into the eye stalk.

Distribution of neurosecretory cell groups in the brain of *M. rosenbergii*

Neurons, which are secretory in nature are present on the median, posterior and anterior and also on the lateral sides of the brain. The secretory neurons in the brain can be divided into four separate groups namely B1, B2, B3 and B4. The position of various cell groups of the brain is presented diagrammatically shown in Fig 9-B.

B_1 Group : This cell group is located on the anterio-dorsomedian side of the brain. They are designated as the anterior group of cells and thus form the largest of four neurosecretory cell groups and the major portion of this group is situated in between protocerebral lobes extending backwards to almost half the length of the brain. On the anterior side this cell group diverges into two horns which move downwards and ends on the ventral side of the brain.

B_2 group : This group of cells is paired and is situated more or less towards the middle of the brain, but more towards the lateral sides than the B_1 group. They are designated as the anterior lateral groups.

B_3 group : This group of cells also is paired. On each side this group envelops the olfactory lobe of its side on all sides except the outer border. The disposition of this group of cells is such that its length lies parallel to the length of the brain with its anterior end extending up to the posterior border of the B_2 group, and the posterior end being wedged in between the lobus parolfactorius and the tritocerebrum. They are designated as the posterio-lateral groups.

B_4 group : This is a posterior median group of cells situated in the posterio-ventral side of the brain. On the anterior side it is bordered by the lobus parolfactorius and posterio laterally by the antennary neuropiles of the tritocerebrum.

Neurosecretory cell types-Brain.

B-type neurosecretory cells : 'B' type neurosecretory cells are the largest of neurosecretory cells in the brain of *M.rosenbergii*. These cells are spherical in shape and without axons and the size ranged from 73.22 to 96.24μ. The nucleus is oval or rounded containing one or two nucleoli. The nucleus shows variation in structure in relation to the secretory activity of the cell. The cytoplasm in the normal course is dense and homogeneous but at certain times vacuoles are formed which acquire large dimensions. These cells display the neurosecretory cycle as manifested by the change in the neurosecretory material (NSM) in their cytoplasm and the nuclear diameter at different periods. The neurosecretory material of these cells is positive to Gomori's chromealum haematoxylin phloxin and Mallory's triple stain. Gomori's chromealum haematoxylin gives deep

blue colour to the neurosecretory cells while triple Mallory's stain gives pinkish violet colour.

'C' type neurosecretory cells : 'C' type neurosecretory cells are smaller than 'B' type cells ranging between 24.97 and 44.96μ. Like 'B' types they are also round to oval in shape but with or without axons. The cytoplasm is lesser in quantity than the 'B' cells and also has vacuoles. The nuclei and the nucleoli of these cells are similar to those of 'B' types. In respect of neurosecretory cycle as well as the staining properties they are similar to 'B' type cells.

'D' type neurosecretory cell : 'D' type neurosecretory cells are smaller than 'C' type, ranging from 11.65 to 21.65μ in diameter. These cells are oval or slightly polygonal in shape with a single nucleolus. They also exhibit neurosecretory cycle. They stain pinkish with Mallory's triple stain and blue with Gomori's chromealum haematoxylin phloxin stain.

'E' type neurosecretory cells : 'E' type neurosecretory cells are the smallest of all having an average size of 10μ and round in shape. The quantity of cytoplasm in these cells is much less and is without vacuoles. Each nucleus contains a single nucleolus. These cells do not exhibit neurosecretory cycle. They respond positively to Gomori's chromealum haematoxylin and triple Mallory's stain.

Distribution of different types of neurosecretory cells in the brain neurosecretory centres.

The position of various cell groups of the brain is diagrammatically shown in Fig 9-B. There is an anterior group of cells

(AN) which is mainly composed of 'D' type neurosecretory cells, whereas type 'B', 'C' and 'E' cells are less prominent. Laterally there are two other neurosecretory cell groups on either side of the brain. They are designated as anterio-lateral groups (AL) and the posterio-lateral groups (PL). In the AL only type 'C', 'D' and 'E' cells are observed while the PL contains 'B', 'C', 'D' and 'E' type cells. There is also a posterior group of cells (PG) which mainly consists of type 'E' cells with a sparse presence of 'C' and 'D' types.

Neurosecretory cell types in the brain of various male morphotypes and their transitional stages of *M.rosenbergii*

Small male : the average cell diameters of 'B', 'C' and 'D' type cells recorded were 88.25, 31.32 and 19.98μ respectively. The nuclear diameter measured on an average size of 14.57, 13.32 and 8.66μ in the 'B', 'C' and 'D' type cells respectively. 'E' type cell and nuclear diameter measured on an average size of 10 and 3.33μ respectively (Fig 9.8).

Weak Orange Clawed Male : For 'B', 'C' and 'D' type cells the cell diameters were 86.58, 31.64 and 19.98μ respectively. The respective cell diameters of 'B', 'C' and 'D' type cells were 14.15, 13.73 and 8.32μ. Type 'E' cells do not exhibit any variation (Fig 9.9).

Strong Orange Clawed Male : The average cell diameters of 73.23, 24.97 and 11.65μ were recorded for 'B', 'C' and 'D' types of cells. The average cell diameters of 12.48, 11.65 and 6.67μ were recorded for 'B', 'C' and 'D' types of cells. Type 'E' cells do not exhibit any variation (Fig 9.10).

Pre-transforming Orange Clawed Male While in t-SOC morphotype the cell diameters were 83.25, 29.97 and 17.48μ

respectively for 'B', 'C' and 'D' type cells, their nuclear diameters were 16.65, 12.48 and 6.67μ respectively for 'B', 'C' and 'D' type cells. Type 'E' cells do not exhibit any variation (Fig 9.11).

Weak Blue Clawed Male : For 'B', 'C' and 'D' type cells, the cell diameters were 91.97, 38.63 and 20.65μ, respective. The respective nuclear diameter of 'B', 'C' and 'D' type cells were 18.32, 12.90 and 8.66μ. Type 'E' cells do not exhibit any variation (Fig 9.12).

Strong Blue Clawed Male : The average cell diameters of 96.24, 44.96 and 21.65μ were recorded for 'B', 'C' and 'D' types of cells. The average nuclear diameters of 19.98, 14.98 and 10μ were recorded for 'B', 'C' and 'D' types of cells. Type 'E' cells do not exhibit any variation (Fig 9.13).

Old Blue Clawed Male : The average cell diameter of the 'B', 'C' and 'D' type cells recorded were 79.92, 33.32 and 18.73μ respectively. The nuclear diameter measured on an average size of 16.65, 12.48 and 7.49μ in the 'B', 'C' and 'D' type cells respectively. Type 'E' cells do not exhibit any variation (Fig 9.14).

Thoracic ganglion and its neurosecretory centres

The ventral nerve cord of *M. rosenbergii* consists of a series of fused ganglia and connectives which are seventeen in number. Though in the subesophageal and thoracic regions the composition of each median ganglion by two units is very clear by the presence of a median furrow, in the abdominal region it is hardly discernible

externally and can be made out only by the presence of two separate neuropiles in the interior.

The ganglia and the connectives forming the ventral nerve cord can be divided into three portions. They are (i) the subesophageal nerve chain consisting of three pairs of post oral ganglia which are cephalic in position and supply the last three cephalic appendages, (ii) the thoracic nerve chain which supplies nerves to the three pairs of maxillipeds and five pairs of peracopods and (iii) the abdominal nerve chain which incorporates the remaining six pairs of ganglia.

The ganglia, their connectives and the nerves to the appendages are covered by a perineurial sheath formed of elongated, dorsoventrally flattened cells with small nuclei. Besides the outer sheath of perineurial cells, the interior of the entire ventral nerve cord contains a lot of interspersed connective tissue nuclei. All the seventeen pairs of ganglia possess cells which appear to be secretory, along with the ordinary neurons. They occupy the ventral and lateral positions of the paired neuropiles of these ganglia. The perineurial as well as endoneurial cells of the ventral nerve cord, like similar cells in the optic lobe peduncle and the brain are secretory and the substances expelled also appear to be identical. They travel along the interaxonal spaces, and in many instances aggregations of these substances have been observed in the neuropiles as well.

Distribution of neurosecretory cell groups in the thoracic ganglion of *M. rosenbergii*

In the ventral nerve cord, groups of cells consisting of both neurosecretory and ordinary neurons are located on the ventral half of each ganglia. In the thoracic ganglion, the neurosecretory cells are arranged in four groups (Fig 9-T) These are a single anterior, a single posterior, a single median and three pairs of lateral groups. The lateral groups are designated as the anterio-lateral, mid-lateral and the posterio-lateral groups.

Neurosecretory cell types- thoracic ganglion

'A' type neurosecretory cells : The 'A' type cells are the largest among the neurosecretory cells of the thoracic ganglion, but they are only very few in number. They are larger than the 'B' type cells of the brain. These are round or ovoid in shape and measure from 81.58 to 159.84μ. The nucleus is large and centrally placed and on an average measures from 13.32 to 26.64μ. The nucleolus is also comparatively large and centrally placed. The cells show variations in size and stainability indicating that it may be taking part in the elaboration of the neurosecretory products. The axons of the 'A' cells are very distinct and homogeneous, always staining bluish with Gomori's chromealum haematoxylin phloxin and pinkish with triple Mallory's stain. The secretory products do not agglomerate to form larger droplets. Larger vacuoles are observed in the cytoplasm, which at certain periods of intense release acquire large dimensions almost filling the whole of the cytoplasm. Though axonal migration of secretory products is observed, peripheral secretion also is suggested

as a mode of release along the periphery of the cells due to the presence of appreciable quantity of the secretory granules in the intercellular spaces between them.

'B' type neurosecretory cells : 'B' type cells are also few in number in the thoracic ganglion neurosecretory cells. They are almost spherical in shape, but because of the presence of three or more dendrites, may often assume a polygonal appearance. The nuclei are large and spherical with a large single nucleolus in the center. These cells also show variations in size suggesting an active participation in the elaboration of the secretory products. The size of the cell ranges from 49.95 to 63.27μ.

'C' type neurosecretory cells : The 'C' type cells are oval or round in shape and the size ranges between 33.33 and 56.61μ. The nuclei are spherical and measure on an average from 6.67 to 10μ in diameter, with a single nucleolus. Vacuoles are absent in the cytoplasm. As the cell reaches its peak of activity, the secretory matter increases in size probably as a result of confluences of smaller granules. Axonal and peripheral secretions are present in these cells. The axons have a pale pink colour and the secretory products can be seen as droplets in them.

'D' type neurosecretory cell : 'D' type neurosecretory cells are smaller than 'c' type cells, ranging between 18.32 and 22.47μ in diameter and round to oval in shape. The cytoplasm in these cells is scarce and without vacuoles. The nuclei are comparatively larger. They exhibit neurosecretory cycle. The staining properties of these cells are similar to those of 'A' type cells.

'E' type neurosecretory cells : 'E' type neurosecretory cells are the smallest of all, having an average diameter of 6.67μ and round in shape. Small size, little cytoplasm relative to the size of the nucleus and scarcity of neurosecretory materials are the characteristics of these cells. The nuclei occupy the greater part of the cell bodies. Each nucleus contains a single nucleolus. These cells do not exhibit sufficient neurosecretory activity. They take pinkish with Mallory's triple stain and is positive to Gomori's chromealum haematoxylin phloxin.

Distribution of different types of neurosecretory cells in the thoracic ganglion neurosecretory centres

All the five types ('A', 'B', 'C', 'D' and 'E') of neurosecretory cells are present in the thoracic ganglion. Type 'C', 'D' and 'E' cells are present in the lateral groups. All the five types are present in the anterior group and the median group of the thoracic ganglion. More 'C' and few 'B', 'D' and 'E' cells are found in the posterior group.

Neurosecretory cell type in the thoracic ganglion of various male morphotypes and their transitional stages of *M. rosenbergii*.

Small male : The average cell diameter of the 'A', 'B', 'C' and 'D' type cells recorded were 93.24, 57.94, 40.39 and 19.98μ respectively. The nuclear diameter measured on an average size of 16.65, 14.65, 8.33 and 6.67μ in the 'A', 'B', 'C' and 'D' type cells respectively. The average cell and nuclear diameter of 'E' type cells were 6.67 and 3.33μ respectively (Fig 9.15)

Weak Orange Clawed male : For 'A', 'B', 'C' and 'D' type cell diameters were 116.55, 59.94, 39.96 and 20.82μ, respectively. The respective cell diameters of 'A', 'B', 'C' and 'D' type cells were 23.31, 14.98, 7.91 and 6.67μ. Type 'E' cells do not exhibit any variation (Fig 9.16).

Strong Orange Clawed Male : The average cell diameters of 81.59, 49.95, 33.33 and 18.32μ were recorded for 'A', 'B', 'C' and 'D' types of cells. The average nuclear diameter respectively are of 13.32, 10.82, 6.67 and 6.67μ were recorded for 'A', 'B', 'C' and 'D' type cells. Type 'E' cells do not exhibit any variation (Fig 9.17).

Pre-transforming Orange Clawed Male : The average cell diameter of the 'A', 'B', 'C' and 'D' type cells recorded were 106.56, 54.94, 41.63 and 21.65μ respectively. The nuclear diameter measured on an average size in the order of 19.15, 11.66, 8.74 and 6.67μ in the 'A', 'B', 'C' and 'D' type cells respectively. Type 'E' cells do not exhibit any variation (Fig 9.18).

Weak Blue Clawed Male : While in WBC morphotype the cell diameters were 113.22, 58.27, 44.96 and 20.76μ respectively for 'A', 'B', 'C' and 'D' type cells, their nuclear diameters were 19.98, 13.32μ, 9.99 and 6.67μ respectively for 'A', 'B', 'C' and 'D' type cells. Type 'E' cells do not exhibit any variation (Fig 9.19)

Strong Blue Clawed Male : The average cell diameters of 159.84, 63.27, 56.61 and 22.47μ were recorded for 'A', 'B', 'C' and 'D' types of cells. The average nuclear diameter of 26.64, 16.65, 13.32 and 6.67μ

were recorded for 'A', 'B', 'C' and 'D' types of cells. Type 'E' cells do not exhibit any variation (Fig 9.20).

Old Blue Clawed Male : For 'A', 'B', 'C' and 'D' type cells the cell diameters were 96.57, 52.45, 42.18 and 19.15μ, respective. The respective cell diameters of 'A', 'B', 'C' and 'D' type cells were 17.48μ, 12.48, 8.33 and 6.67μ. Type 'E' cells do not exhibit any variation (Fig 9.21).

Discussion

The decapodan neurosecretory systems located in the eye stalk, brain and the thoracic ganglion are known to contain a variety of hormones or factors apparently governing such diverse functions as growth, moulting, metabolic rate, metabolism of sugars and protein, water balance, dispersion of pigment and sexual activity (Lockwood, 1968; Adiyodi and Adiyodi, 1970). With the view to investigate the structure of these neurosecretory systems and the role of neurohormones produced by them on morphotypic transformation these investigations have been carried out in three main male morphotypes and their four transitional stages of *M. rosenbergii* and the results are presented in the Tables 1 to 21.

Detailed examination of the serial sections of the eye stalk, brain and the thoracic ganglion revealed that they contain certain specialized cells. These cells differ from the ordinary nerve cells by having neurosecretory material in them and hence postulated as neurosecretory cells. Depending on their cytological characteristics, such as the shape, size, presence of axons, condition of cytoplasm, the shape and size of the

nuclei and the staining properties, the neurosecretory cells of the eye stalk neurosecretory system (optic ganglion), brain (cerebral ganglion) and the thoracic ganglion of *M. rosenbergii* are differentiated and designated as types 'A', 'B', 'C', 'D' and 'E'.

Eye stalk : The optic ganglion of *M. rosenbergii* is composed of four parts, namely Lamina ganglionaris (LG), Medualla externa (ME), Medulla interna (MI) and Medulla terminalis (MT) situated one behind the other in anterio-posterior sequence. Only three types, ('C', 'D' and 'E') of NSC are observed to be distributed in the optic ganglion.

The gross anatomy of the eye stalk ganglia conforms to the descriptions of Hanstrom(1947), Mayrot (1956), Pillay (1961b), Satiya and and Bajaj (1967), Thampy and John (1972) in the well developed condition of the LG, ME, MI and MT and in the respective shapes of these structures. Thus the optic ganglia show more or less the same morphological features as in the case of other crustaceans except in certain caverniculous forms like *Cambarus setoses* (Fingerman *et al.*, 1964) where this ganglion is very much reduced.

Regarding the nomenclatures used for describing some secretory centers in the eye stalk of Crustacea, there appears to be no uniformity. The distribution of the neurosecretory cell groups in the eye stalk of various male morphotypes and their transitional stages of *M. rosenbergii* has been described and the NSC are found scattered in the LG while they are found to form specific cell groups in the ME, MI, and MT. Carlisle (1953c, 1959c) described the location of NSC groups in the eye stalk of other Natantians like *Lysmata seticaudata*

and *Pandalus borealis*. In the former, he described two cell groups, the X-organs and in the latter, four cell groups, two associated with medulla terminalis and one each in the medulla interna and externa. The distribution of the neurosecretory cell groups in the eye stalk of *Caridina* has been described by Pillai (1961). He observed five cell groups of which three were found in the medulla terminalis, one in between the medulla terminalis and medulla interna and another in between meduallа interna and externa. Orientation of NSC groups of the optic ganglion of various male morphotypes of *M.rosenbergii* are identical to those of *M. lamarrei* as described by Nagabhushanam (1986). The occurrence of two groups of the NSC in the ME of *M.rosenbergii* is similar to those as reported by Rangnekar and Madhyastha (1974) in *Metapenaeus monoceros* and Joshi (1980) in *Parapenaeopsis hardwickii*. However, in *Parapenaeopsis stylifera*, only one NSC group is reported in its ME (Joshi, 1980). The NSC group is reported to be absent in the MI of *Palaemon serratus* (Van Herp *et al.*, 1977) and *P. stylifera* and *P. harwickii* (Joshi 1980). However, a single group of NSC is present in the MI of *M. rosenbergii* as reported by Nagabhushanam and Vasantha (1972) in *Caridina weberi*; Hisano (1974) in *Palaemon paucidens*; Sarojini and Victor (1985) in *Caridina rajadhari;* Nagabhushanam (1986) in *M. lamarrei* and Nanda and Ghosh (1985) in *Penaeus. monodon*. The occurrence of two NSC groups in the MT of *M.rosenbergii* conforms to the observation of Nagabhushanam (1986) in *M. lamarrei*. However, it does not agree with the finding of Joshi(1980) who reported three NSC groups in the MT of *P. stylifera* and *P. hardwickii*.

Though a substantial volume of data is available on the neurosecretory system in crustaceans, classification of neurosecretory cells is found incomplete and even the identification and localization of cell types forming groups in the optic ganglion are not always unequivocal. Furthermore, there is parallelism on the basis of classification adopted by several investigators like Enami (1951), Durand (1956), Miyawaki (1956), Matsumoto (1958), Pillai (1961), Nagabhushanam and Rao (1966), Nagabhushanam and Vasantha (1972), Smith and Naylor (1972), Hisano (1974) and Bellon-Humbert *et al.* (1981), who worked on multiple species of the suborders Reptantia and Natantia of the order Decapoda. In all the above cases, priority has been attributed to the shape, size and the staining criteria for the classification of unique identifiable neurons.

The present study revealed that three cell types contributed to the formation of groups of secretory neurons in the optic ganglia of various male morphotypes and their transitional stages of *M. rosenbergii*. Involvement of these three cell types in respective groups resulted in the orientation of the optic ganglionic components in various male morphotypes of *M. rosenbergii* as postulated by a number of investigators like Smith and Naylor (1972) in *Carcinus maenas*, Hisano (1974) in *Palaemon paucidens* and Bellon-Humbert *et al.*1981) in *Penaeus serratus.*

various authors have described differently the presence of neurosecretory cell types in the optic ganglion of various species of craustaceans. Only one type of NSC in the optic ganglion of *Rivulogammarus syriacus* has been reported by Baid and Dabbagh

(1972). Thampy and John (1972) reported three types of cells in the optic ganglia of *Caridina natarajani* Thiwari Pillai. Nagabhushanam (1986) reported the presence of two types of NSC in the optic ganglion of *Macrobrachium lamarrei*. Three types of size variations have been observed in the NSC of the optic ganglion of *Caridina rajadhari* (Sarojini and Victor, 1985), *Metapenaeus affinis* (Sambasiva Rao *et al.*, 1986) and *Macrobrachium kistnensis* (Sarojini *et al.*, 1987). Joshi (1980) observed five types of NSC in the optic ganglion of *P. stylifera* and *P. hardwickii*. On the contrary, in *M. rosenbergii* only three types of NSC are recorded in its optic ganglia and this finding corroborates the view expressed by Thampy and John (1972), Sarojini and Victor (1985), Sambasiva Rao *et al.* (1988) and Sarojini *et al.* (1987).

The 'C' type cells of the present investigation closely resemble or comparable with Hisano's (1974) type 'III' cells in *Palaemon spp*, 'V' type cells of *P. stylifera* reported by Joshi (1980), type '2' cells of *M. affinis* described by Sambasiva Rao *et al.* (1986), and 'C' type cells of *M. lamarrei* described by Nagabhushanam (1986). Type 'D' cells are comparable with type 'V' cells of *Palaemon spp* of Hisano's (1974), 'VIII' type cells of *P. stylifera* of Joshi (1980); 'I' type cells of *M. affinis* of Sambasiva Rao *et al.* (1986) and 'C' type cells of *M. kistnensis of* Sarojini *et al.* (1987). 'E' type cell, the smallest one, corresponds to or is comparable with Enami's (1951) type 'Y' cell, Pillai's (1960) 'IVth' type cell, Hisano's (1974) type 'VI' cells and type 'I' cells of Smith and Naylor (1972).

As evidenced by cell diameter, nuclear diameter and vacuoles in their cytoplasm, 'C' and 'D' type NSC of *M. rosenbergii* can

be considered in the active state. On the other hand, the 'E' type NSC show only less amount of the neurosecretory materials and therefore, they may be classified into less active stage.

Brain : Distribution of the neurosecretory cells in the brain of various morphotypes and their transitional stages of *M. rosenbergii*, in the present study, revealed the presence of four groups of neurosecretory cells which are located in the anterior (one), posterior (one) and lateral groups (two). This generally agrees with those described for *Orconectes virilis* (Durand, 1956); *Cambarus setosus* (Fingerman, *et al.*, 1964); *M. kistnensis* (Mirajkar, 1980); *M. lamarrei* (Gyananath, 1982) but differs from that of the marine prawn, *Metapenaeus affinis* (sambasiva Rao *et al.*, 1988).

Four types of NSC viz. 'B', 'C', 'D' and 'E' were recorded from the brain of *M. rosenbergii* in the present investigation. In a few crustaceans, only two types of cells have been reported in the brain like that of the crab, *Potamon magnum magnum* (Baid *et al.*, 1967). In most of the decapod crustaceans, only three types of NSC are reported from the brain, such as the crabs, *Paratelphusa hydrodromous* (Parameswaran, 1956), *Scylla serrata* (Nagabhushanam and Ranga Rao, 1966), *Paragrapsus gaimardii* (Lake, 1970a), *Barytlephusa cunicularis* (Diwan and Nagabhushanam, 1975) and the fresh water prawn *Caridina rajadhari* (Victor and Sarojini, 1985). Four types of NSC were described in *Caridina* (Pillai, 1961) and in *Orconectes virilis* (Durand, 1956). Similarly, four types of NSC were reported in the prawn *Parapenaeopsis stylifera* (Joshi, 1980) and in *Macrobrachium lamarrei* (Gyananath, 1982). Out of the four types of the NSC found in

the brain of various male morphotypes and their transitional stages of *M. rosenbergii*, only three types i.e, 'B', 'C' and 'D' have shown the cyclic secretory activity in the present study.

'B' cells of the brain of *M.rosenbergii* do not show any appreciable amount of axonal migration of secretory material and thus resembling A-cells of *Sesarma* and *Eriocheir* (Matsumoto, 1958). Axonal release of the secretory materials is very limited in the 'C' cells of the brain of *M. rosenbergii* resembling the condition described for *Paratelphusa* (Adiyodi, 1967). In this respect, these cells differ from the 'B' cells of the five species of crabs described by Matsumoto (1958). The presence of the neurosecretory materials in 'C' type cells indicates that they are functionally active.

Thoracic ganglion : The neurosecretory cells (NSC) of the thoracic ganglion have been described to a considerable extent in reptantians (Enami 1951; Matsumoto 1954, 1958; Parameswaran 1956; Miyawaki 1960; Nagabhushanam and Ranga Rao 1956; Gorgees and Rashan 1977). However, similar studies on the natantian decapodes are meager (Nagabhushanam and *Vasantha, 1972; Ramadan and Matta, 1976; Narasimha Rao et al.*, 1981). In brachyuran crustaceans, morphological (Matsumoto 1958, 1962) and experimental (Otsu, 1963; Gomez, 1965; Hinsch and Bennett, 1979) evidences have indicated that the thoracic ganglion is the major site of neuroendocrine factors with gonad accelerating effect and this principle is generally termed the gonad stimulating hormone (GSH). Among the natantians even though there are indications of the presence of neurosecretory cells on the ventral cord as reported by

Carlisle and Knowles (1959) and Pillai (1958), no detailed information on the histology of the system is available. It has been reported that NSC are restricted to the ventral half of each ganglia as in the case of *Homarus vulgaris* (Johanson and Schreiner, 1965). Parameswaran (1956) has described three types of NSC for the thoracic ganglion of *Paratelphusa hydrodromous.*

In the present study the five types of NSC viz. 'A', 'B', 'C', 'D' and 'E' were recorded from the thoracic ganglion of various male morphotypes and their transitional stages of *M. rosenbergii.* Type 'A' cells are the largest NSC encountered in the whole neurosecretory system. Because of their exceptionally large size, they are termed as giant cells. They are comparable to the 'B' type cells found in the thoracic ganglion of *Metapenaeus affinis* (Srojini *et al.*, 1987).

In the thoracic ganglion of *M. rosenbergii,* the axonal migration of the secretory product is not very pronounced. In most of the cells which are located in the periphery of the ganglia, the products of secretion are liberated directly into the body fluid. On the whole peripheral secretion is predominant and the secretory products both basophil and acidophil discharged from the periphery of the cells, reach the tissue fluid.

A detailed study of the histophysiology of the neurosecretory cells in the eye stalk, brain and thoracic ganglion of various male morphotypes and their transitional stages of *M. rosenbergii* revealed that concomitant with the different stages in their developmental pathway from one morphotype to another

morphotype (SM →WOC →SOC →t-SOC →WBC →SBC →OBC), the neurosecretory cell types also demonstrate cytological changes as evidenced by the variation in cell diameter, nuclear diameter and intensity of neurosecretory material.

Variations in the cell diameter and nuclear diameter could be observed in the 'C' and 'D' type cells in the eye stalk of various male morphotypes and their transitional stages of *M. rosenbergii*. The highest value of cell diameter and nuclear diameter could be observed in the 'C' and 'D' type cells in the eye stalk of SOC morphotype whereas the lowest value of cell diameter and nuclear diameter could be observed in the 'C' and 'D' type cells of the eye stalk of SBC morphotype. The reproductive activity of these morphotypes has been studied by various researchers and reported that these male morphotypes differ from each other in their reproductive behavior (Ra'anan and Sagi 1985; Sureshkumar and Kurup, 1998) and growth rate (Ra'anan and Cohen, 1985). Orange clawed male morphotype represents a sexually inactive intermediary stage characterised by faster somatic growth (Ra'anan and Sagi, 1985; Sureshkumar and Kurup, 1998; Joseph and Kurup, 2001). On the other hand, SBC morphotypes are characterised by a highly reproductive activity and slow in growth rate (Sagi *et al.*, 1988; Sureshkumar and Kurup, 1998; Joseph and Kurup, 2001).

The crustacean eye stalks contain an endocrine organ, the X-organ sinus gland complex, hormonal secretion of which has been found to be responsible for varied physiological functions of the body (Adiyodi and Adiyodi, 1970). This has been amply supported by the observation that the removal of the eye stalk brings about many

functional disturbances (Abramowitz and Abramowitz, 1939; Abramowitz and Abramowitz, 1940; Kleinholz and Bourquin, 1941; Bliss, 1951; Passano, 1951). Several reports postulate that ablation of the eye stalk resulted in enhanced gonadal development (Hard, 1942; Brown and Jones, 1949; Vernet cornubert, 1964; Weitzman, 1964; De Leersnyder, 1967; Adiyodi and Adiyodi, 1970). Two antagonistic factors (inhibitory and stimulatory) control gonad maturation and is evident from knowledge of reproductive endocrinology (Demousy and Adiyodi, 1967). The inhibitory factor is produced by the X-organ neurosecretory cell and stimulatory factor is by thoracic ganglion and brain (Sarojini and Jhagirdar,1972). Gonadal enlargement occurs in juvenile females of *Paratelphusa* after implantation of brain (Gomez and Nayar, 1965), but other parts of the nervous system appear to be ineffective (Gomez, 1965). By these procedures gonadal development could be accelerated to nearly the same extent as with eye stalk ablation, and it is thus possible that GSH originates in the central nervous system.

The increased secretory activity of neurosecretory cells in the eye stalk of SOC resulted in the production of large amount of inhibitory hormones which in turn prevented the gonadal development and reproductive activity in SOC morphotype. On the contrary, the low amount of inhibiting hormones produced by the neurosecretory cells in the eye stalk resulted in the high reproductive activity in SBC morphotype. The present observation is in agreement with the findings the marine crab, *Scylla serrata* (John and Sivadas, 1978); the fresh water crab, *Barytelphusa cunicularis*

(Nagabhushanam and Diwan, 1974) and Shrimp *Crangon crangon* (Bomirski and Klekkavirska, 1974). These work showed presence of inhibitory gonadotropins from the eye stalks of various crustaceans, while its removal caused incessant gonadal growth whereas its replacement resulted in gonadal development suppression.

The neurosecretory cells of the brain also exhibit alterations in their structure in relation to various morphotypic stages of *M. rosenbergii*. Type 'B' 'C' and 'D' cells become more active in SBC morphotype as manifested by the increase in cell diameter and nuclear diameter where these types of cells have been found less active in SOC morphotype as evidenced by the decrease in cell diameter and nuclear diameter. The 'E' type cells do not show any variations in their size and secretory activity.

Type 'A', 'B' and 'C' cells of the thoracic ganglion are found active in the SBC morphotype. In this morphotype, the increase in the cell diameter and nuclear diameter of NSC was recorded maximum and the lowest values were recorded in SOC morphotype. The 'D' and 'E' type cells of the thoracic ganglion are not found to be significantly active in these morphotypes.

The brain and thoracic ganglion of various male morphotypes of *M. rosenbergii*, like the optic lobe peduncle, are the seats of a variety of neurosecretory cells assembled into fairly well defined groups or clusters (Parameswaran, 1955, 1956; Durand, 1956; Matsumoto, 1958, 1962; Szudarski, 1963; Kurup, 1964; Adiyodi, 1967). The NSC activity observed in the brain and thoracic

ganglion of the various male morphotypes of *M. rosenbergii* reflect the nature of their elaboration and perhaps the production of gonad stimulating hormone. From the above data, it is evident that there is considerable production of GSH by the NSC of thoracic ganglion of the SBC morphotypes, in contrast, least production of NSC hormone in SOC morphotype. The GSH produced in the thoracic ganglion might be acting indirectly on the testes through the androgenic gland and bringing about gonad maturation. The results of the present study also supported by the first histological statement of Otsu (1963). Otsu (1960, 1964) demonstrated that repeated implantation of thoracic ganglia into the sexually quiescent female crab, *Potamon dehaani*, caused considerable ovarian development. Gomez and Nayar (1965) reported that in addition to the thoracic ganglia, the brain also secretes a gonad stimulating hormone in the fresh water crab, *Paratelphusa hydrodromous*. The present observations in various male morphotypes of *M. rosenbergii* strongly corroborate the findings of Adiyodi and Adiyodi (1970) that GSH from the brain promotes gonadal growth. It has been found that repeated injections of brain and thoracic ganglia into intact male hermit crabs caused considerable testicular developments in *Paratelphusa kulkarni*. This provides sufficient proof that testicular stimulatory factors are fabricated by the brain and thoracic ganglia of *P. kulkarni*. Similar stimulatory gonadotropins released from the brain were reported by Berreur Bonnenfant (1967) in the amphipod, *Orchestia gammarella*; and (Adiyodi and Adiyodi, 1970) in the crab *P. hydrodomous*. A similar observation was made by Narasimha Rao *et al.* (1981) in *Macrobrachium lanchesteri* that GSH is produced in the thoracic

ganglion during the reproductive season. Further, the GSH elaborated in the brain and thoracic ganglion might be acting on the secondary sexual characters of the SBC morphotypes probably through a sex hormone produced in the androgenic gland through the testes. The synchronism between gonadal activity and development of the secondary sex characters suggests that these transformations may be under the influence of the hormonal mechanisms governing reproductive activity and behavior among these male morphotypes of *M. rosenbergii*. The androgenic gland hormone (AGH) controls not only differentiation of the male reproductive system, but also its functioning and development of the male sexual characters (Charniaux –Cotton, 1964; Charniaux-Cotton and Payen, 1985; Nagamine *et al.*, 1980; Payen, 1973; Puckett, 1964). There is no evidence for a testicular hormone in crustaceans (Fingerman, 1997). But there is evidence for an ovarian hormone that induces development of ovipositing setae and brood chambers (Charniaux-Cotton, 1955; Nagamine and Knight, 1987). In this connection it is interesting to note that an ovarian hormone (OH) produced in the amphipod, *Orchestia gammarella*, is known to control secondary sexual characters (Charniaux-Cotton, 1952, 1957).

Therefore, it can reasonably be asserted that the variation in the reproductive activity of various male morphotypes of *M. rosenbergii* can be presumed to be due to the elaborate production of GSH from the thoracic ganglion and brain and reduction in the gonad inhibiting hormone from the eye stalk. In short, it can be concluded that the type 'C' and 'D' cells of the eye stalk, type 'B', 'C' and 'D' cells

of brain and the type 'A', 'B' and 'C' cells of the thoracic ganglion play active role in the morphotypic transformation of various male morphotypes and their transitional stages of *M. rosenbergii* since each morphotype characterised with specific reproductive behavior and growth pattern.

TABLE 9.1

Cytological details of neurosecretory cell types in the eye stalk of Small male morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
C	Polygonal	21.31	9.99	Pinkish	Blue
D	Round to Oval	13.32	6.67	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.2

Cytological details of neurosecretory cell types in the eye stalk of Weak orange clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
C	Polygonal	22.64	10.00	Pinkish	Blue
D	Round to Oval	14.69	6.67	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.3

Cytological details of neurosecretory cell types in the eye stalk of strong orange clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
C	Polygonal	29.30	15.32	Pinkish	Blue
D	Round to Oval	17.32	10.00	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.4

Cytological details of neurosecretory cell types in the eye stalk of pre-transforming orange clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
C	Polygonal	27.97	13.32	Pinkish	Blue
D	Round to Oval	16.65	8.66	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.5

Cytological details of neurosecretory cell types in the eye stalk of Weak blue clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
C	Polygonal	24.64	10:00	Pinkish	Blue
D	Round to Oval	15.98	7.99	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.6

Cytological details of neurosecretory cell types in the eye stalk of strong blue clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
C	Polygonal	18.65	6.67	Pinkish	Blue
D	Round to Oval	11.32	3.33	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.7

Cytological details of neurosecretory cell types in the eye stalk of old blue clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
C	Polygonal	17.33	6.67	Pinkish	Blue
D	Round to Oval	9.99	3.33	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.8

Cytological details of neurosecretory cell types in the brain of small male morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
B	Round to oval	88.25	14.57	Pinkish	Blue
C	Round	31.32	13.32	Pinkish	Blue
D	Oval	19.98	8.66	Pinkish	Blue
E	Spherical	10.00	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.9

Cytological details of neurosecretory cell types in the brain of weak orange clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
B	Round to oval	86.58	14.15	Pinkish	Blue
C	Round	31.64	13.73	Pinkish	Blue
D	Oval	19.98	8.32	Pinkish	Blue
E	Spherical	10.00	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.10

Cytological details of neurosecretory cell types in the brain of strong Orange clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
B	Round to oval	73.23	12.48	Pinkish	Blue
C	Round	24.97	11.65	Pinkish	Blue
D	Oval	11.65	6.67	Pinkish	Blue
E	Spherical	10.00	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.11

Cytological details of neurosecretory cell types in the brain of Pre-transforming Orange Clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
B	Round to oval	83.25	16.65	Pinkish	Blue
C	Round	29.97	12.48	Pinkish	Blue
D	Oval	17.48	6.67	Pinkish	Blue
E	Spherical	10.00	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.12

Cytological details of neurosecretory cell types in the brain of weak blue clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
B	Round to oval	91.97	18.32	Pinkish	Blue
C	Round	38.63	12.90	Pinkish	Blue
D	Oval	20.65	8.66	Pinkish	Blue
E	Spherical	10.00	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.13

Cytological details of neurosecretory cell types in the brain of Strong blue clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
B	Round to oval	96.24	19.98	Pinkish	Blue
C	Round	44.96	14.98	Pinkish	Blue
D	Oval	21.65	10.00	Pinkish	Blue
E	Spherical	10.00	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.14

Cytological details of neurosecretory cell types in the brain of old blue clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
B	Round to oval	79.92	16.65	Pinkish	Blue
C	Round	33.30	12.48	Pinkish	Blue
D	Oval	18.73	7.49	Pinkish	Blue
E	Spherical	9.99	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.15

Cytological details of neurosecretory cell types in the thoracic ganglion of small male morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
A	Round to oval	93.24	16.65	Pinkish	Blue
B	Round	57.94	14.65	Pinkish	Blue
C	Round to oval	40.39	8.33	Pinkish	Blue
D	Round to oval	19.98	6.67	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.16

Cytological details of neurosecretory cell types in the thoracic ganglion of weak orange clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
A	Round to oval	116.55	23.31	Pinkish	Blue
B	Round	59.94	14.98	Pinkish	Blue
C	Round to oval	39.96	7.91	Pinkish	Blue
D	Round to oval	20.82	6.67	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.17

Cytological details of neurosecretory cell types in the thoracic ganglion of strong orange clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
A	Round to oval	81.59	13.32	Pinkish	Blue
B	Round	49.95	10.82	Pinkish	Blue
C	Round to oval	33.33	6.67	Pinkish	Blue
D	Round to oval	18.32	6.67	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.18

Cytological details of neurosecretory cell types in the thoracic ganglion of Pre-transforming orange clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
A	Round to oval	106.56	19.15	Pinkish	Blue
B	Round	54.94	11.66	Pinkish	Blue
C	Round to oval	41.63	8.74	Pinkish	Blue
D	Round to oval	21.65	6.67	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.19

Cytological details of neurosecretory cell types in the thoracic ganglion of weak blue clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
A	Round to oval	113.22	19.98	Pinkish	Blue
B	Round	58.27	13.32	Pinkish	Blue
C	Round to oval	44.96	9.99	Pinkish	Blue
D	Round to oval	20.76	6.67	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

* Gomori's chromealum haemotoxylin phloxin

TABLE 9.20

Cytological details of neurosecretory cell types in the thoracic ganglion of Strong blue clawed morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
A	Round to oval	159.84	26.64	Pinkish	Blue
B	Round	63.27	16.65	Pinkish	Blue
C	Round to oval	56.61	13.32	Pinkish	Blue
D	Round to oval	22.47	6.67	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

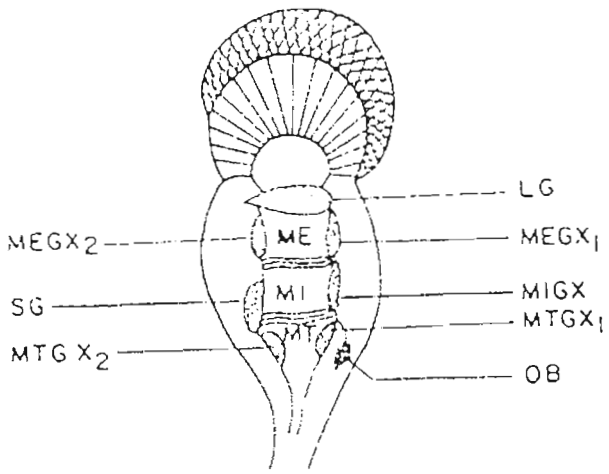
* Gomori's chromealum haemotoxylin phloxin

TABLE 9.21

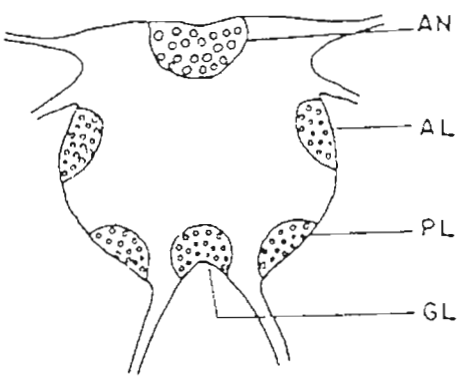
Cytological details of neurosecretory cell types in the thoracic ganglion of old blue clawed male morphotype of *M. rosenbergii*

Cell type	Shape of the cell	Average cell diameter (μ)	Average Nuclear diameter (μ)	Staining property	
				Mallory's triple	GCHP *
A	Round to oval	96.57	17.48	Pinkish	Blue
B	Round	52.45	12.48	Pinkish	Blue
C	Round to oval	42.18	8.33	Pinkish	Blue
D	Round to oval	19.15	6.67	Pinkish	Blue
E	Spherical	6.67	3.33	Pinkish	Blue

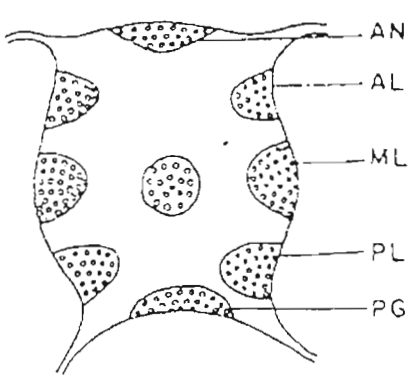
* Gomori's chromealum haemotoxylin phloxin



Schematic diagram of the neurosecretory cell groups in the Eye stalk of Macrobrachium rosenbergii (de Man)



Schematic diagram of the neurosecretory cell groups in the brain of Macrobrachium rosenbergii (de Man)



Schematic diagram of neurosecretory cell groups in the Thoracic ganglion of Macrobrachium rosenbergii (de Man)

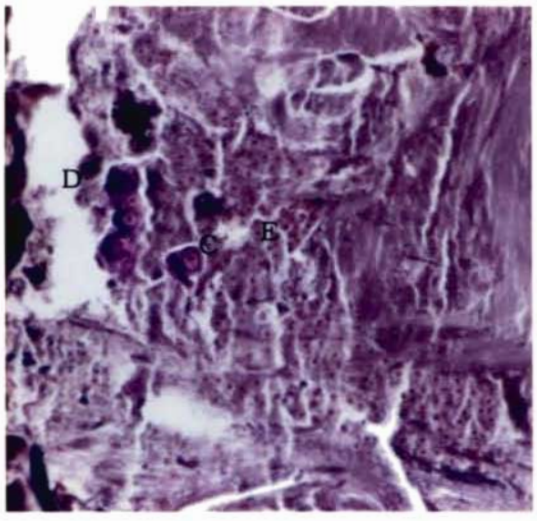


Fig 9.1 : LS of eyestalk of SM showing NSC types C, D and E (x100)

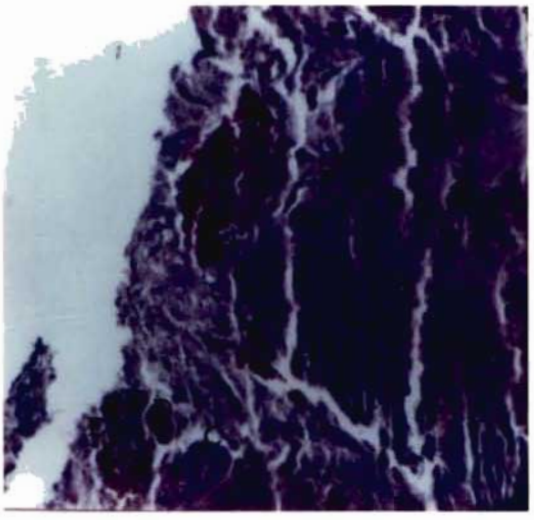


Fig 9.2 : LS of eyestalk of WOC showing NSC types C, D and E (x100)

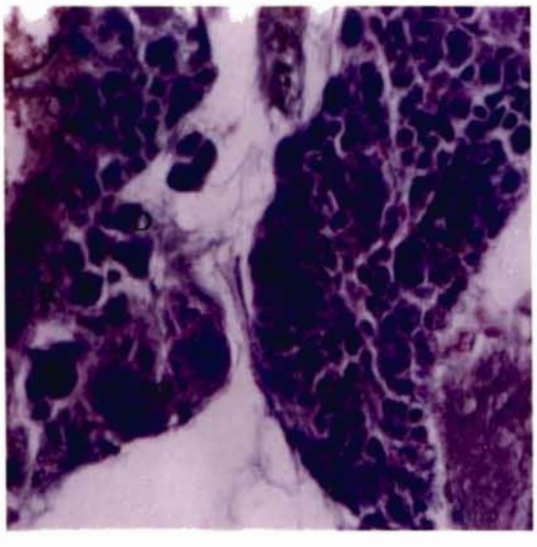


Fig 9.3 : LS of eyestalk of SOC showing NSC types C, D and E (x100)

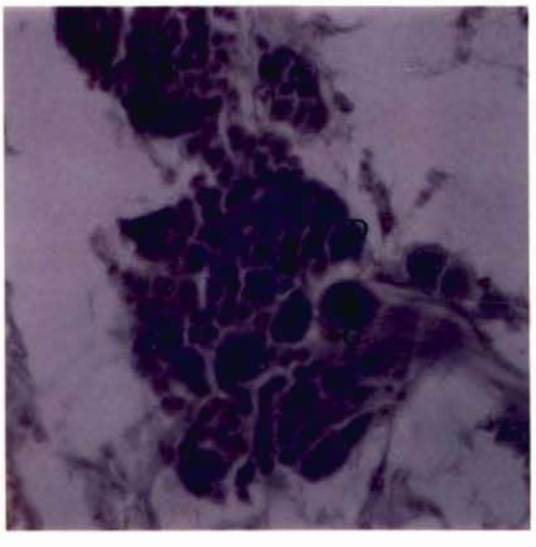


Fig 9.4 : LS of eyestalk of t-SOC showing NSC types C, D and E (x100)

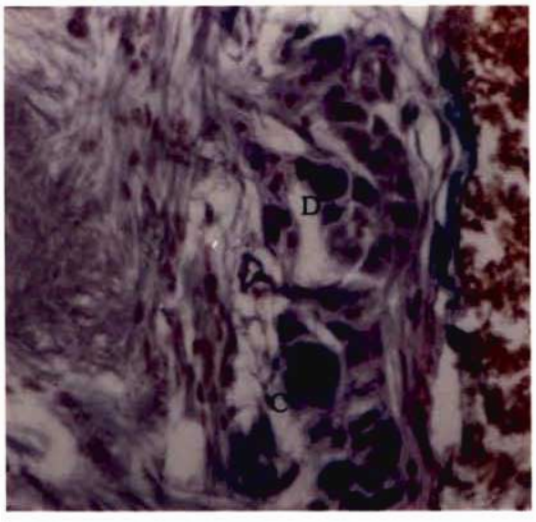


Fig 9.5 : LS of eyestalk of WBC showing NSC types C, D and E (x100)

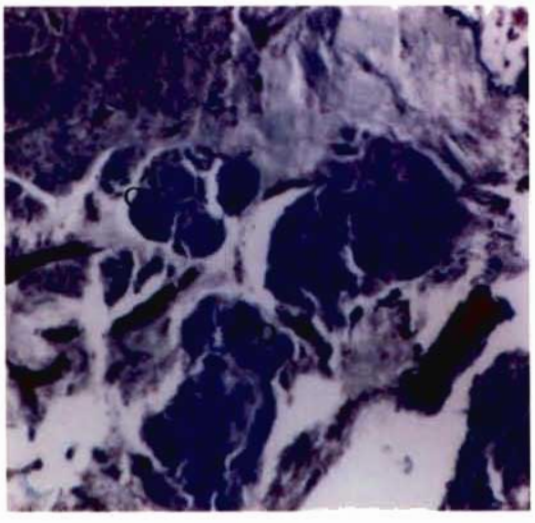


Fig 9.6 : LS of eyestalk of SBC showing NSC types C, D and E (x100)

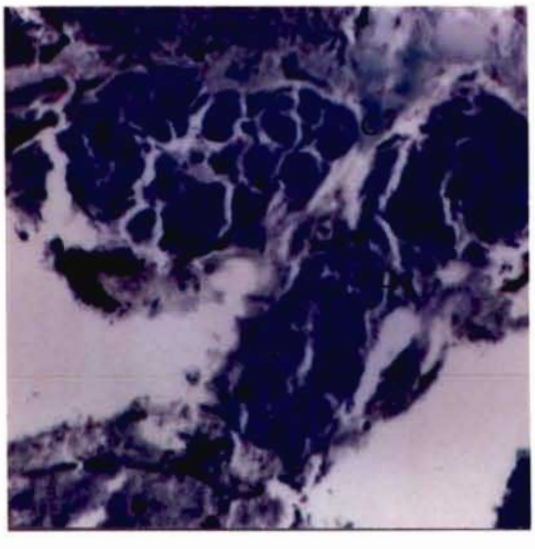


Fig 9.7 : LS of eyestalk of OBC showing NSC types C, D and E (x100)

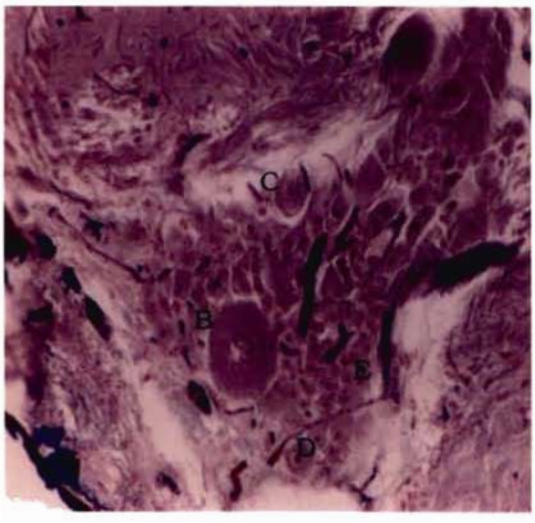


Fig 9.8 : TS of brain of SM showing NSC types B, C, D and E

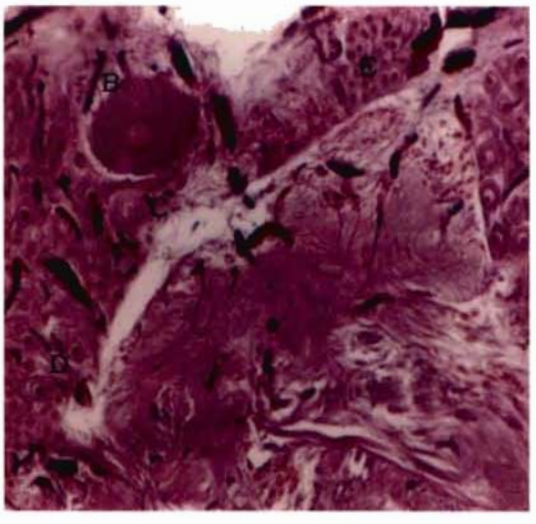


Fig 9.9 : TS of brain of WOC showing NSC types B, C, D and E

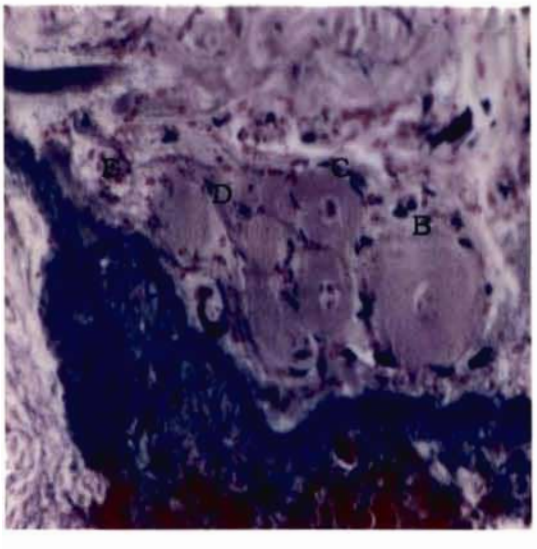


Fig 9.10 : TS of brain of SOC showing NSC types B, C, D and E

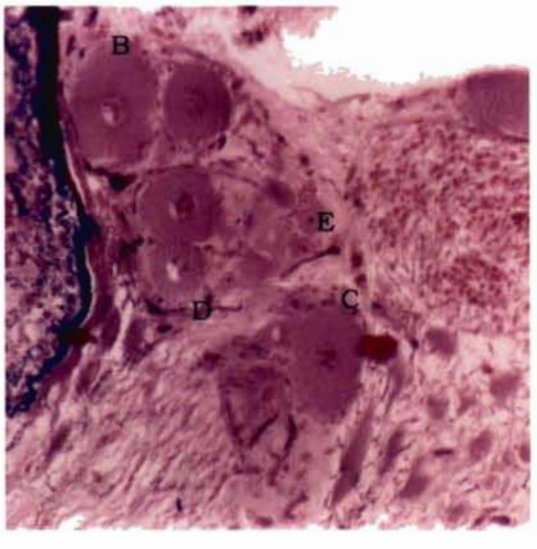


Fig 9.11 : TS of brain of t-SOC showing NSC types B, C, D and E

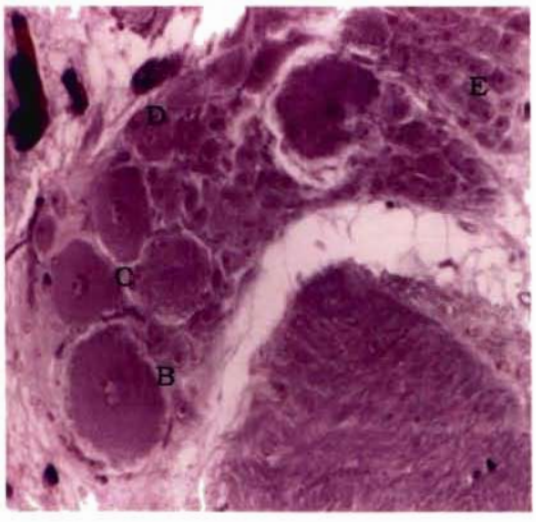


Fig 9.12 : TS of brain of WBC showing NSC types B, C, D and E

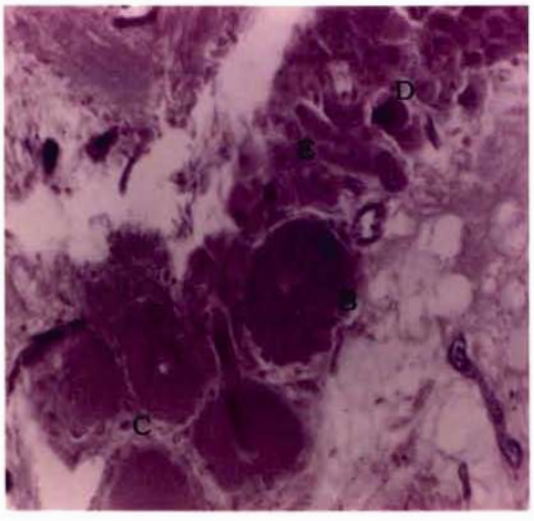


Fig 9.13 : TS of brain of SBC showing NSC types B, C, D and E

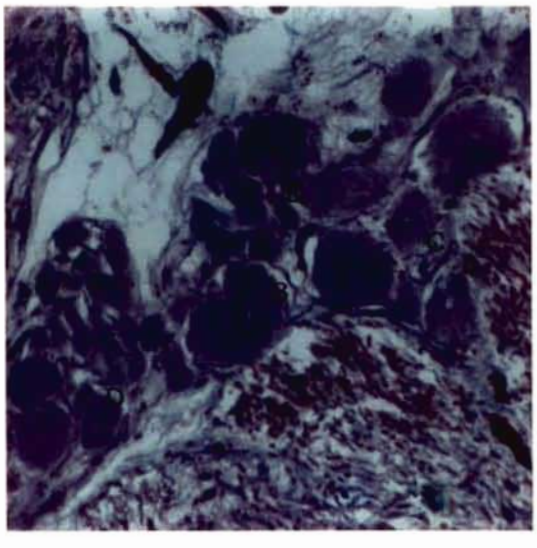


Fig 9.14 : LS of eyestalk of OBC showing NSC types C, D and E (x100)

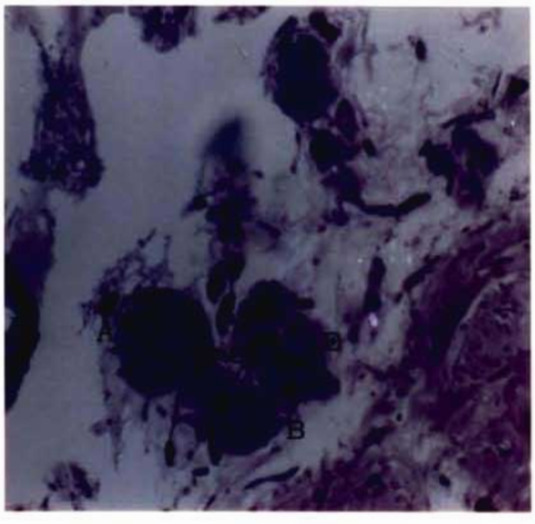


Fig 9.15 : TS of thoracic ganglion of SM showing NSC types A, B, C, D and E

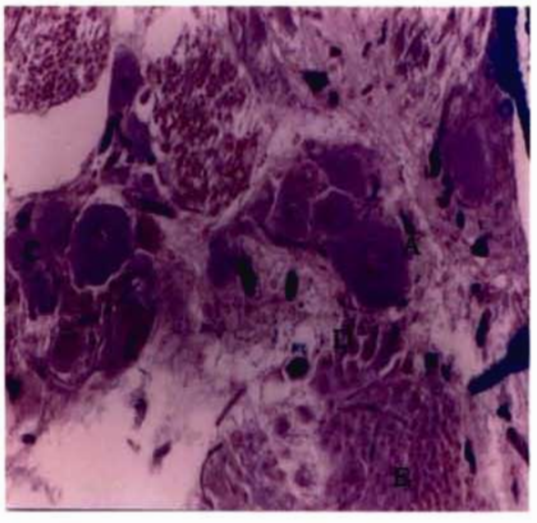


Fig 9.16 : TS of thoracic ganglion of WOC showing NSC types A, B, C, D and E

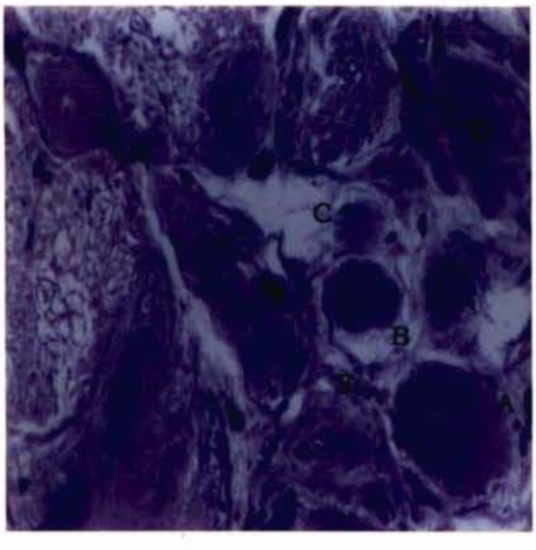


Fig 9.17 : TS of thoracic ganglion of SOC showing NSC types A, B, C, D and E

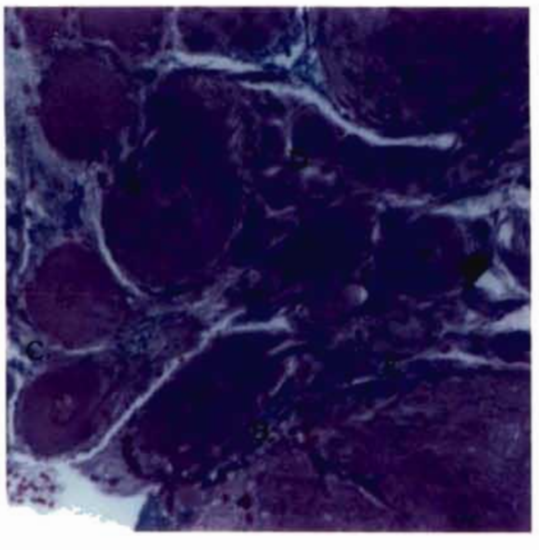


Fig 9.18 : TS of thoracic ganglion of t-SOC showing NSC types A, B, C, D and E

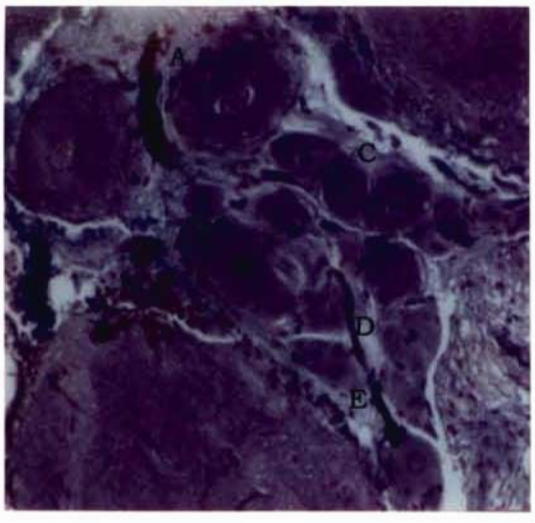


Fig 9.19 : TS of thoracic ganglion of WBC showing NSC types A, B, C, D and E

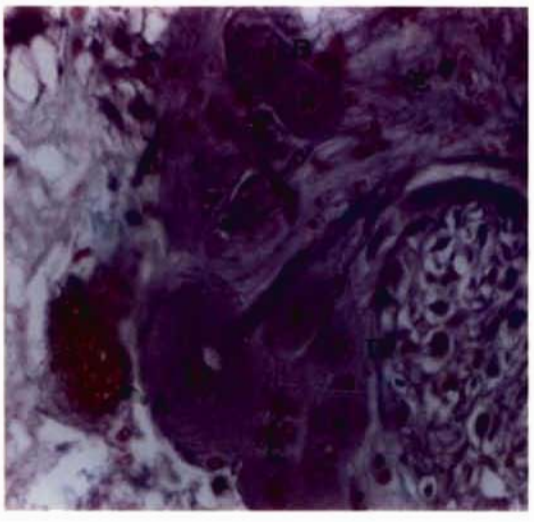


Fig 9.20 : TS of thoracic ganglion of SBC showing NSC types A, B, C, D and E

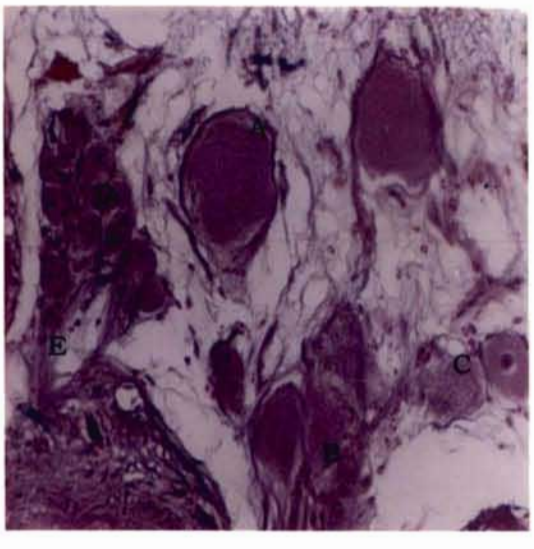


Fig 9.21 : TS of thoracic ganglion of OBC showing NSC types A, B, C, D and E

SECTION 3

HISTOCHEMICAL CHARACTERISATION OF MALE MORPHOTYPES AND THEIR TRANSITIONAL STAGES OF *Macrobrachium rosenbergii* (de Man)

CHAPTER 10

HISTOCHEMISTRY OF REPRODUCTIVE SYSTEM

Histochemistry of testes

Introduction

The structure of male reproductive organs in crustacea was described by Spalding (1942), Cronin (1947), King (1948), Ryan (1967), Langreth (1969), Reger and Fain-Maurel (1973), Wolfe (1971), Chiba and Honma (1971), Diwan and Nagabhushanam (1974), Gupta and Chatterji (1976), Wielgus (1976), Joshi and Khanna (1982), Sarojini and Gyananath (1984),Butcher and Fielder (1994). In contrast, there is very little information on the histochemical aspects of male reproductive organs of fresh water prawns.

Materials and methods

Collection, acclimatization and segregation of various male morphotypes and their transitional stages of *M. rosenbergii* were carried out the same as described in chapter 4. For histochemical studies of protein, lipid, glycogen, RNA and DNA, the techniques used were the same as in chapter 4.

Protein : In primary and secondary spermatogonial cells of the testes of SM, WOC and t-SOC morphotypes, the chromatin material of the nucleus appeared to be in the form of discrete bodies which stain positively to Bromephenol blue. Similarly, the testes of SOC and WBC morphotypes which mainly consist of primary and secondary spermatocytes, the thread like chromatin material gathers on one

side of nucleus and takes deep stain which indicate the presence of protein. In SBC and OBC morphotypes which are characterised by the presence of mature sperm, deep positive colours could be observed in the nucleus of spermatozoa (Figs10.1.1 to 10.1.7).

Lipids : The Sudan Black B test was applied on the fixed sections of the testes of various male morphotypes. viz. SM, SOC and SBC and their transitional stages viz. WOC, t-SOC, WBC and OBC of *M. rosenbergii*.It was found that in SM, WOC, SOC, t-SOC, WBC and OBC morphotypes, the cytoplasm of all the spermatogenic components have diffusely distributed sudanophilic lipids.(Figs 10. 2.1 to 10.2.7). But the intensity of the reactions varies considerably with the development stages as the cytoplasm of the secondary spermatogonia stain more deeply with Sudan Black B than that of primary spermatogonia. However, Sudan Black B positive diffused lipids were in higher concentration in primary and secondary spermatocytes than those of primary and secondary spermatogonia. In SM, WOC, t-SOC, SBC and OBC, a moderately positive diffuse Sudan Black B reactions is shown by the mature spermatozoa. Besides the Sudan Black B positive diffuse lipids some sudanophilic lipids granules were also observed in the cytoplasm of all the spermatogenic cells which were perhaps mitochondrial granules. Interlobular elements such as connective tissue also react positively during these treatments.

Glycogen : The PAS technique when employed indicated the negative activity in all the constituent parts of testes except the lobular wall connective tissue of all the male morphotypes viz. SM, SOC and SBC

and their transitional stages viz. WOC, t-SOC, WBC and OBC of *M. rosenbergii* (Figs 10.3.1 to 10.3.7).

RNA and DNA : The nucleolus and cytoplasm of primary and secondary spermatogonia of the SM, WOC and t-SOC morphotypes are rich in RNA (Figs 10.4.1, 10.4.2 and 10.4.4) and revealed by the pink or red colouration with Methyl green Pyronin G Test. Cytoplasmic RNA indicating colouration is also seen in the spermatocytes of the SOC and WBC morphotypes. In spermatocytes, the thread like chromatin material gathers on one side of nucleus and takes stain for DNA. The homogeneous positive colour for DNA is also found in the nucleus of spermatids (Figs 10.4.3 and 10.4.5). It was observed that DNA is present in all the spermatozoan cells in the testes of SM, WOC, t-SOC, SBC and OBC morphotypes (Figs 10.4.1, 10.4.2, 10.4.4, 10.4.6 and 10.4.7) The highest RNA and DNA content was observed in SOC and SBC morphotypes respectively.

Histochemistry of vas deferens

Introduction

Although the reproductive anatomy of the crustaceans has received considerable attention, relatively few studies have been done in terms of histology and histochemistry of male reproductive system (Spalding, 1942; Cronin, 1947; Matthews, 1953,1954; Ryan, 1967; Wolfe, 1971; Hinsch and Walker, 1974; 1975; Deecaraman and Subramoniam, 1980; Lane, 1980; Uma and Subramoniam, 1979; Radha and Subramoniam, 1985). The present report gives the

histochemical nature of the vas deferens of various male morphotypes and their transitional stages of *M. rosenbergii.*

Materials and methods

Collection, acclimatization and segregation of various male morphotypes and their transitional stages of *M. rosenbergii* were carried out the same as described in chapter 4. For histochemical studies of protein, lipid and glycogen, the technique used were the same as in chapter 4.

Results

The histochemical tests on some important staining reactions of metabolites viz. protein, lipid and glycogen of the vas deferens of various male morphotypes viz. SM, WOC, SOC, t-SOC, WBC, SBC and OBC are illustrated in (Figs10.5.1 to 10.5.7- Protein; 10.6.1 to 10.6.7- lipid; 10.7.1 to 10.7.7- glycogen) according to the visually estimated intensities. As reported earlier, based on the functional morphpology, the vas deferens is categorized into four regions; the proximal, the median and distal vas deferens and seminal vesicle. The proximal vas deferens is coiled and has a translucent wall. Its wall gradually becomes thicker and the lumen narrower towards the distal region. Spermatozoa can be seen in the fluid here floating and mixed with fluid. This fluid gives a positive reaction to glycogen, i.e. positive to Periodic acid Schiff.

A unique fold of the medial layer of the vas deferens rises from the middle of the median vas deferens and extends up to distal vas

deferens and is called the 'typhlosole'. The typhlosole, whose proximal portion extends into the lumen as a tongue like intrusion. Here, the columnar epithelial cells are narrow and tall. The typhlosole epithelium blends into the general epithelium. The lumen of the median vas deferens is filled with spermatophore capsules, which are embedded in the matrix. The viscous fluid matrix seen in the lumen of the median vas deferens, which reacts like the proximal vas deferens fluid to Periodic acid Schiff. The wall of the spermatophoric capsule was found to be elastin and is intensely positive in response to Periodic acid Schiff and also to Bromphenol blue. In the median vas deferens, positive reactions to elastin are given by the wall of the spermatophoric capsule, fibrous elements in the matrix, the thinner layer on the surface of the general epithelium and the granular cells in the typhlosole. There is no trace of elastin cells in the typhlosole of the distal vas deferens. The typhlosole in the region represents a large muscular structure. It is a concentration of muscle bundles and is not the characteristic loosely arranged connective tissue interspersed with elastin cells as observed in median vas deferens.

Spermatozoans develop in the testicular lobes. When they enter the vas deferens, an accessory material is secreted. Fully formed spermatozoa find their way into the proximal vas deferens. Here, the undifferentiated mass of sperm cells mix with a mucoid fluid. The wall of the proximal vas deferens plays an important role in secreting this fluid. In the distal part of the proximal vas deferens, distinct ampullae are formed, which as they pass into the median vas deferens, secret elastin that envelopes the spermatozoan mass

forming the spermatophoric capsules. The general wall and the typhlosole elastin cells contribute the elastin. The lumen of the distal vas deferens is filled with a viscous fluid, permeated by elastin fibres. The distal vas deferens is packed with spermatotophores when they attained sexual maturity.

Results of the histochemical study showed that the vas deferens of various male morphotypes and four transitional stages of *M. rosenbergii* has three distinct regions which can be distinguished morphologically as well as functionally. As in many other crustaceans, the anterior part of the vas deferens has a secretory function (Cronin, 1947; Deecaraman and Subramoniam, 1980; Ryan, 1967). In all the three male morphotypes and their four tansitional stages of *M. rosenbergii*, the histological study revealed that the typhlosole rises from the middle of the median vas deferens, becomes thicker towards the distal vas deferens and is bifurcated in the distal vas deferens as observed in *Dardanes asper* (Matthews, 1953; Berry and Heydorn, 1970). The histochemical tests showed that the wall of the median vas deferens and the characteristic granule cells of the typhlosole in the median vas deferens secrets elastin. This typhlosole is comprised of loosely packed connective tissue elements interspersed with characteristically distinct oval glandular cells, which were shown to be the elastin cells. The general epithelium adjoining the typhlosole is also involved in the secretion of elastin. A correlation exists between the abundance of elastin provided by the general epithelium and the absence or presence of elastin cells in the typhlosole connective tissue. In the median vas deferens where

elastin cells occur in the typhlosole, the general epithelium secrets relatively smaller amounts of elastin. The secretion from the median vas deferens help in the formation of the sperms ampullae (Spermatophore). The spaces between the ampullae are filled with a matrix secreted by the proximal median vas deferens; encapsulation of the ampullae results from elastin secreted by the distal median vas deferens. Similar observations were made in many crustaceans that the typhlosole secrets a gelatinous matrix (Matthews, 1953; 975; Uma and Subramanyam, 1979). The fluid matrix in the spermatophore capsule is positive to the histochemical tests viz. Periodic acid Schiff, Bromphenol blue and Sudan Black Test. The spermatophore wall shows positive reactions to PAS test suggesting that it is mainly composed of a neutral polysaccharide. It also includes basic proteins as shown by its positivity with Mercury Bromephenol blue. The sperm cells contain a significant quantity of glycogen, possibly for endogeneous energy metabolism. Polysaccharides form the main component of the spermatophores in the various male morphotypes and their transitional stages of *M. rosenbergii* as the case in the spermatophores of *Penaeus indicus, Albunea symnista and Emerita asiatica.* The predominance of mucosubstances in the spertmatophore components may be correlated to their protective and structural functions. The functional significance of polysaccharides is related to its role in spertmatophore hardening and protection of the delicate spermatozoa during their storage on the sternum of the female (Radha and Subramoniam, 1985). The presence of protein in the spermatophore in *C. maenas,* and the protein conjugated with chitin similarto the chitin protein

matrix of the arthropod exoskeleton were already reported (Spalding, 1942). Again in *Penaeus setiferus* a chitinous layer forming an outer covering to the spermatophoric sheath was identified (King, 1948). The possible occurrence of phenolic tanning in the spermatophoric wall of *Penaeus. trisulcatus* (Malek and Bawab, 1971) and a chitin-protein like lamellar pattern for the spermatophore wall in copepodes (Gharagozlove-Van Ginneken, 1978) are likewise reported. Based on these observations a similarity between the crustacean spermatophore and arthropod cuticle has been proposed.

Histochemistry of androgenic gland

Introdution

The androgenic gland plays an important role in crustacean sex determination as well as in the regulation of primary and secondary sexual characteristics. The importance of this gland for aquaculture research is attributed to the fact that, in some crustaceans, males and females defer in their growth patterns. In *M. rosenbergii,* the male growth rate is considerably higher than that of the female (Sagi *et al.,* 1986). However, male growth rates vary greatly (Fujimura and Okamoto, 1972; Smith *et al.,* 1978; Brody *et al.,* 1980; Malecha *et al.,* 1984; Harikrishnan and Kurup, 1997; Sureshkumar and Kurup, 1998) due to the existence of male morphotypes within the prawn population. The objective of present work is to study the histochemical nature of the androgenic gland of various male morphotypes and their transitional stages of *M. rosenbergii* with a focus on the nature and function of this endocrine gland.

The androgenic gland (AG) was first described in the crab *Callinectes sapidus* by Cronin (1947), while the first insight into its function was provided by Charniaux-Cotton (1954) in the amphipod *Orchestia*. The glands are usually located at the sub terminal portion of the sperm duct. The cells are arranged as a thin compact lobed structure (Kleinholz and Keller, 1979). Histological and histochemical studies of the crustacean androgenic gland have been carried out by Cronin (1947); Charniaux-Cotton (1954); Kleinholz and Keller (1979); Mirajkar (1986); Joshi and Khanna (1987); Fowler and Leonard (1999); Awari and Dubey (2000) and Sun *et al.* (2000). The ultra structural study of the crustacean androgenic gland includes that of King (1964); Veith and Malecha (1983); Taketomi (1986) and Sagi *et al.* (1997). The biochemical nature of the crustacean androgenic gland secretion was demonstrated by King (1964); Gilgan Idler (1967); Berreur-Bonnenfant *et al.* (1973); Ferezou *et al.* (1978); Katakura *et al.* (1975); Jachault *et al.* (1978);Katakura and Hasegawa (1983); Hasegawa *et al.* (1987); Martin *et al.* (1996); Sun *et al.* (2000).

Materials and Methods

Collection, acclimatization and segregation of various male morphotypes and their transitional stages of *M. rosenbergii* were carried out the same as described in chapter 4. For histochemical studies of protein, lipid and glycogen, the technique used were the same as in chapter 4.

Results

Histochemical characterization of the androgenic gland of various male morphotypes of *M. rosenbergii* viz. SM, SOC and SBC and their transitional stages viz. WOC, t-SOC, WBC and OBC was carried out to study the histochemical localisation and possible significance of some metabolites viz.protien, lipid and glycogen in the androgenic gland. Histological studies of the androgenic gland revealed that Weak Orange Clawed male, pre-transforming Orange Clawed male, Weak Blue Clawed male and Old Blue clawed male exhibited medium activity where as Small male and Strong Blue clawed male exhibited very high activity. On the contrary, the androgenic gland of Strong Orange clawed male exhibited very low activity. Histochemical tests revealed that cell type I of the androgenic gland of SM and WOC male morphotypes as well as cell type II of the androgenic gland of t-SOC, WBC, SBC and OBC male morphotypes were strongly positive to Mercury Bromphenol blue test (Figs10.8.1 to 10.8.7). The result of the Mercury Bromphenol blue reaction and the pyrinophilous nature of the cytoplasm is in agreement with the observations made by King (1964), Taketomi (1986), Hasegawa *et al.* (1987), Mirajkar *et al.* (1984), Sagi (1991), Dubey and Kiran (2000) and Sun *et al.* (2000). The ultra structure of the AG in the crab, *P. crassipes* resembles that of a vertebrate protein producing cell rather than a steroid producing cell (King, 1964), since it is characterised by a well developed granular endoplasmic reticulum and abundant mitochondria. The presence of considerable protein in the secretory vesicles of the cytoplasm suggest that the androgenic hormone may

be protein or polypeptide (King, 1964). The proteinaceous nature of secretion was confirmed by Taketomi (1986) who reported the existence of two kinds of AG cells in *Procambarus clarkii*, type A which resembles protein-secreting cells and type B which do not. Veith and Malecha (1983) have also observed more than one type of cell in the AG of *M. rosenbergii*. Juchault *et al.* (1978) have extracted a sizable water soluble substance which is still active at 125°c from the AG of *Armadillidium vulgare*. A single injection of this substance into female *A. vulgare* induced the appearance of all the external male characteristics Katakura *et al.* (1975) extracted an active water-soluble substance from the reproductive system of *Armadillidium*. Injection of the active extract into young females induced masculanization of the external sexual characteristics and transformations of the internal female reproductive organs into testes and sperm ducts (Katakura *et al.*, 1975; Katakura and Hasegawa *et al.*, 1983). Hasegawa *et al.* (1987) isolated and characterised two proteinaceous androgenic hormones from the reproductive system of *A. vulgare*. Sun *et al.* (2000) carried out total protein analysis of androgenic glands from three male morphotypes (Orange Claw, Orange Blue Claw and Blue Claw) of the fresh water prawn *M. rosenbergii* and revealed that total AG protein content increased quantitatively from sexually immature OC to sexually mature BC.

In the present study the cells of the androgenic gland are positive with reference to lipid content. The Sudan Black B reveals a fine granular nature of the cytoplasm of the androgenic gland cells. Greatest concentration of lipids was seen in the epithelial cells lining

the lumen of the ejaculatory duct. Regarding the presence of lipids in androgenic gland, results were different in Crustaceans. Lipids appear to be evenly distributed throughout the gland and were not confined to any of the two cell types viz. type I and type II. The present result is in agreement with that of Veith and Malecha (1983) who also observed a positive staining for lipid in the androgenic gland of *M. rosenbergii*. Berreur-Bonnenfaunt *et al.* (1973) reported that they could extract a lipoidal substance with a molecular weight of 200 to 250 daltons, from the androgenic gland of crab, *Carcinus maenas*. Injection of the substance every second day inhibited vitellogenesis in sexually active female *Orchestia*. Carotenoid pigments on the second antennae, a secondary male characteristic, appeared as early as the sixth day after similar injections in *Talitrus* females. The active molecule has been characterised by Ferezou *et al.* (1978) as farnesylacetone and was shown to be synthesised by the androgenic gland. The action of farnesylacetone at a low concentration is rapid, organ specific, being expressed in the gonads and did not exhibit any species specificity. The farnesylacetone affects protein and RNA synthesis in its target organs. Contrary to the findings reported above Mirajkar *et al.* (1984) reported that the presence of lipid positive substances are doubtful in *M. kistnensis*. Dubey and Kiran (2000) also reported that the absence of lipid positive reactions in the androgenic gland of *M. rosenbergii*.

Gilgan Idler (1967) reported the conversion of androstendione to testosterone in *Homarus americanus* testes and AG. The tissues of these organs contain 17 Beta-hydroxysteriod de-dehydrogenase (HSD). A comparison of the ability of the different

tissues to synthesize testosterone indicated that the AG was the most active. 17 Beta-HSD activity was demonstrated in the AG of the blue crab *Callinectes sapidus* (Tcholakian and Eik-Ness, 1971). The AG tissue converted progesterone to hydroxyprogesterone, androstendione, testosterone and deoxycorticosterone. The conversion was demonstrated invitro and invivo. On a weight basis, the AG converted more progesterone of the H-Y antigen; male primary and secondary sex characteristics are induced by the subsequent stimulation of testosterone. The regression of the Mullerian ducts (the fetal duct from which the female reproductive duct developed) is induced by a glycoprotein, the anti-Mullerian hormone (AMH) (Josso, 1986). Sagi (1988,1997) opined that the AG may be involved in the secretion of more than one hormone. These may control different aspects of the wide spectrum of male biological functions including sex determination in post larval stages, primary and secondary sex characteristics, pigmentation and behavior, morphotypic differentiation and growth rate in the adult males.

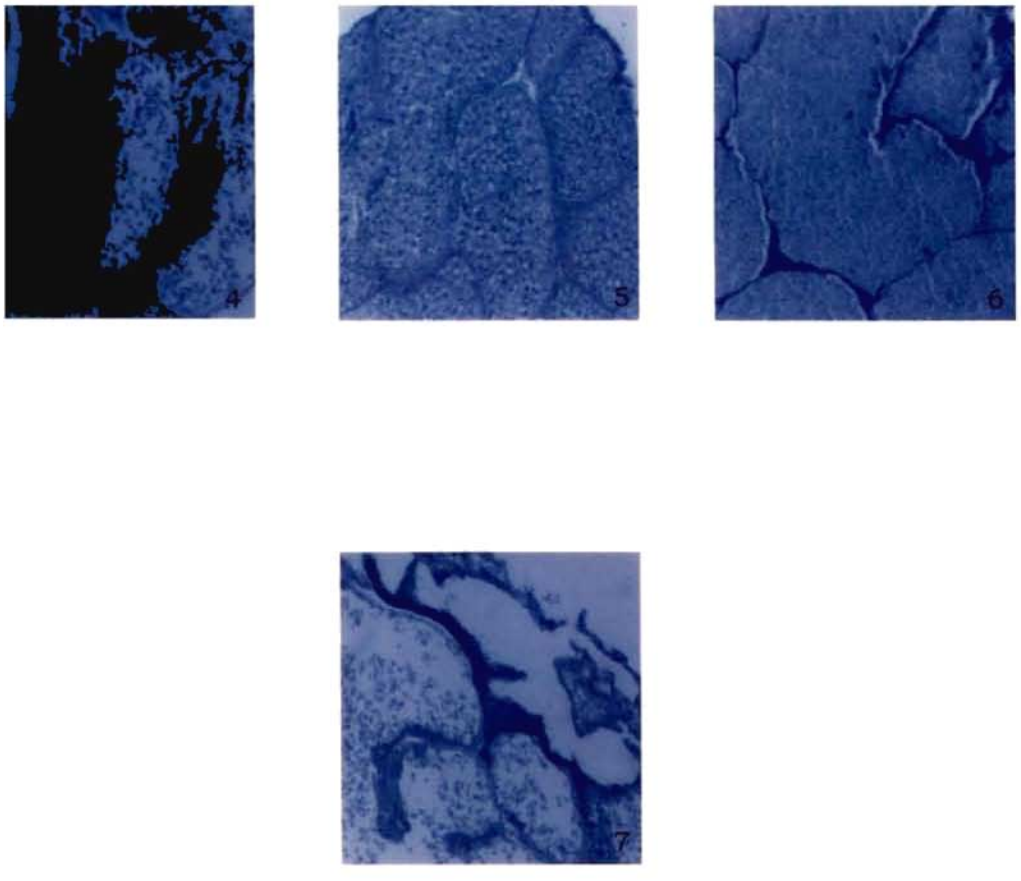


Fig 10. 1 : Photo micrographs of the TS of the testis treated with Mercury bromphenol blue (x100) showing proteinous nature of testicular wall and distribution of proteins in the nucleus and cytoplasm of primary and secondary spermatogonia, spermatocytes and mature spermatozoa
1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

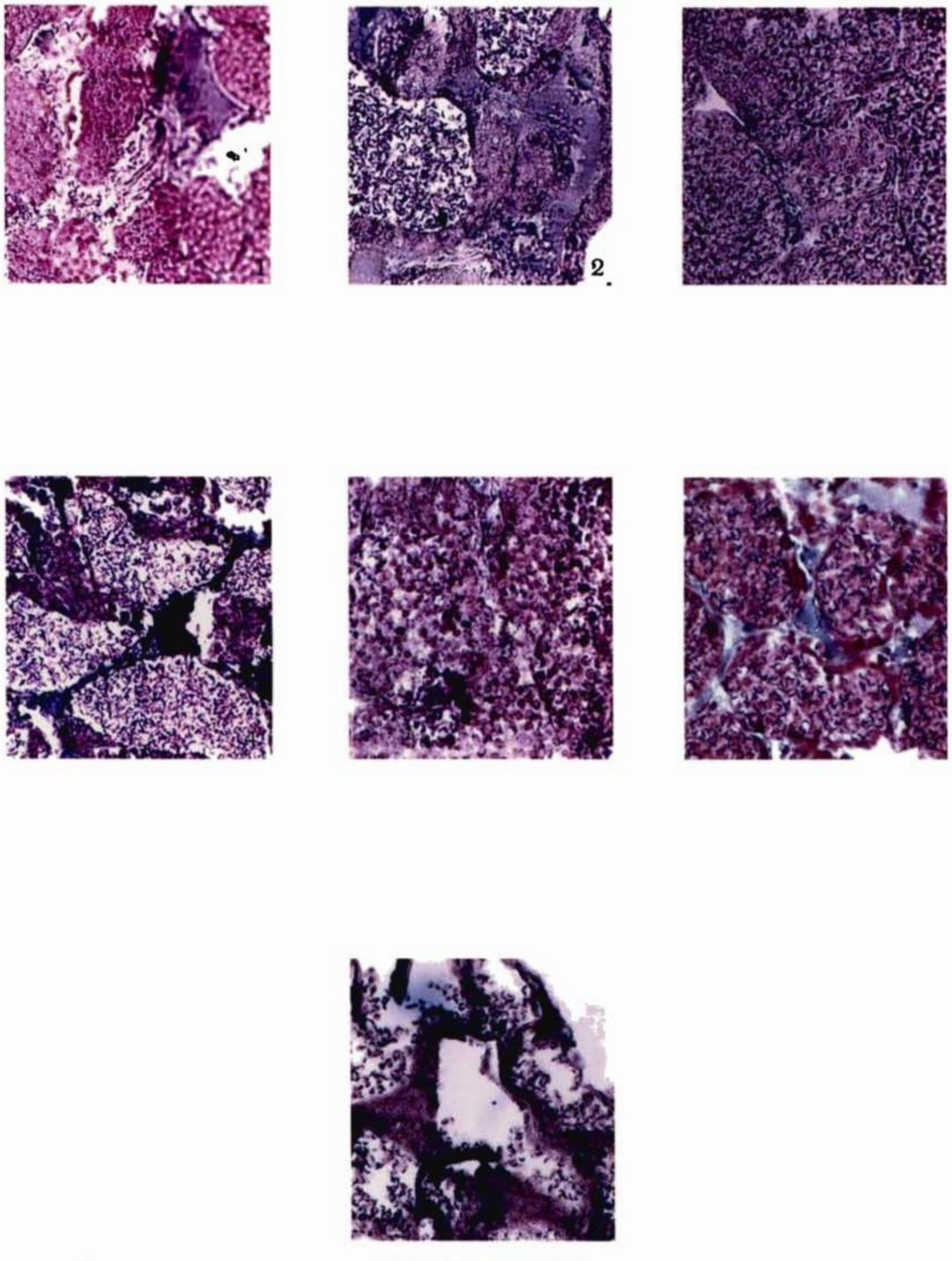


Fig 10. 2 : Photo micrographs of the TS of the testis treated with Sudan Black B (x100) showing the distribution of lipids in the spermatogonia spermatocytes, spermatids and mature spermatozoa
1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

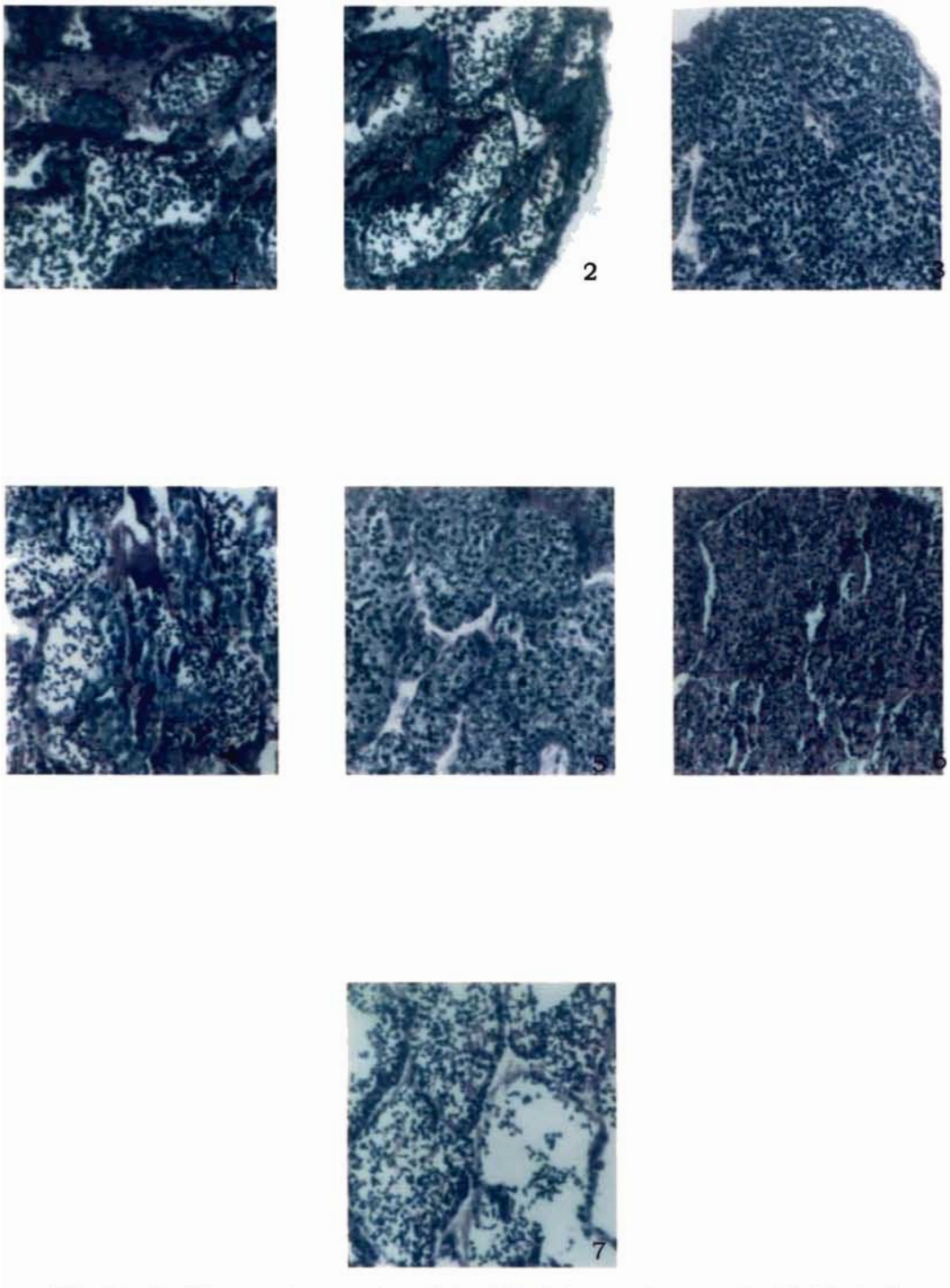


Fig 10. 3 : Photo micrographs of the TS of the testis treated with Periodic Acid Schiff (x100) showing the presence of glycogen in the nucleus and cytoplasm of primary and secondary spermatogonia, spermatocytes and mature spermatozoa 1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

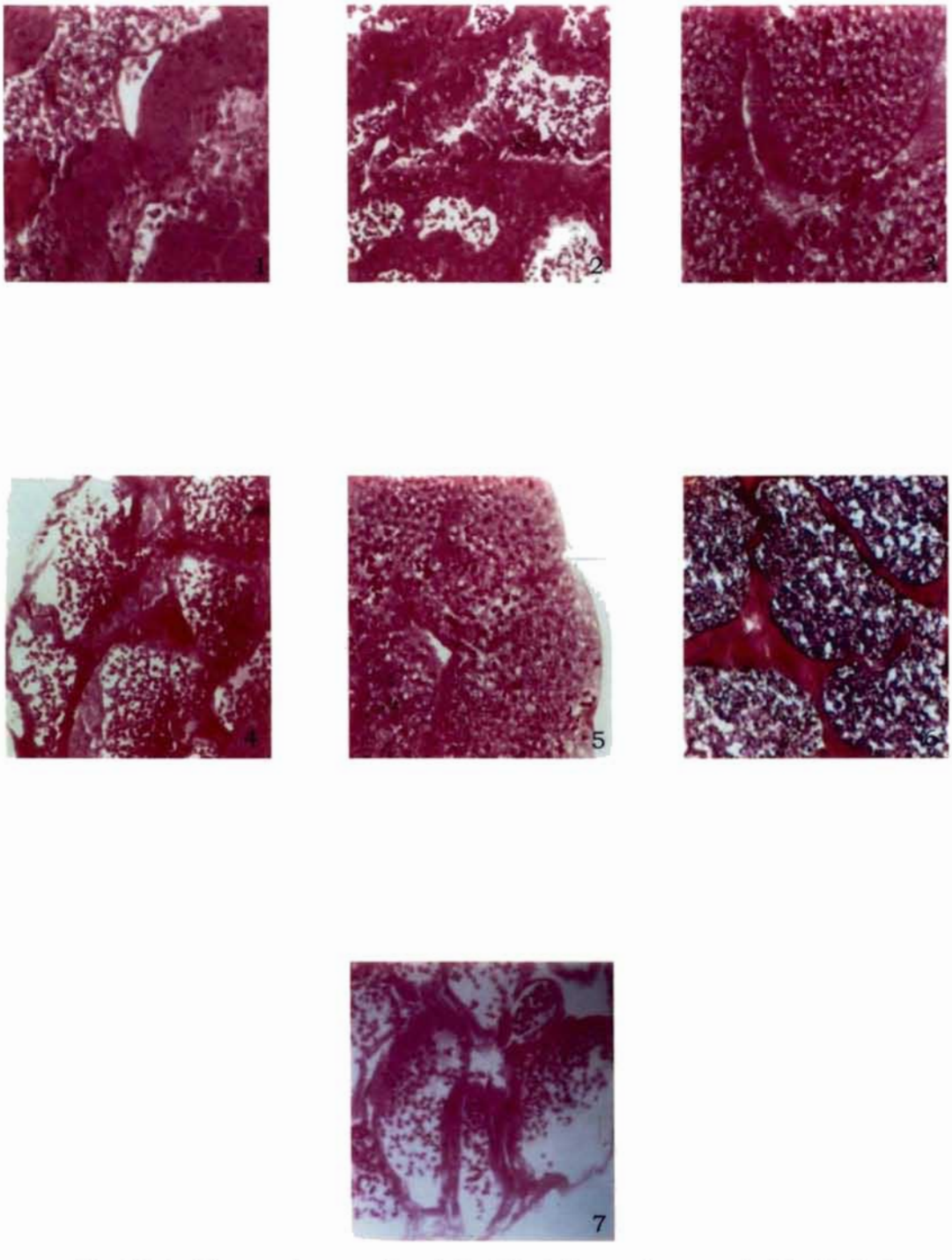


Fig 10.4 : Photo micrographs of the TS of the testis treated with Methyl Green Pyronin G (x100) showing the presence of RNA in the nucleolus and cytoplasm of spermatogonia, spermatocytes and DNA in the mature spermatozoa 1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

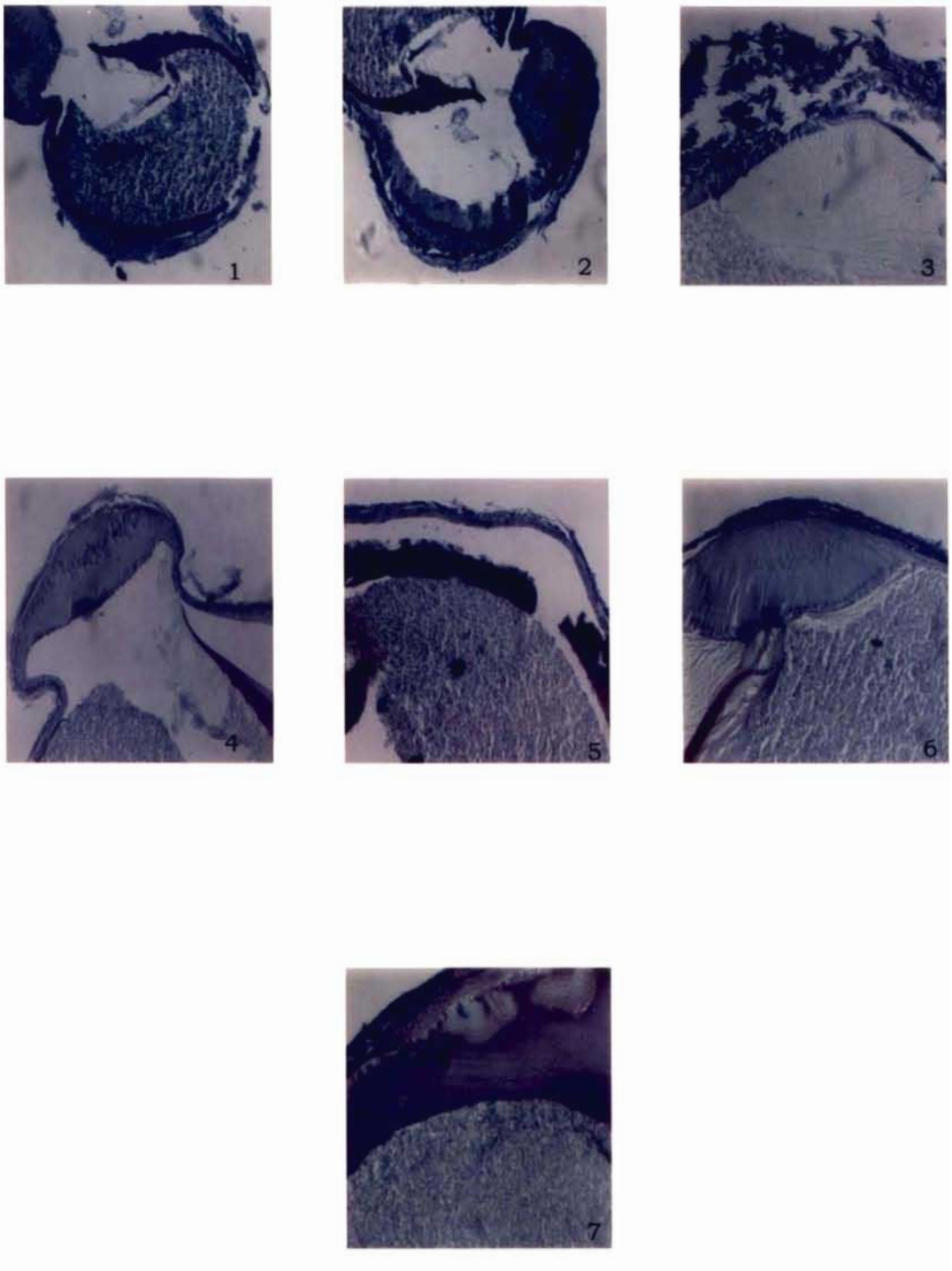


Fig 10. 5 : Photo micrographs of the TS of the vas deferens treated with Mercury bromphenol blue (x200, a portion) showing positive reaction
1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

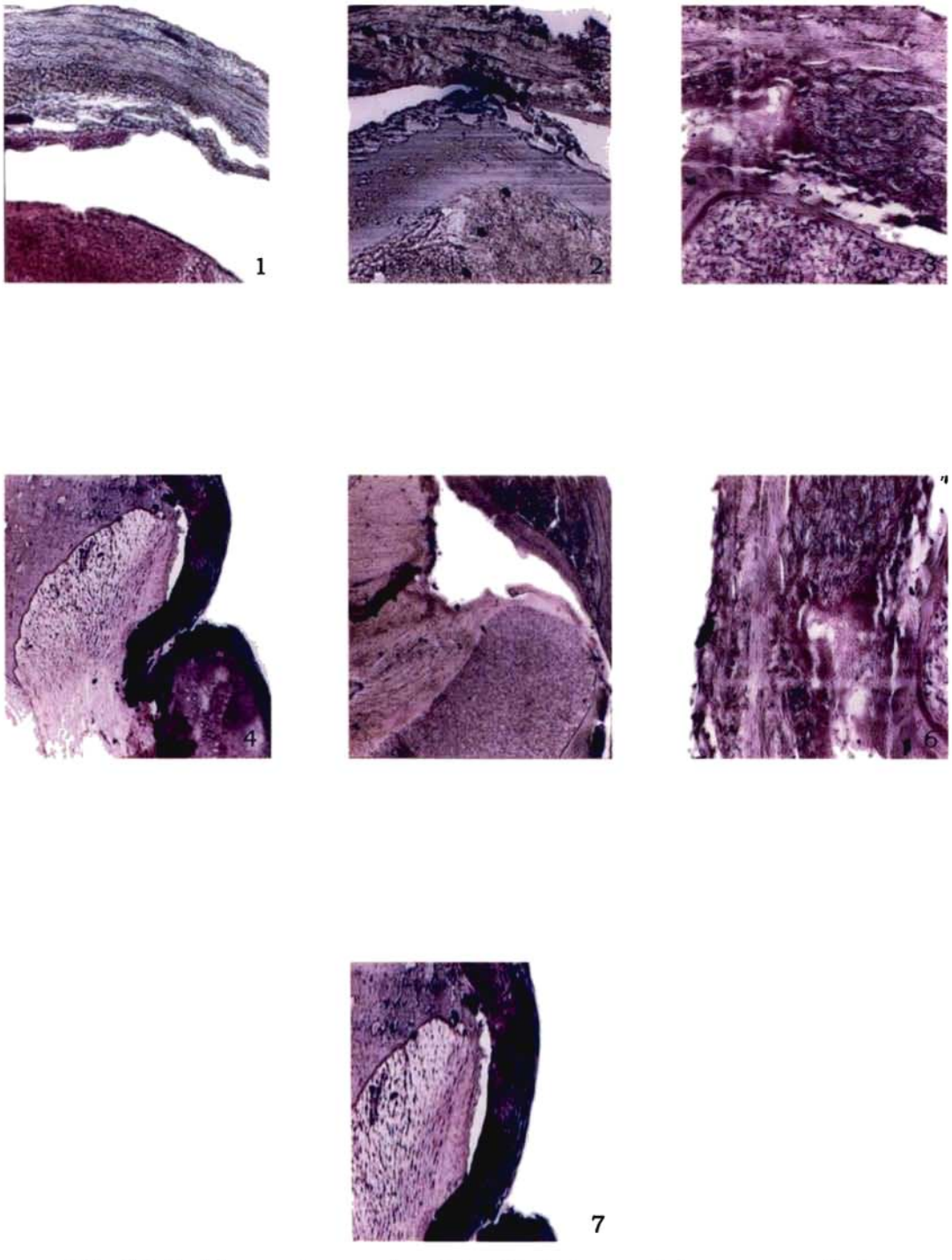


Fig 10. 6 : Photo micrographs of the TS of the vas deferens treated with Sudan Black B (x200, a portion) showing positive reaction
1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

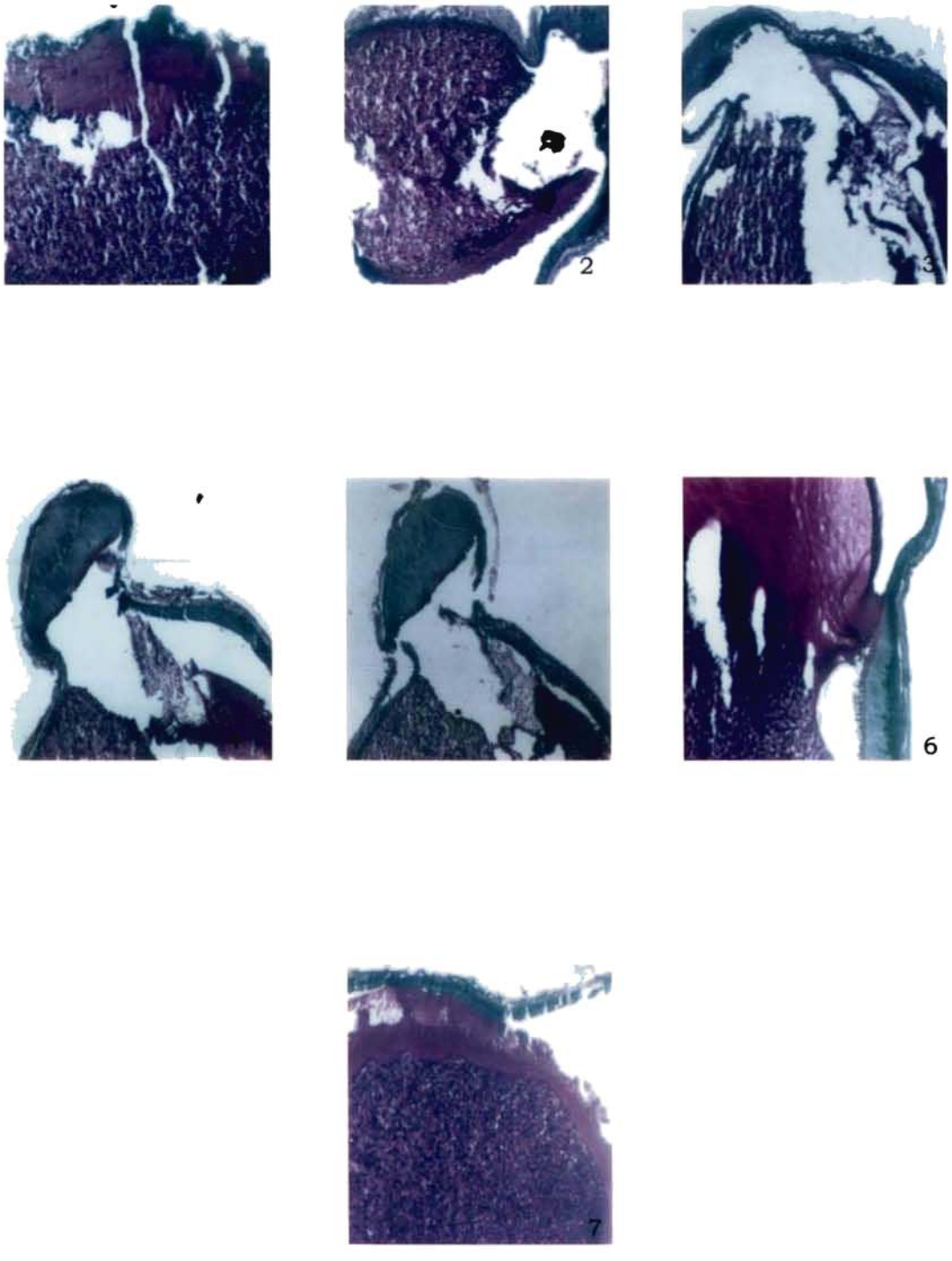


Fig 10. 7 : Photo micrographs of the TS of the vas deferens treated with Periodic Acid Schiff (x200, a portion) showing positive reaction
1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

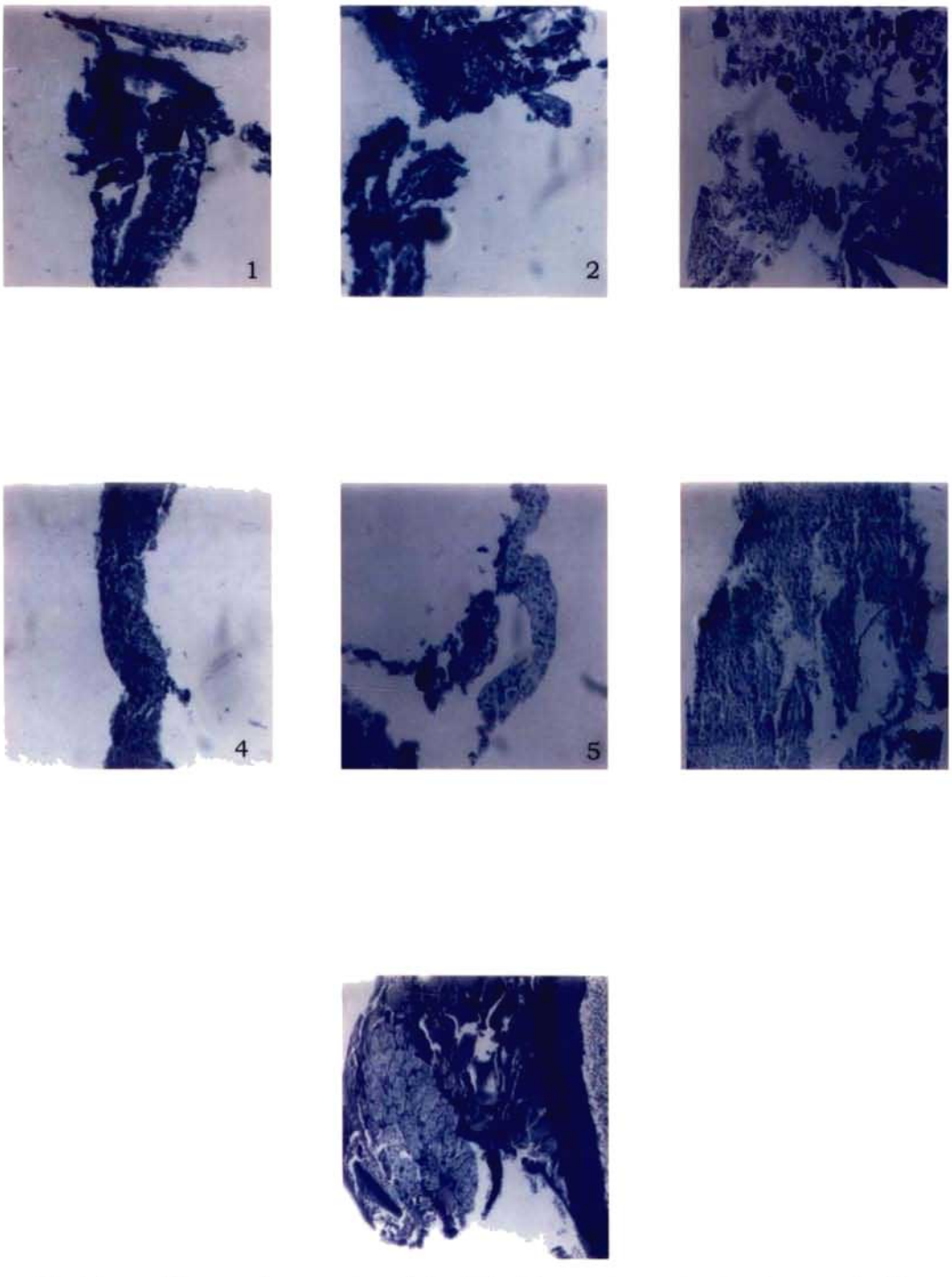


Fig 10. 8 : Photo micrographs of the TS of the androgenic gland treated with Mercury Brom Phenol Blue B (x200) showing positive reaction
1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

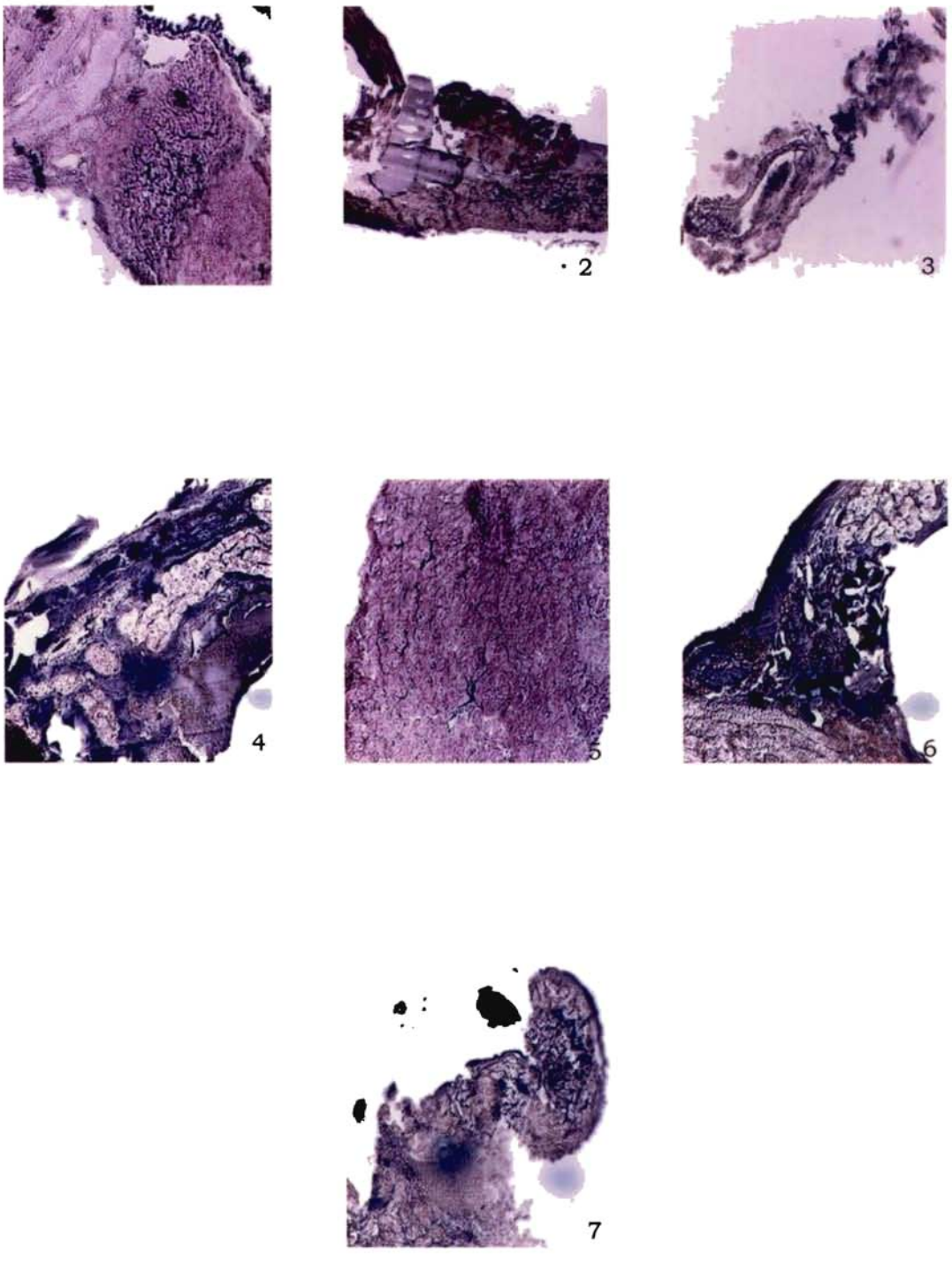


Fig 10. 9 : Photo micrographs of the TS of the androgenic gland treated with Sudan Balck B (x200) showing positive reaction
1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

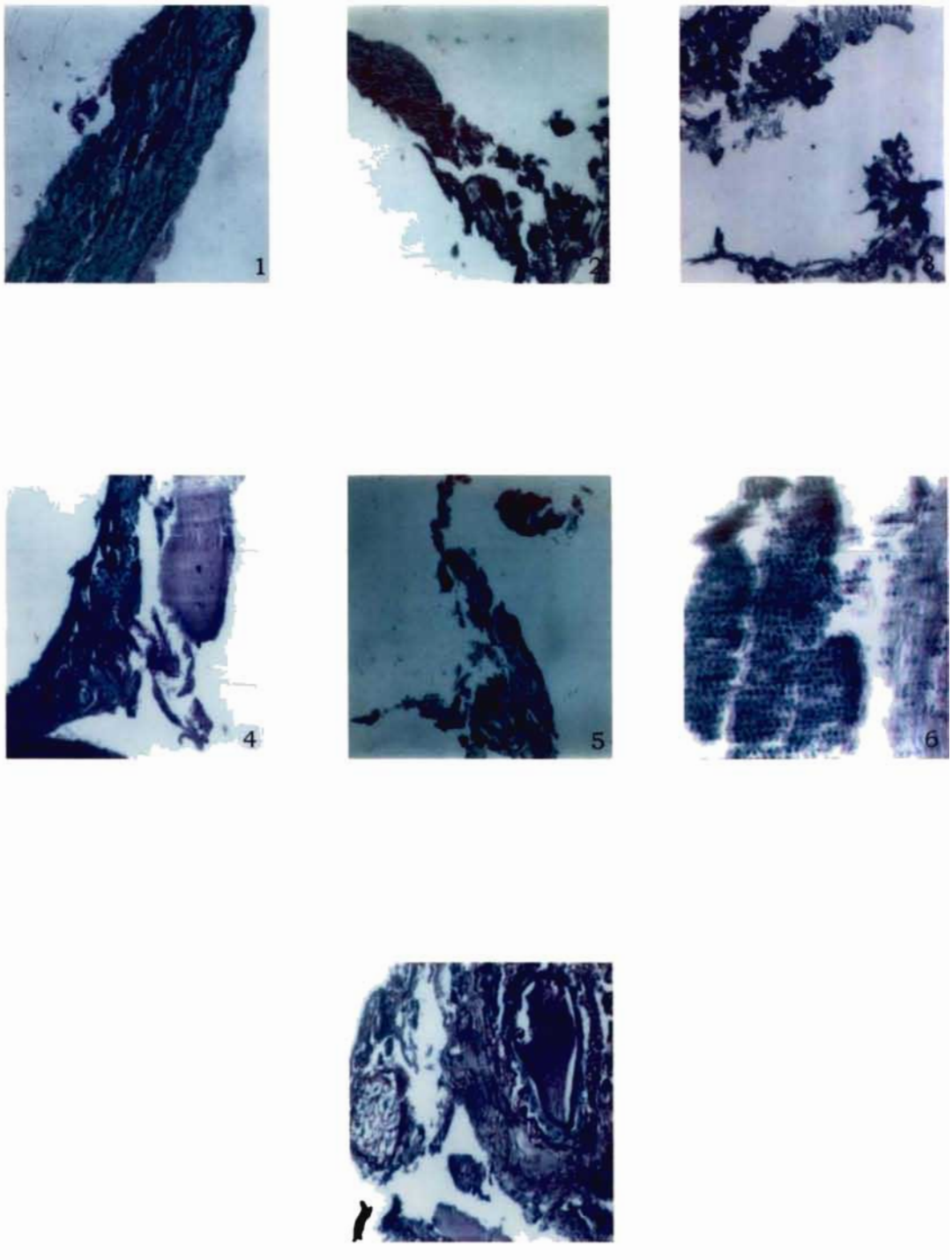


Fig 10. 10 : Photo micrographs of the TS of the androgenic gland treated with Periodic Acid Schiff (x200) showing positive reaction
1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

CHAPTER 11

HISTOCHEMISTRY OF HEPATOPANCREAS

Introduction

A major seat of bulk storage, synthesis and transformations of variety of organic and inorganic substances is taking place in the hepatopancreas of crustaceans which is analogous to the liver of vertebrates and the fat body of insects (Lockwood, 1968; Huggins and Munday, 1968; Adiyodi and Adiyodi, 1970). Hepatopancreas is the primary organ for the storage of reserves and the haemolymph play a secondary role. The organic reserves constituted of carbohydrates, lipids and proteins and are of important for many metabolic functions.

Studies on accumulation of organic reserves in *Cancer pagurus* (Renaud, 1949), histological and histochmical changes in the hepatopancreas and integumental tissues of *Panulirus argus* (Travis, 1955; 1957), histochemical localisation of lipid and glycogen in the integument and hepatopancreas of the crab *Ocypoda macrocera* (Nagabhushanum and Rao, 1967), Cyclic fluctuations in the levels of organic and inorganic reserves in the hepatopancreas of *Paratelphusa* (Adiyodi and Adiyodi, 1972), changes in epidermal DNA and protein synthesis in the Crayfish *Orconectes sanborni* (Humphreys and Stevenson, 1973) and cyclic histological and histochemical changes in the hepatopancreas of *Metapenaeus monoceros* (Madhyastha and Rangnekar, 1974) during the moult cycle have been reported. In this chapter an attempt is made to bring out the histochemical

localization of various metabolites viz. protein, lipid and glycogen content in the hepatopancreas of various male morphotypes viz. SM, SOC and SBC and their transitional stages viz. WOC, t-SOC, WBC and OBC of *M. rosenbergii.*

Materials and Methods

Collection, acclimatization and segregation of various male morphotypes and their transitional stages of *M. rosenbergii* were carried out in the same way as described in chapter 4. For histochemical studies of protein, lipid and glycogen, the technique used were similar as in chapter 4.

Results

The histochemical localisation of some important staining reactions of protein, lipids and glycogen, are illustrated in Figs 11.1.1- to 11.1.7- protein; 11.2.1 to 11. 2.7 –lipid; 11.3.1 to 11.3.7 – Glycogen according to the visually estimated intensities. As reported earlier, the hepatopancreas of various male morphotypes of *M. rosenbergii* under study, four cell types viz. Embryonic (E), Absorptive (R), Fibrillar (F) and Secretory (B) cells are discernible. At a comparative level, the distribution of these metabolites are variable in the various cells elements in the hepatopancreas of these male morphotypes. In SM and WOC morphotypes, the epithelial tissue of the hepatopancreatic tubules are predominantly of the 'F' cells with a basal nucleolus having one or two nuclei. Secretory 'B' and Absorptive 'R' cells are less numerous. In SOC, t-SOC and WBC, the Secretory 'B' cells become more numerous and they form the

predominant type of cells in the epithelium of hepatopancreatic tubules. These cells become larger and swollen with the apical complex coalescing with the vacuolar contents. The vacuoles increases in size, distending the cell and pushing the nucleus to the base. These large vacuoles discharging their contents plus adjacent cytoplasm into the lumen leaving only the basal region and nucleus of the cell intact, secretion occuring in an apocrine fashion. In SBC and OBC morphotypes, the hepatopancreatic tubules become more columnar and Absorptive 'R' cells become more numerous than secretory cells.

The PAS technique gave positive reaction at the luminal borders of the hepatopancreatic acini. The 'E' cells were noticed to be PAS negative. The 'F' cells showed a uniform PAS positive reaction. The PAS positivity was found to be associated with the 'B' cells mainly around the vacuoles and vacuolar contents. The 'R' cells revealed a higher degree of intensity of PAS staining at their striated borders. Moreover, PAS positive fine granules were localized in the cytoplasm of these cells.

They also give positive test with Mercury Bromphenol blue showing the presence of protein The epithelial tissue of the hepatopancreas give positive test with Sudan Black B Lipid droplets are found in considerable quantities in all the cell types in the hepatopancreatic tubules.

Discussion

The presence of various chemical substances in the different cell elements and connective tissue of the hepatopancreas of various male morphotypes and their transitional stages of *M. rosenbergii*, is noteworthy. The brush borders of the 'F', 'B' and 'R' cells showed presence of glycogen. The granular staining for glycogen was obtained only in the cytoplasm of the 'R' cells. In the 'E' cells on the other hand, glycogen was undetectable. Glycolipids and glycoproteins were found mainly at the brush border of the tubule and free globules associated with the connective tissue. Previous histochemical studies on crustacean hepatopancreas suggest that PAS positive material is mostly confined to the brush border of the hepatopancreatic tubule (Travis, 1955, 1957; Davis and Burnett, 1964; Stainer *et al.*, 1968). The light and electron microscopical observations revealed that the absorptive cells of the hepatopancreas are responsible for the storage of glycogen (Miyawaki *et al.*, 1961; Davis and Burnett, 1964; Bunt, 1968; Stainer *et al.*, 1968; Loizzi, 1971).

The knowledge regarding the physiological role of the mucosubstances in the crustacean digestive gland is far from complete. It is known that glycogen forms the substrate for energy reactions in the cell. Thus, its presence at the brush borders of 'F', 'B', 'R', cells and in the cytoplasm of the latter cells suggest that it may be utilized for such a purpose. The present observations suggest that the localization of glycogen in the different cell types of the hepatopancreas might be associated with their functional diversity. The 'E' cells, which are the precursors of the other cell types did not show the presence of glycogen.

This suggests that these cell elements are not concerned with carbohydrate storage. The 'R' cells appear to serve as store houses of glycogen and lipids. The 'F' cells and 'B' Cells also act as a storage place of lipids as evidenced by the histochemical test. In nutshell, the hepatopancreas of crustaceans is the major site for biosynthetic and degradatory reactions in the metabolism of cabohydrate, fat proteins. Absorption and storage of various organic and inorganic substances also takes place in this gland. Considering the diversity of the hepatopancreatic cellular elements and their functional behavior in each male morphotypes, there is difference in glycogen, protein, lipid contents in their cellular elements of *M. rosenbergii.*

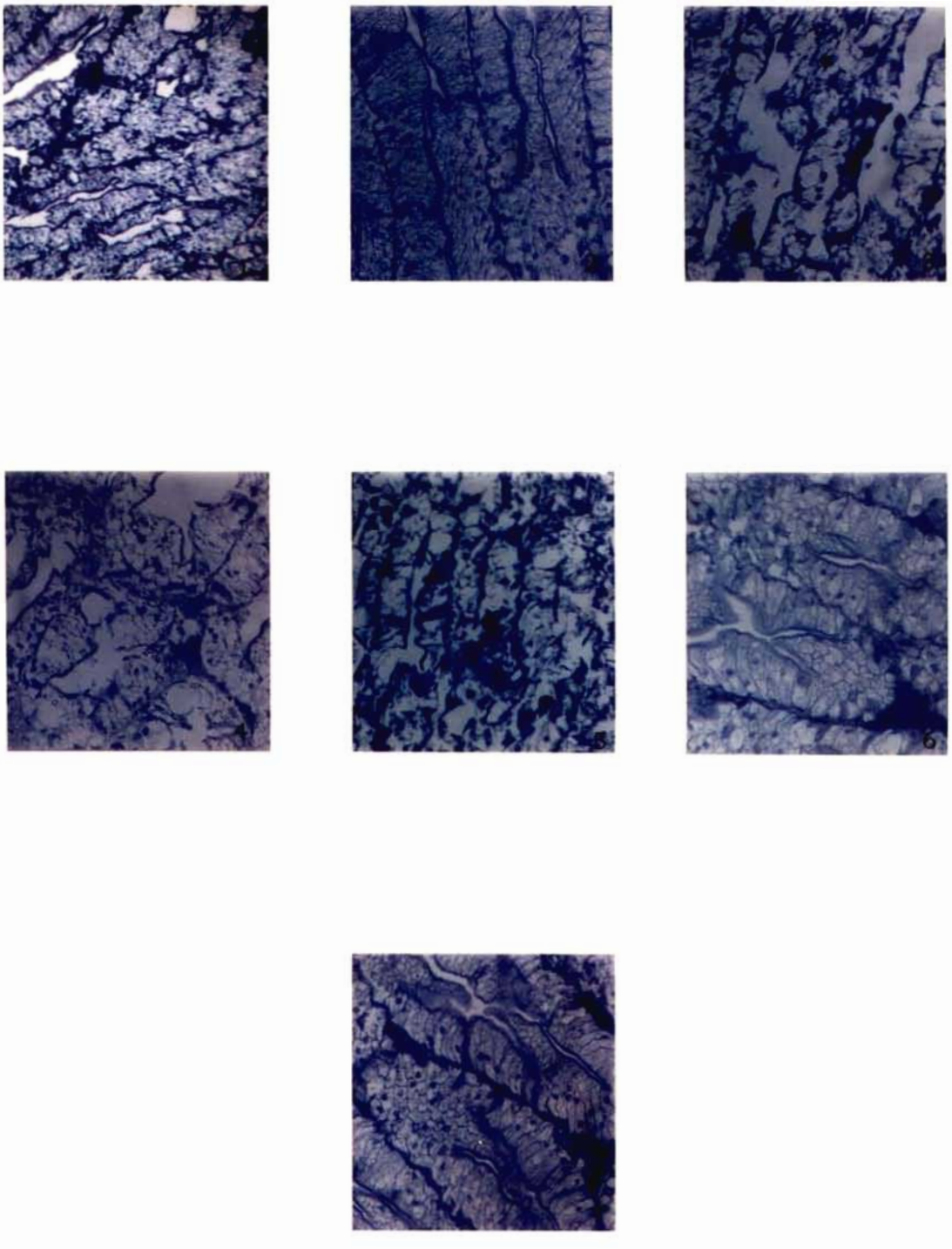


Fig 11 3 : Photo micrographs of the TS of the hepatopancreas treated with Mercurybromphenol blue (x200) showing positive reaction
1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

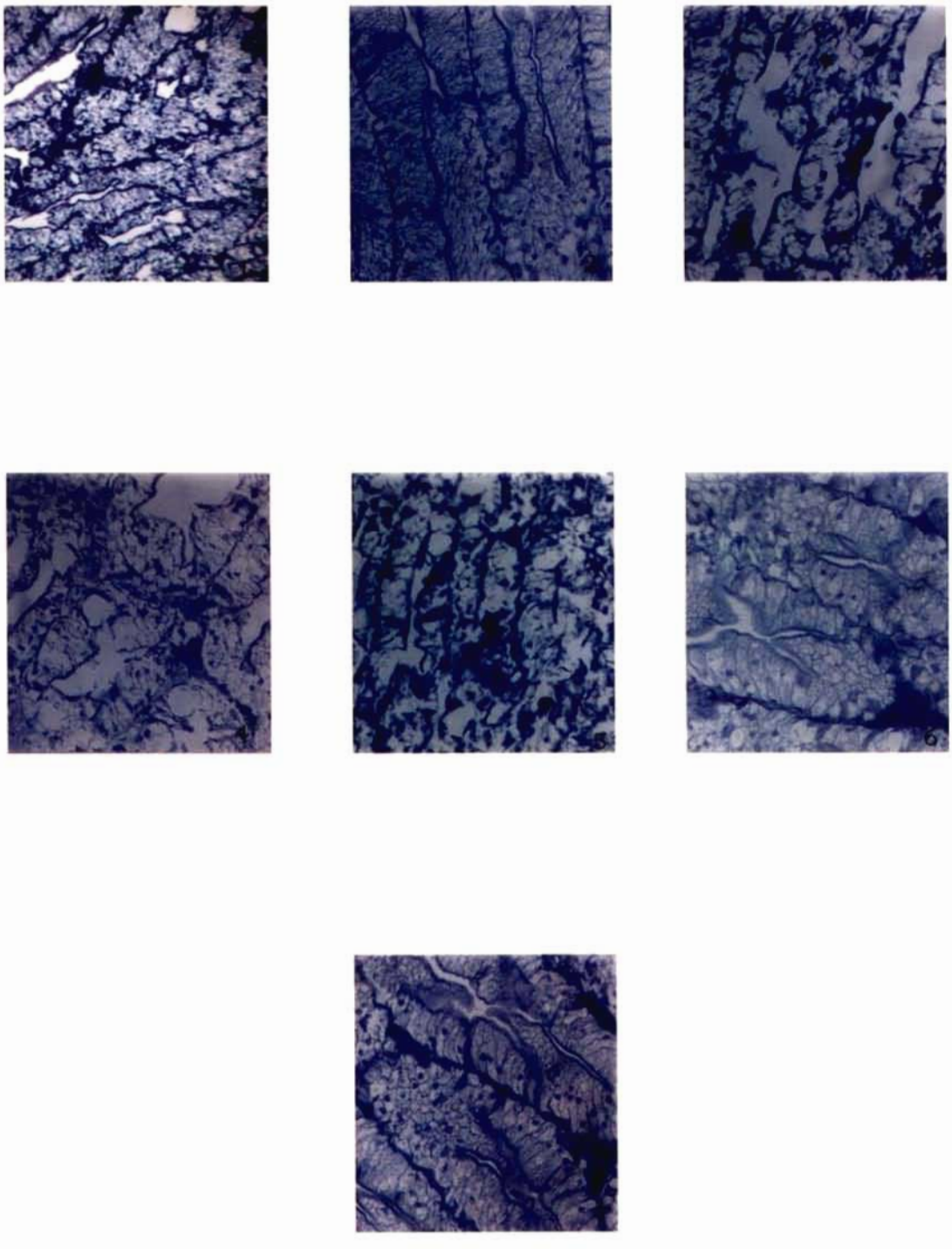


Fig 11 3 : Photo micrographs of the TS of the hepatopancreas treated with Mercurybromphenol blue (x200) showing positive reaction
1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

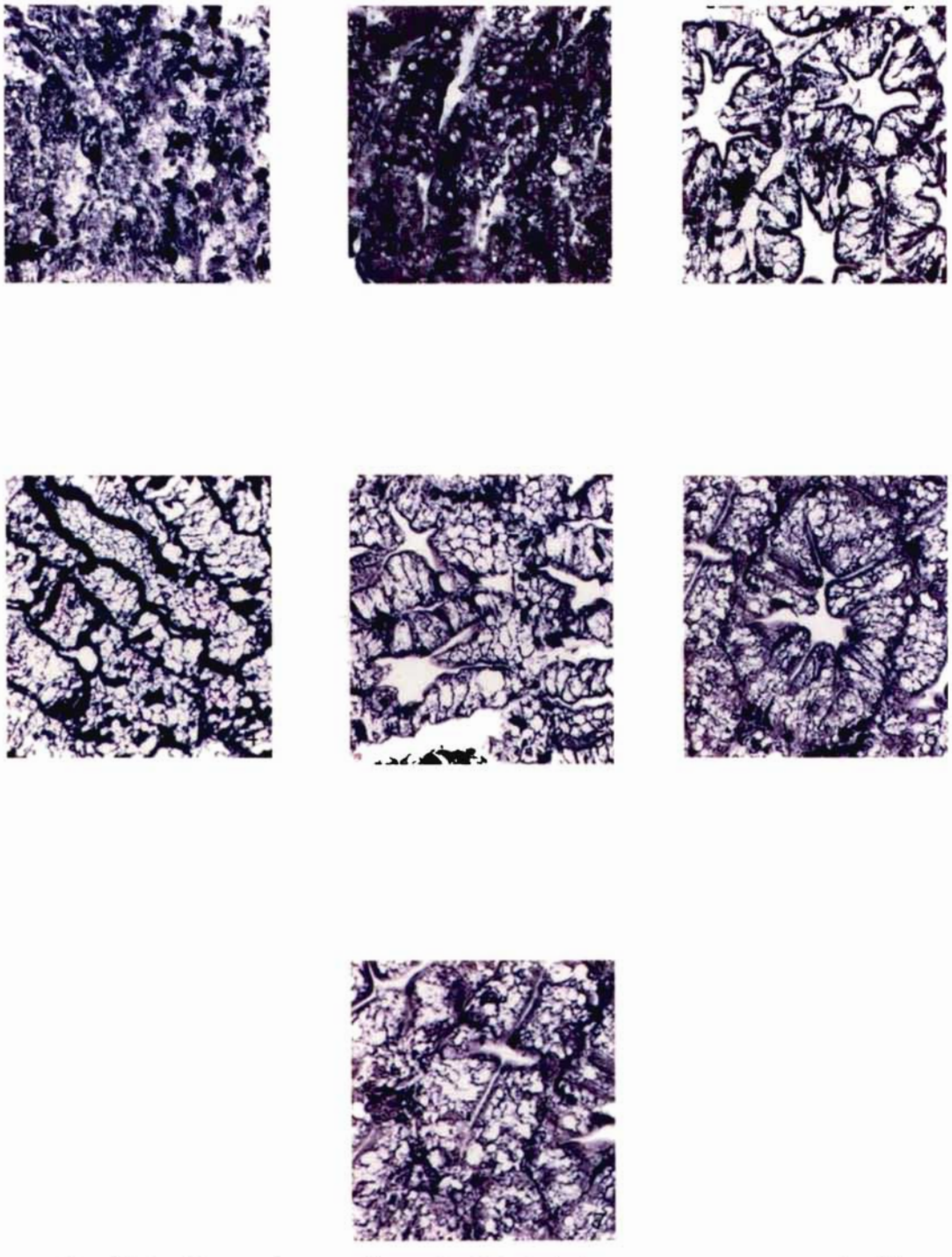


Fig 11 2 : Photo micrographs of the TS of the hepatopancreas treated with Sudan Black B (x200) showing positive reaction
1-SM, 2-WOC, 3-SOC, 4-t-SOC, 5-WBC, 6-SBC, 7-OBC

SECTION4

BIOCHEMICAL CHARACTERISATION OF MALE MORPHOTYPES AND THEIR TRANSITIONAL STAGES OF *Macrobrachium rosenbergii* (de Man)

CHAPTER 12

BIOCHEMICAL COMPOSITION AND DETERMINATION OF AMINOACIDS PROFILE

Introduction

Sexually mature male population of *M. rosenbergii* belonging to same age group has been differentiated into three distinct mrophotypes viz. Small males (SM), Orange Clawed male (OC) and Blue Clawed males (BC) representing three phases in the developmental pathway of males (Brody *et al.*, 1980; Cohen *et al.*, 1981). Besides, the two transitional stages of both OC and BC are also distinguishable from the fully differentiated males (Kuris *et al.*, 1987; Harikrishnan and Kurup, 1997a). The above morphotypes and their transitional stages are phenotypically and behaviorly distinct and are well characterized and documented (Ra'anan and Sagi, 1985; Sagi and Ra'anan, 1988; Harikrishnan and Kurup, 1997; Kurup *et al.*, 1997; Sureshkumar and Kurup, 1998; Harikrishnan and Kurup, 1999) from growout and natural systems. Biochemical characterisation could also be established among the various male morphotypes and their transitional stages of *Macrobrachium rosenbergii* from growouts (Sureshkumar and Kurup, 1998; Sureshkumar and Kurup, 1999; Ranjeet and Kurup, 2001). However, information on biochemical characterization of different male morphotypes and their transitional stages of *M. rosenbergii* from the wild is lacking. The present study is aimed to evaluate the significance and involvement of some important biochemical

components like protein, lipid, carbohydrate, ash and water content in the organ tissues viz. muscle tissue and hepatopancreas of various male morphotypes and their transitional stages from natural environment. An attempt was also made to determine the aminoacid profile of various male morphotypes and their transitional stages from growouts.

Studies on the biochemical changes in relation to reproductive cycle in invertebrates have been investigated by several workers. Seasonal variation in different organic constituents of oysters had been shown by Russel (1923); Okazaki and Kohagashi (1929); Sekine *et al.*, 1930; Tully (1936), Humbrey (1941); and Durve and Bal (1960). Pearse and Giese (1966) have worked on the biochemical changes in relation to reproductive cycle in gastropod, *Neobuccinum eatoni* and in bivalve, *Limaluta hodgsoni.*

Among crustaceans, most of these studies are centered on the changes in biochemical components like protein, lipid and carbohydrate in relation to different stages of maturity. In brachyuran crabs the studies of Rahman (1967), Chandran(1968), Adiyodi (1968), Pillay and Nair (1973), Diwan and Nagabhushanam (1974) and Subramoniam (1982) made significant contribution in this direction.

Similar investigations on penaeid prawns have been carried out by several investigators. Pillai and Nair (1973) studied the variation in biochemical components of ovary, muscle and hepatopancreas in relation to reproductive phases in *M. affinis.* Lawrence *et al.* (1979) reported the percentage composition of protein,

carbohydrate and lipid in ovary and hepatopancreas of ablated and unablated females of *P. vannamei*, *P. stylirostris* and *P. setiferus*. Read and caulton (1980) reported the changes in body composition in relation to moulting and ovarian development in *P. indicus* from the South African coast. Castille and Lawrence (1989) studied the relationship between maturation and biochemical composition of gonad and digestive gland in *P. aztecus* and *P. setiferus*. The variation in lipid profile of ovary and hepatopancreas during maturation in *P. japonicus* has been delineated by Teshima *et al.* (1989). Biochemical changes in shrimps have been reported by Kulkarni and Nagabhushanam (1979) in *P. hardwickii*, Achuthankutty and Parulekar (1984) in *M. affinis*, *M. dobsoni*, P. *merguiensis* and *P. stylifera*. However in freshwater prawns, these types of information are scanty. A very few studies are on record in fresh water prawns. Information is also very sparse on the variations in muscle protein during maturation. Carbohydrate is also a reserve food material in crustaceans and a number of reports are available describing its changes in relation to maturation (Florkin, 1960; Diwan and Nagabhushanam, 1974).

Moisture forms the major constituent of all the tissues and an antagonistic relationship between moisture and protein or lipid content has been reported in gonad and hepatopancreas of certain crustaceans (Pillai and Nair, 1973). Biochemical studies on pond reared *M. rosenbergii* have been carried out by Sherief *et al.* (1990). Effects of unilateral eyestalk ablation and feeding frequencies on the growth, survival and body composition of Juvenile fresh water prawn

Macrobrachium rosenbergii has been studied by Koshio *et al.* (1992). Nutrient compostion of hepatopancreas of *M. rosenbergii* has been carried out by Sherief and Xavier (1994). The biochemical characterization of different male morphotypes of *M. rosenbergii* from growout having single aged population has been carried out (Sureshkumar and Kurup, (1998). Sureshkumar and Kurup (1999) also studied the variations in hepato somatic index and biochemical profiles among the male morphotypes of *M. rosenbergii.*

Crustaceans require food protein in the form of essential amino acids for maintenance of life, growth and reproduction. The requirement for protein depends on animal characteristics, ie, species, physiological stage, size as well as dietary characteristics ie. protein quality (digestibility and biological value), energy level, etc. and also biotic factors, ie. temperature, salinity (D'Abramo and Sheen, 1993; Reed and D'Abramo, 1989). Proteins are composed of amino acids linked with peptide bonds and cross-linked between chains with sulphydryl and hydrogen bonds. There are twenty amino acids and of these the ten which can not be synthesised by shrimp are termed essential amino acids. The amino acid composition of proteins from different sources varies widely and while some proteins are good sources of the essential amino acids whereas others may have none. The availability of amino acids from a protein is affected to a great extent by its digestion and metabolism. The amino acid profile of a muscle tissue describes the relative proportions of the essential amino acids requirements of the cultured animals.The quantitative distribution of the free amino acids in certain species of

prawn in India has already been reported by Velankar and Iyer(1961). Amino acid requirements of some of the species have also been predicted through analysis of body tissues (Tidwell, 1998). Few studies have addressed amino acid requirements and metabolism in the fresh water prawn, *Macrobrachium rosenbergii* (de Man) (D' Abramo and Sheen, 1993; Reed and D' Abramo, 1989). The tail muscle amino acid profile of *Macrobrachium rosenbergii*, raised in fertilizer ponds, unfertilized ponds and fed a formulated pelleted feed has been reported by Tidwell *et al.* (1998). However, no information is available with regard to the quantitative distribution of amino acids of various male morphotypes and their transitional stages of *M. rosenbergii* except that of Ranjeet and Kurup (2001).

In the present study an attempt is made to examine the biochemical composition of various male morphotypes and their transitional stages of *M. rosenbergii* collected from natural environment. This also includes the study to determine the aminoacid profile of various male morphotypes and their transitional stages from growouts.

Materials and methods

Details regarding the collection and segregation of samples and methods follwed are described in chapter 4.

Results

The data for the various biochemical components viz. moisture, protein, lipid, carboydrate and ash content of the muscle tissue and hepatopancreas of various male morphotypes and their

transitional stages of *M.rosenbergii* are presented in Table 12.1 and 12.2 The aminoacid profile of various male morphotypes and their transitional stages of Macrobrachium rosenbergii are presented in the Table 12.3.

Ash content

The data for the ash content of the muscle tissue and hepatopancreas are given in Table 12.1 and 12.2. The ash content of the muscle tissue ranged from 0.666 to 0.705%, the lowest in OBC while it was highest in SOC and in the hepatopancreas the lowest was in SM with 0.67% and the highest in OBC with 0.795%. Any striking variation in the ash content in the muscle and hepatopancreas tissue among different male morphotypes and their transitional stages of *M. rosenbergii* have not been observed. (Table 12.4 and12.5).

Moisture

Moisture content of the muscle tissue and hepatopancreas are given in Table 12.1 and 12.2. Moisture content in the muscle tissue varied from 73.055 to 78.937%, lowest value in SOC morphotype while highest in OBC morphotype (Table 12.1). Where as moisture content in the hepatopancreas ranged between 53.352 to 57.785% (Table12.2). The lowest value of moisture content was found in SOC morphotype while it was highest in SBC morphotype. Significant variations in the values of moisture content in the muscle tissue and hepatopancreas was observed among the various male

morphotypes and their transitional stages of *M.rosenbergii* (Table12.6 and 12.7).

Protein

The values of protein in the muscle tissue and hepatopancreas ranged between 18.07 to 22.966% and 9.85 to 11.386% respectively (Table12.1and 12.2). In both cases, the lowest value was recorded in OBC and the highest value in SOC morphotype followed by t-SOC. Results of the analysis of variance showed that the protein content in the muscle tissue exhibits significant difference among vaious male morphotypes and their transitional stages of *M. rosenbergii* (Table 12.8). While significant variations in the protein content of hepatopancreas could' not be noticed among these morphotypes (Table12.9).

Lipid

The total lipid content in the muscle tissue was found to be very low when compared to that in hepatopancreas. The lipid content of muscle tissue ranged from 1.163 to 1.660% in OBC and SOC morphotype, respectively (Table 12.1). Where as in hepatopancreas the lowest in OBC with 28.79% and the highest in SOC with 33.173% (Table 12.2). No significant difference was observed in the lipid content of muscle tissue among the morphotypes (Table12.10). In contrast, significant difference in the values of lipid content in the hepatopancreas was observed among the various male morphotypes and their transitional stages of *M. rosenbergii* (Table 12.11).

Carbohydrate

The data for the carbohydrate content of the muscle tissue and hepatopancreas are given in Table 12.1 and 12.2. The carbohydrate of muscle tissue showed the lowest value in SM with 0.996% while it was highest in SOC with 1.616% and the hepatopancreas carbohydrate showed the lowest value of 1.75% in SBC and the highest value of 2.2% in SOC. Results of the anlysis of variance showed significant variations in muscle tissue as well as in hepatopancreas among the various male morphotypes and their transitional stages of *M. rosenbergii* (Table 12.12 and 12.13).

Except for variations in protein, carbohydrate and moisture contents, other biochemical constituents like lipid and ash content of the muscle tissue do not significantly vary among the various male mophotypes and their transitional stages of *M. rosenbergii.* Where as in the case of hepatopancreas, moisture, lipid and carbohydrate contents showed significant variations among the various male morphotypes and their transitional stages of *M. rosenbergii.*

Aminoacid profile

The results of the analysis of amino acids in the tail muscle tissue of various male morphotypes and their transitional stages are presented in Table12.3. The amino acid composition of the tail muscle tissue ranged between 79. 93 g/16 gn to 97.91 g/16 gn. The lowest value was observed in SBC while the highest value was recorded in SOC.

Discussion

Muscle tissue

The changes in the biochemical constituents in the muscle tissue are not so pronounced except that in protein, carbohydrate and moisture. The lipid and ash content of the muscle tissue do not show any significant fluctuations. The muscles are rich in protein and poor in fat and carbohydrate. From the tables 12.1 and 12.2 it can be seen that highest values of both protein and carbohydrate are in SOC motphotype and the lowest value in SBC morphotype. These morphotypes exhibit distinct variations in their reproductive activity and in growth rate. (Sagi *et al.,* 1988; Sureshkumar and Kurup, 1998). SOC morphotypes are reproductively inactive and exhibit faster growth rate. In contrast, the SBC morphotypes showed high reproductive activity while the growth rate is slow (Sagi *et al.*, 1988; Sureshkumar and Kurup, 1998; Joseph and Kurup, 2000, 2001). There exists an antagonistic relation exist between the reproduction and growth in crustaceans (Demousy, 1967). The significant difference in muscle protein can be taken as an index of higher cellular activity (Lemmens, 1995). Faster somatic growth observed in SOC males can be correlated with the high protein content recorded in the muscle tissue and this manifests the possibilities of higher protein synthesis (Anger and Hirche, 1990). Among the male morphotypes, SOC and its transitional stages are reported to be fastest growing animals (Sagi and Ra'anan, 1988; Kurup *et al.,* (unpublished) and the highest protein content of the body muscle recorded in this morphotype fully explains the biochemical basis of

faster growth rate. The results of the present study is in agreement with the observations made by Sureshkumar and Kurup, (1998, 1999) who had conducted studies on biochemical characterisation of various male morphotypes from growouts. Ranjeet and Kurup (2001) also found similar observation while conducting investigations on the primary intrinsic factors governing the heterogenous individual growth in various male morphotypes of *Macrobrachium rosenbergii*. They opined that the difference in the protein contents could be linked to differences in phenotypic triats which ultimately show a difference in somatic growth. The protein and glycogen content in the muscle tissue were found to be lower in SM, which occupies the initial stage of morphogenesis pathway. Growth of SM is also reported to be very slow as they convert a large part of their energy for mating attempts using a 'sneak mating' behaviour (Ra'anan and Sagi, 1985; Telecky, 1984).The low level of carbohydrate in the muscle tissue of SM as observed in the present study may be due to high energy expenditure used for mating behaviour as shown by SM.

Carbohydrate level probably represents storage of food, mainly glycogen in tissues and might be expected to vary during the reproductive period. Significant difference in the carbohydrate level as observed in the present study revealed that the glycogen levels are low in SBC and higher levels in SOC. This suggests that the stored carbohydrate might be utilized for the formation of reproductive elements and reproductive activities in SBC where as the higher carbohydrate content in the SOC morphotype manifests its reproductive submissiveness. It is further suggested that the organic

material may be transferred from the storage organ to gonad as the animal matures (Ferguson, 1964). Okazaki and Kobayashi(1929) while working on the Japanese oysters have stated that glycogen was at minimum level when breeding occurred. Humphrey (1941) and Hatanka (1940) also suggested that glycogen acted as a reserve food material and was utilized for the formation of gonad products. Similar observation was made by Giese *et al.*(1958) on purple sea urchin, *Strongylocentrotus purpuratus* and Durve and Bal (1960) on oyster *Crassostrea gryphoides*.The gonads of *Kathorina tunicata* showed lowest value of glycogen during the spawning period and the level rose again after spawning period (Giese, 1969).

The fat appeared to be one of the most important sources of the energy metabolism during breeding season. Even though conspicuous variations have not been noticed in the lipid content of muscle tissue of various male morphotypes and their transitional stages of *M.rosenbergii,* the highest value was observed in SOC and the lowest value in SBC morphotype.

Hepatopancreas

Hepatopancreas plays a significant role in food assimilation and mobilization of energy during moulting, pigmentation, gluconeogenesis and carbohydrate storage (Dhall and Moraity, 1984; Ghidalia, 1985; Skinner, 1985). The hepatopancreas is known to be a storage organ in decapod crustaceans and it is evident that considerable quantities of lipid are stored in the organ (Yonge, 1924). It appears that some glycogen is also stored in the hepatopancreas.

The hepatopancreas is rich in fat, where as the protein content is comparatively low and the glycogen content is higher than that of the muscle. The present study reveals that there is significant variation in the lipid content of hepatopancreas of various male morphotypes and their transitional stages of *M. rosenbergii.* The results of analysis of variance showed that significant variation (Table 12.11) in the lipid content of hepatopancreas of various male morphotypes and their transitional stages. The results of the present study is in contradictory to the observation made by Sureshkumar and Kurup (1998) who did not observe any significant variation in the lipid content of the hepatopancreas. The difference observed in the present study might be due to the variation in their habitat in which they live and their feeding strategies. Wild male morphotypes solely depends on natural feeds where as growout systems exclusively depends on prepared artificial feeds. This fact is in agreement with the observations made by Ackman (1974), Sidwell (1971) and Rubbi et al. (1985). Ackman (1974) has pointed out that cultured fishes have higher levels of lipid content as they are usually fed with prepared artificial feeds. Sidwell (1977) also reported that the composition of sea food can vary with the animal age, size, diet, location and season of catch. From the table 12.2, it can be seen that highest values of both lipid and glycogen are recorded in SOC morphotype while it was lowest in SBC morphotype. These morphotypes exhibit distinct variations in their reproductive activity and growth rate. SOC morphotypes are reproductively inactive and they are fast growing animals. In contrast, SBC morphotypes are reproductively very active while growth rate is low (Sagi *et al.*, 1988; Sureshkumar and kurup,

1998; Joseph and Kurup, 2000, 2001). The SBC morphotypes may require more organic materials than available directly from the food supply for reproductive activities. At this time the much needed lipid for developing gamates may be mobilised from the hepatopancreas which stores considerable quantities of lipid. This is in agreement with the reports of Pillay and Nair (1970) and Diwan and Nagabhushanam (1974). Pillay and Nair (1973) reported that in males of *Metapenaeus affinis* the lipid content of liver shows marked fluctuations in individuals at different stages of gonadal maturity during certain months. There is less fat in the liver of ripe individual than in unripe specimens.

Carbohydrate level probably represents storage of food, mainly glycogen, in the hepatopancreas and might be expected to vary during the reproductive period. Results of analysis of variance showed that carbohydrate level in hepatopancreas were found significantly different in various male morphotypes and their transitional stages. Lowest carbohydrate value was found in SBC and highest in SOC. This would postulate that the stored carbohydrate might be utilized for the formation of reproductive elements and reproductive activities in SBC whereas the higher carbohydrate content in the SBC morphotype is due to the inactivity in reproduction and submissive nature (Sagi and Ra'anan, 1988; Kurup *et al.,* unpublished). Significant difference in carbohydrate and lipid content of the hepatopancreas in the present study will lend to support the difference in growth among various male morphotypes as reported by Cohen *et al.* (1981); Kuris *et al.* (1987); Sagi and Ra'anan

(1983). Among the various male morphotypes, SOC and its transitional stages are reported to be fastest growing animals. The size of hepatopancreas of SOC is larger (Sureshkumar and Kurup, 1999) when compared to other morphotypes and this finding would suggest the possibility of better food assimilation and higher carbohydrate storage (Dhall and Moraity, 1984). The growth of SM is also reported to be very low (Cohen *et al.*, 1981; Kuris *et al.*, 1987; Sagi and Ra'anan, 1983; Kurup et al unpublished) as they convert a large part of their energy for mating attempt using a 'sneak mating' behaviour (Telecky, 1984). The low growth rate observed in SM morphotype may be due to the high amount of energy spent for reproductive activities, that might be spared by the lipid and carbohydrate in the hepatopancreas. Sherief *et al.* (1992) reported lower levels lipid in stunted males and this finding fully conforms to the present observation. The results of the present study strongly corroborates with that of Sureshkumar and Kurup (1998, 1999) who carried out studies on biochemical characterization of various male morphotypes and their transitional stages of *M. rosenbergii* collected from grow out system.

Moisture content also showed significant fluctuation in both muscle and hepatopancreas of various male morphotypes and their transitional stages of *M. rosenbergii*. This is in agreement with the results of Pillay and Nair (1973) who observed an inverse relationship between the water content and gonad development in *Metapanaeus affinis* and *Portunus pelagicus*. Moisture and lipid are inversely

related in the hepatopancreas as observed in the case of fish and prawn (Mukundan *et al.*, 1981; Sherief *et al.*, 1992).

In conclusion, there is strong indications of possible transfer of organic substances such as protein and carbohydrate from the muscle, lipid and carbohydrate from the hepatopancreas for various metabolic processes connected with reproduction and growth among the various male morphotypes and their transitional stages of *M. rosenbergii.*

Aminoacid profile

Among the amino acids, glutamic acid registered the highest concentration followed by Aspartic acid, Arginine, Leucine, Alanine and Glycine in the order. The lowest value was recorded by Tryptophan. The abundance of Glutamic acid, glycine, alanine and arginine was reported also in Brazelian Shrimps by De Almidia (1954) and in Penaeid shrimps by James (1972). Hashimoto (1964) demonstrated that the higher amount of free glycine, proline, valine and methionine in the shrimp muscle contributes to flavour. Tryptophan content was found to be negligible in all the morphotypes and their transitional stages of *M. rosenbergii.* The results of the present study also corroborates with the work of Tidwell *et al.* (1998), they also reported a similar pattern of amino acid profile of the tail muscle of *M. rosenbergii*, except that of glutamic acid and aspartic acid. These two amino acids were not included in their study since their work solely concentrated on essential amino acids. Tidwell *et al.* (1998) also reported that amino acid profiles of tail muscle was not

treatment dependent. Comparison of amino acids profile of the diet with that of tissues of animals fed that diet revealed that dietary levels of arginine, histidine, methionine and especially, lysine may be suboptimal. However, under practical culture conditions, prawn growth may not be significantly reduced because of relatively low dietary requirements and contributions from natural foods in the ponds. They also reported that though there were some differences, the actual magnitude of these differences was quite small, and they were probably not having biological significance. As reported by Tidwell,(1998), amino acid requirements for some species could be predicted through analysis of body tissue. The present study reveals that the muscle tissue showed highest concentration of amino acid profile in SOC morphotype where as it was least in SBC morphotype (Table 12.3). These two morphotypes differ in their growth rate (Sagi *et al.*, 1988; Kurup., unpublished) and therefore, it can reasonably be inferred that the difference observed in their amino acid profile might have a direct influence on the growth difference manifested by these morphotypes. This is in agreement with the observation made by Ranjeet and Kurup (2001) that a sudden decline of total and free aminoacids from SM to t-SOC and subsequently a gradual rise till OBC manifest the utilisation of aminoacids by SM and OBC for somatic growth insignificantly and reciprocally the aminoacids get accumulated in the cytoplasm whereby increasing their overall concentration.

Table 12.1

Biochemical composition of muscle tissue of various male morphotypes and their transitional stages of *Macrobrachium rosenbergii* collected from the wild

Biochemical composition / Morphotypes	* Moisture %	* Protein %	Lipid %	* Carbohydrate %	Ash %
Small Male (SM)	76.765	19.985	1.486	0.997	0.685
Weak Orange Clawed Male (WOC)	76.134	20.568	1.573	1.045	0.680
Strong Orange Clawed Male (SOC)	73.055	22.966	1.660	1.610	0.705
Pre-transforming Orange Clawed male (t-SOC)	75.420	21.053	1.630	1.196	0.701
Weak Blue Clawed Male (WBC)	76.130	20.268	1.616	1.300	0.685
Strong Weak Clawed Male (SBC)	78.397	18.466	1.356	1.108	0.675
Old Blue Clawed Male (OBC)	78.937	18.073	1.163	1.150	0.666

* Significant at 5% level

Table 12.2

Biochemical composition of hepatopancreas of various male morphotypes and their transitional stages of *Macrobrachium rosenbergii* collected from the wild

Biochemical composition / Morphotypes	* Moisture %	Protein %	* Lipid %	* Carbohydrate %	Ash %
Small Male (SM)	57.86	9.63	30.43	1.81	0.670
Weak Orange Clawed Male (WOC)	56.50	9.88	31.00	1.95	0.675
Strong Orange Clawed Male (SOC)	53.35	10.55	33.17	2.20	0.725
Pre-transforming Orange Clawed male (t-SOC)	54.75	10.54	31.90	2.11	0.695
Weak Blue Clawed Male (WBC)	56.18	10.22	30.85	2.06	0.695
Strong Weak Clawed Male (SBC)	57.78	10.41	29.28	1.75	0.775
Old Blue Clawed Male (OBC)	57.69	10.75	28.79	1.97	0.795

* Significant at 5% level

TABLE 12.3

Amino acid profile of tail muscle of various male morphotypes and their transitional stages of *Macrobrachium rosenbergii* collected from the growout

Morphotypes / Amino acids	SM	WOC	SOC	t - SOC	WBC	SBC	OBC
	g/16gn	g/16gn	g/16gn	g/16gn	g/16gn	g/16gn	g/16gn
Aspartic acid	10.84	10.19	11.32	9.63	9.92	9.38	9.68
*Threorine	3.43	3.49	3.86	3.16	3.51	3.26	3.32
Serine	3.83	3.5	3.82	3.25	3.46	3.19	3.43
Glutamic acid	16.72	16.07	17.65	14.44	16.10	14.34	15.83
Proline	2.91	2.18	3.75	3.57	3.14	2.53	3.17
Glycine	5.94	6.01	6.13	4.42	5.48	4.27	5.58
Alanine	5.70	6.4	6.66	5.16	6.70	6.26	6.01
Cystine	1.15	1.41	1.83	1.10	1.31	1.47	0.93
*Valine	3.63	4.15	4.36	3.72	4.06	4.05	3.78
*Methionine	1.78	2.06	1.99	1.29	2.04	1.74	1.56
*Isoleucine	3.04	3.44	3.66	3.05	3.43	3.31	3.40
*Leucine	6.53	6.76	7.36	6.17	6.92	6.30	6.63
Tyrosine	3.06	3.14	3.44	2.65	3.06	2.99	2.62
*Phenylalanine	3.36	4.11	3.83	3.20	3.50	3.31	3.19
*Histidine	2.5	2.96	3.26	2.52	2.99	2.44	2.56
*Lysine	4.41	5.31	5.13	4.40	4.77	3.51	4.23
*Arginine	8.51	9.92	9.64	9.04	8.87	7.41	9.68
*Tryptophan	0.95	0.75	1.20	1.50	1.01	1.18	0.70
Total	87.65	91.24	97.91	80.94	89.48	79.93	85.77

* Essential Amino acids.

SM – Small Males

WOC – Weak Orange Clawed Males

SOC – Strong Orange Clawed Males

t – SOC – Pre-transforming Orange Clawed Mlaes

WBC – Weak Blue Clawed Males

SBC – Strong Blue Clawed Males

OBC – Old Blue Clawed Males

Table 12.4

Details of ANOVA of ash content in the muscle tissue of various male morphotypes and their transitional stages collected from the wild

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
0.71	0.74	0.72	0.73	0.72	0.66	0.64
0.66	0.64	0.69	0.67	0.65	0.69	0.69

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	1.37	0.685	0.00125
Column 2	2	1.38	0.69	0.005
Column 3	2	1.41	0.705	0.00045
Column 4	2	1.4	0.7	0.0018
Column 5	2	1.37	0.685	0.00245
Column 6	2	1.35	0.675	0.00045
Column 7	2	1.33	0.665	0.00125

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.002271	6	0.000379	0.209486	0.962309	3.86597776
Within Groups	0.01265	7	0.001807			
Total	0.014921	13				

Table 12.5

Details of ANOVA of ash content in the hepatopancreas of various male morphotypes and their transitional stages collected from the wild

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
0.65	0.66	0.77	0.64	0.71	0.82	0.88
0.69	0.68	0.68	0.75	0.68	0.73	0.71

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	1.34	0.67	0.0008
Column 2	2	1.34	0.67	0.0002
Column 3	2	1.45	0.725	0.00405
Column 4	2	1.39	0.695	0.00605
Column 5	2	1.39	0.695	0.00045
Column 6	2	1.55	0.775	0.00405
Column 7	2	1.59	0.795	0.01445

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.029786	6	0.004964	1.156406	0.421314	3.865978
Within Groups	0.03005	7	0.004293			
Total	0.059836	13				

Table 12.6

Details of ANOVA of moisture content in the muscle tissue of various male morphotypes and their transitional stages collected from the wild

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
75.948	75.6337	73.479	75.42	76.499	78.53	78.2046
76.849	76.6337	73.062	74.72	75.752	77.142	79.0046
77.498	76.1337	72.624	76.12	76.139	79.52	79.6046

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	230.295	76.765	0.605917
Column 2	3	228.4011	76.1337	0.25
Column 3	3	219.165	73.055	0.182793
Column 4	3	226.26	75.42	0.49
Column 5	3	228.39	76.13	0.139563
Column 6	3	235.192	78.39733	1.426921
Column 7	3	236.8138	78.93793	0.493333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	68.57008	6	11.42835	22.29283	2.08E-06	2.847727
Within Groups	7.177055	14	0.512647			
Total	75.74714	20				

Table 12.7

Details of ANOVA of moisture content in the hepatopancreas of various male morphotypes and their transitional stages collected from the wild

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
57.06	55.7	53.28	54.75	56.18	57.785	57.692
57.46	56.5	54.14	54.15	56.88	57.035	56.792
57.86	57.3	52.64	55.35	55.48	58.535	58.592

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	172.38	57.46	0.16
Column 2	3	169.5	56.5	0.64
Column 3	3	160.06	53.35333	0.566533
Column 4	3	164.25	54.75	0.36
Column 5	3	168.54	56.18	0.49
Column 6	3	173.355	57.785	0.5625
Column 7	3	173.076	57.692	0.81

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	49.82292	6	8.303821	16.19565	1.43E-05	2.847727
Within Groups	7.178067	14	0.512719			
Total	57.00099	20				

Table 12.8

Details of ANOVA of protein content in the muscle tissue of various male morphotypes and their transitional stages collected from the wild

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
19.485	20.985	22.815	22.97	21.185	18.75	18.61
21.485	19.485	22.515	20.97	21.435	19.28	17.43
18.985	21.235	23.568	19.22	18.185	17.37	18.18

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	59.955	19.985	1.75
Column 2	3	61.705	20.56833	0.895833
Column 3	3	68.898	22.966	0.294303
Column 4	3	63.16	21.05333	3.520833
Column 5	3	60.805	20.26833	3.270833
Column 6	3	55.4	18.46667	0.972233
Column 7	3	54.22	18.07333	0.356633

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	48.2778	6	8.046301	5.092287	0.005738	2.847727
Within Groups	22.12134	14	1.580096			
Total	70.39914	20				

Table 12.9

Details of ANOVA of protein content in the hepatopancreas of various male morphotypes and their transitional stages collected from the wild

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
9.5	9.88	12.08	12.067	9	9.166	10.061
8.66	10.72	10.424	8.737	11.16	12.08	9.645
10.75	9.053	9.174	10.817	10.5	10	12.555

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	28.91	9.636667	1.106033
Column 2	3	29.653	9.884333	0.694736
Column 3	3	31.678	10.55933	2.124945
Column 4	3	31.621	10.54033	2.829633
Column 5	3	30.66	10.22	1.2252
Column 6	3	31.246	10.41533	2.252225
Column 7	3	32.261	10.75367	2.476865

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.88646	6	0.481077	0.264959	0.944349	2.847727
Within Groups	25.41928	14	1.815663			
Total	28.30574	20				

Table 12.10

Details of ANOVA of lipid content in the muscle tissue of various male morphotypes and their transitional stages collected from the wild

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
1.2	1.44	1.43	1.37	1.52	1.2	1.21
1.52	1.54	1.67	1.72	1.71	1.54	0.98
1.74	1.74	1.88	1.8	1.62	1.33	1.3

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	4.46	1.486667	0.073733
Column 2	3	4.72	1.573333	0.023333
Column 3	3	4.98	1.66	0.0507
Column 4	3	4.89	1.63	0.0523
Column 5	3	4.85	1.616667	0.009033
Column 6	3	4.07	1.356667	0.029433
Column 7	3	3.49	1.163333	0.027233

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.586590476	6	0.097765	2.575024	0.067914	2.847727
Within Groups	0.531533333	14	0.037967			
Total	1.11812381	20				

Table 12.11

Details of ANOVA of lipid content in the hepatopancreas of various male morphotypes and their transitional stages collected from the wild

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
30.43	29.2	32.83	31.71	30.743	29.21	27.405
30.03	31.8	32.78	33.21	32.173	31.15	30.615
30.83	32	33.91	30.8	29.643	27.48	28.355

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	91.29	30.43	0.16
Column 2	3	93	31	2.44
Column 3	3	99.52	33.17333	0.407633
Column 4	3	95.72	31.90667	1.481033
Column 5	3	92.559	30.853	1.6093
Column 6	3	87.84	29.28	3.3709
Column 7	3	86.375	28.79167	2.719033

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	40.13108695	6	6.688514	3.841482	0.017814	2.847727
Within Groups	24.3758	14	1.741129			
Total	64.50688695	20				

Table 12.12

Details of ANOVA of carbohydrate content in the muscle tissue of various male morphotypes and their transitional stages collected from the wild

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
0.98	0.92	1.62	1.21	1.23	1.02	1.14
1.12	1.03	1.71	1.25	1.29	1.12	1.09
0.89	1.17	1.52	1.13	1.38	1.18	1.22

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	2.99	0.996667	0.013433
Column 2	3	3.12	1.04	0.0157
Column 3	3	4.85	1.616667	0.009033
Column 4	3	3.59	1.196667	0.003733
Column 5	3	3.9	1.3	0.0057
Column 6	3	3.32	1.106667	0.006533
Column 7	3	3.45	1.15	0.0043

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.785314	6	0.130886	15.67941	1.73E-05	2.847727
Within Groups	0.116867	14	0.008348			
Total	0.902181	20				

Table 12.13

Details of ANOVA of carbohydrate content in the hepatopancreas of various male morphotypes and their transitional stages collected from the wild

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
1.7	2	2.19	2.16	2.16	1.837	2.12
1.81	2.05	2.34	2.18	2.08	1.647	1.88
1.92	1.8	2.07	2	1.93	1.767	1.92

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	5.43	1.81	0.0121
Column 2	3	5.85	1.95	0.0175
Column 3	3	6.6	2.2	0.0183
Column 4	3	6.34	2.113333	0.009733
Column 5	3	6.17	2.056667	0.013633
Column 6	3	5.251	1.750333	0.009233
Column 7	3	5.92	1.973333	0.016533

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.463923	6	0.077321	5.577915	0.003855	2.847727
Within Groups	0.194067	14	0.013862			
Total	0.65799	20				

CHAPTER 13

LIPID FRACTIONISATION

Introduction

Lipids are indispensable nutrients for growth and survival of shrimps and prawns (Kanazawa, *et al.*, 1985). These are important source of energy reserve for cellular constituents during their developmental stages (Harrison, 1990). In crustaceans, organic reserves in the form of lipid are stored in the hepatopancreas and act as the sole storage depot (O'connor and Gilbert, 1968). The uptake of hepatopancreatic lipid during maturation has been investigated in crabs (Anilkumar, 1980; Varadarajan and Subramoniam, 1982) and prawns (Kulkarni and Nagabhushanam, 1979; Mohamed and Diwan, 1992). Information is scanty on the mobilization of muscle lipid during maturation, but for the report of Ajmalkhan and Natrajan (1980) in crab.

Several studies have been carried out to quantify the total lipid and lipid components viz. cholesterol, phospholipid and triglycerides in crustaceans. The cholestrol is a very important constituent in the body of crustaceans since it forms the major component of steroid hormones (Rao *et al.*, 1981a). Cholesterol is an essential precursor of bile acids, steroid hormones, moulting hormones, vitamin D_3 and prostaglandins (Steffens, 1989; Tacon, 1995). Crustaceans are reported to have no ability to synthesise cholesrterol (Huggins and Munday, 1968). However more recently it has been observed that some decapods have the ability to synthesise

cholesterol (Krishnamurthy *et al.,* 1980). Appropriate phospholipid and cholesterol supplementation is known to promote growth and survival of many marine and fresh water crustaceans (Castell *et al.*, 1979; D'Abramo *et al.,* 1981,1984, Kanazawa *et al.*, 1985; Briggs *et al.,* 1988). Most studies have focussed on the requirements of dietary phospholipid and cholesterol on juvenile and larger shrimp rather than their composition in the total lipid in various organ tissues. Idler *et al.* (1964) reported seasonal variations in the unsaponifiable components of scallop muscle. Addison *et al.* (1972) studied the lipid composition of the queen crab, *Chionoecetes opilio.* Guary *et al.* (1974) reported lipid class distribution and fatty acid composition of prawn *Penaeus japonicus.* Gopakumar and Nair (1975) reported lipid composition of five species of Indian prawns. Lipid class and fatty acid composition of *Chorismus antarcticus* (Pfeffer) from South Georgia was reported by Clarke (1977). Lipid characterization and distribution among tissues of the fresh water prawn *Macrobrachium borelli* during an annual cycle has been studied by Gonzalez Baro and Pollero (1988).

Studies have also been carried out to bring out the lipids and fatty acids compositions of the various molluscan spp. Seasonal changes of the lipids of the mollusc *Chlamys tehuelcha* has been reported by pollero *et al.* (1979). Pollero and Brenner. (1981) observed the effect of enviornment and fastening on the lipid and fatty acid composition of *Diplodon patagonicus.* Pollero *et al.* (1983) also reported sterol composition of two freshwater molluscs of genus *Diplodon.* The effect of sexual stages on lipids and fatty acids of *Diplodon delodontus*

has been reported by Pollero *et al.* (1983). Rao *et al.* (1981a) correlated the variations in cholesterol content of the ovary, hepatopancreas and muscle with stages of ovarian development in *Macrobrachium lanchesteri.* The variations in cholesterol content in ovary and hepatopancreas in relation to reproduction have been studied by Sunilkumar (1989) in *Penaeus indicus.* Ando *et al.*, (1987) studied the variation in the lipids of tissues during the moulting cycle of prawn. development Triacylglycerol content as a condition index for fish, bivalve and crustacean larvae has been studied by Fraser (1989). Phospholipids of aquatic animals have been carried out by Zama (1963). The present study aims at analysing the various lipid components viz., cholesterol, phospholipd and triglyceride in the muscle tissue and hepatopancreas of various male morphotypes and theit transitional stages of *M. rosenbergii* collected from the growout.S

Materials and Methods

Details regarding the collection, segregation of morphotypes, prepartion of samples and methods followed for the analysis are described in chapter 4.

Results

The data for the various lipid components such as cholesterol, phospholipid and triglyceride content of the muscle tissue and hepatopancreas of various male morphotypes and their transitional stages of *M. rosenbergii* are presented in Table 13.1 and 13.2.

Cholesterol : The amounts of total cholesterol in the muscle tissue and hepatopancreas of various male morphotypes and their transitional stages of *M.rosenbergii* are shown in Table 13.1 and 13.2. Total cholesterol content in the muscle tissue ranged between 105.175 to 164.13 mg%, the lowest was found in SM and the highest in SOC followed by WBC morphotypes. The cholesterol content in the hepatopancreas ranged between 222.81 to 260.205 mg%. The lowest was observed in SBC and the highest in SOC. Results of statistical analysis of cholesterol content in the muscle tissue and hepatopancreas are given in Table13.3 and 13.4. Significant differences were noted in the cholesterol content of muscle tissues among the various male morphotypes and their transitional stages of *M.rosenbergii* (Table 13.3). The hepatopancreas cholesterol content was found non-significant among the various male morphotypes and their transitional stages of *M.rosenbergii* (Table 13.4)..

Phospholipid : The phospholipid content of the muscle tissue and hepatopancreas lipids of vaious male morphotypes and their transitional stages of *M. rosenbergii* are shown in Table 13.1 and 13.2. Phospholipid content of the muscle tissue ranged between 40.975 to 90.3437 mg%, the lowest and highest value were observed in SBC and SOC respectively. The hepatopancreas phospholipid content varied from 327.867 to 870.917 mg%. The lowest and highest value were observed in OBC and SOC respectively. Results of the statistical analysis of phospholipid contents in the muscle tissue and hepatopancreas are given in Table 13.5 and 13.6. Significant differences could be observed

in the phospholipid content in both the tissues of various male morphotypes and their transitional stages of *M. rosenebergii*.

Triglyceride : The quantity of triglyceride in the muscle tissue and hepatopancreas ranged between 0.5151 to 1.477 g% and 13.9916 to 18.4669 g%, respectively. Lowest value of triglyceride in muscle tissue was observed in SBC and the highest in SOC morphotypes. The lowest and highest value of triglyceride in the hepatopancreas were in SBC and SOC respectively. Results of statistical analysis of triglyceride contents in the muscle tissue and hepatopancreas are shown in Table 13.7 and 13.8. Significant difference was noted in the triglyceride content in the muscle tissue while it was insignificant in the hepatopancreas of various male morphotypes and their transitional stages of *M. rosenbergii*.

Discussion

In general, lipid content is low in the muscle tissue where as in the case of hepatopancreas, the lipid forms the major nutrient in all the male morphotypes and their transitional stages of *M. rosenbergii*. Similar observation was reported in prawn meat by Mukundan *et al.* (1981) and Sherief *et al.* (1992) and Sureshkumar and Kurup (1998) and in hepatopancreas by sherief and Xavier (1994). The present study reveals that though the hepatopancreas of these morphotypes and their transitional stages are rich in lipid content,but low in cholesterol content than that of the muscle tissue. This is in agreement with the observations made by Sherief and Xavier (1994), who carried out the biochemical analysis of

hepatopancreas of *M. rosenbergii*. The phospholipid content is more in muscle tissue than to hepatopancreas. Both the tissues contained higher amounts of triglyceride content in their lipids. Chanmugam *et al.* (1988) reported that the contribution of triglyceride in the lipid composition in the whole *M. rosenbergii* is more than seventy percent. Ackman (1992) reported on the cholesterol contents of three species of crabs in the range of 71 to 78 mg%. Connor and Lin (1982) have reported that the cholesterol content of shellfish are generally low when compared to fish. Higher muscle cholesterol content in the range of 118 to 163mg% has been reported by Mathew *et al.* (1999). In the present study also comparatively higher concentrations of cholesterol were observed in the muscle tissue of various male morphotypes and their transitional stages and this finding is comparable with that of Mathew *et al.*(1999). In the present study it was found that the cholesterol content is very low in hepatopancreas in contrast to the high lipid content in it. This findings show strong corroboration with that of Sherief and Xavier (1994). Idler *et al.* (1964) reported that no obvious difference existed in the composition of male and female Scallops. On the contrary, Mathew *et al.* (1999) observed variation in the cholesterol content in the case of female Mackeral. They are of the view that the increase may not be due to sexual difference but due to the months of their collection of the samples. Variations in the cholesterol content of the muscle tissue, during different seasons has been reported by Idler *et al.* (1964), suggesting a relationship between the metabolism and biosynthesis of these sterols. The role of sterols in crustaceans is still not clearly understood, although a part of cholesterol, an exclusively

major sterol in the crustacean tissue has been shown to be converted to steroid hormones (Kanasawa and Teshima,1971). In the present study also, significant variation in the cholesterol content could be observed in the muscle tissue of various male morphotypes and their transitional stages of *M. rosenbergii.* Therefore, it can be stated that the difference in cholesterol content is due to the variations in reproductive activity of these morphotypes as expressed by Sagi *et al.* (1988); Sureshkumar and Kurup (1998) and Joseph and Kurup (2001).

Triglyceride content in the hepatopancreas was found to be higher in SOC and low in SBC morphotype. Since triglycerols are mainly used as energy store, their increase during gametogenesis would denote that the principal aim of this accumulation is to provide energy rich material for subsequent consumption during gamete development. The present result is also in agreement with the finding in marine prawn *Penaeus* japonicus (Fraser, 1989) who reported an increase in triglyceride content during oogenesis. Higher percentage of triglyceride distribution in the hepatopancreas of SOC may be due to an enlarged food intake by SOC morphotype. Sureshkumar and Kurup (1999) also reported that comparatively higher hepatosomatic index in SOC morphotype when compared to other morphotypes and emphasized that there could be an increased food consumption by the SOC morphotype than other morphotypes.

Table 13.1

Lipid fractions of muscle tissue of various male morphotypes and their transitional stages of *Macrobrachium rosenbergii* from

Lipid fractions / Morphotypes	* Cholesterol mg%	* Phospholipid mg%	* Triglyceride g %
Small Male (SM)	105.175	80.275	0.982
Weak Orange Clawed Male (WOC)	108.290	62.975	1.213
Strong Orange Clawed Male (SOC)	164.130	90.344	1.477
Pre-transforming Orange Clawed male (t-SOC)	134.400	41.417	1.164
Weak Blue Clawed Male (WBC)	140.605	58.025	1.147
Strong Weak Clawed Male (SBC)	123.545	40.975	0.515
Old Blue Clawed Male (OBC)	135.745	60.310	0.836

* Significant at 5% level

Table 13.2

Lipid fractions of hepatopancereas of various male morphotypes and their transitional stages of *Macrobrachium rosenbergii*

Lipid fractions / Morphotypes	Cholesterol mg%	* Phospholipid mg%	Triglyceride g %
Small Male (SM)	226.195	701. 417	15.305
Weak Orange Clawed Male (WOC)	243. 965	762.750	13.992
Strong Orange Clawed Male (SOC)	260.205	870.917	18.469
Pre-transforming Orange Clawed male (t-SOC)	256.025	515.625	16.820
Weak Blue Clawed Male (WBC)	245.125	586.900	17.185
Strong Weak Clawed Male (SBC)	222. 810	407.367	13.815
Old Blue Clawed Male (OBC)	257.395	327.867	15.687

* Singnificant at 5% level

Table 13.3

Details of ANOVA of cholesterol content in the muscle tissue of various male morphotypes and their transitional stages collected from the growout

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
99.46	104.29	169.79	130.59	135.17	119.28	140.27
110.89	112.29	158.47	138.21	146.04	127.81	131.22

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	210.35	105.175	65.32245
Column 2	2	216.58	108.29	32
Column 3	2	328.26	164.13	64.0712
Column 4	2	268.8	134.4	29.0322
Column 5	2	281.21	140.605	59.07845
Column 6	2	247.09	123.545	36.38045
Column 7	2	271.49	135.745	40.95125

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4916.899	6	819.4831	17.55125	0.000677	3.865978
Within Groups	326.836	7	46.69086			
Total	5243.735	13				

Table 13.4

Details of ANOVA of cholesterol content in the hepatopancreas of various male morphotypes and their transitional stages collected from the growout

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
245.23	254.72	269.29	263.93	265.93	232.48	273.01
207.16	233.21	251.12	248.12	224.32	213.14	241.78

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	452.39	226.195	724.6625
Column 2	2	487.93	243.965	231.3401
Column 3	2	520.41	260.205	165.0744
Column 4	2	512.05	256.025	124.9781
Column 5	2	490.25	245.125	865.696
Column 6	2	445.62	222.81	187.0178
Column 7	2	514.79	257.395	487.6565

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2703.905	6	450.6509	1.132116	0.431519	3.865978
Within Groups	2786.425	7	398.0608			
Total	5490.331	13				

Table 13.5

Details of ANOVA of phospholipid content in the muscle tissue of various male morphotypes and their transitional stages collected from the growout

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
82.45	62.975	81.7	37.95	57.2	39.1	55.32
78.1	51.85	90.344	43.5	74.4	48.125	65.3
80.275	74.1	98.987	42.8	42.475	35.7	60.31

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	240.825	80.275	4.730625
Column 2	3	188.925	62.975	123.7656
Column 3	3	271.031	90.34367	74.71009
Column 4	3	124.25	41.41667	9.135833
Column 5	3	174.075	58.025	255.3119
Column 6	3	122.925	40.975	41.23188
Column 7	3	180.93	60.31	24.9001

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6067.968	6	1011.328	13.26242	4.56E-05	2.847727
Within Groups	1067.572	14	76.25515			
Total	7135.54	20				

Table 13.6

Details of ANOVA of phospholipid content in the hepatopancreas of various male morphotypes and their transitional stages collected from the growout

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
704.25	744	785.25	568.75	583.5	431.25	335.75
674.2	781.5	822	462.5	572.2	383.5	320
725.8	762.75	1005.5	515.625	605	407.35	327.85

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	2104.25	701.4167	671.6608
Column 2	3	2288.25	762.75	351.5625
Column 3	3	2612.75	870.9167	13922.15
Column 4	3	1546.875	515.625	2822.266
Column 5	3	1760.7	586.9	277.63
Column 6	3	1222.1	407.3667	570.0158
Column 7	3	983.6	327.8667	62.01583

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	685554.8	6	114259.1	42.82279	3.23E-08	2.847727
Within Groups	37354.59	14	2668.185			
Total	722909.4	20				

Table 13.7

Details of ANOVA of triglyceride content in the muscle tissue of various male morphotypes and their transitional stages collected from the growout

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
0.936	1.5612	1.4852	1.0473	1.0108	0.6236	0.9301
1.0285	0.8653	1.5153	1.0122	1.2228	0.5155	0.8611
0.9817	1.2133	1.4313	1.4313	1.2085	0.4061	0.7169

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	2.9462	0.982067	0.002139
Column 2	3	3.6398	1.213267	0.121069
Column 3	3	4.4318	1.477267	0.001811
Column 4	3	3.4908	1.1636	0.054055
Column 5	3	3.4421	1.147367	0.014039
Column 6	3	1.5452	0.515067	0.011827
Column 7	3	2.5081	0.836033	0.011835

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.704344	6	0.284057	9.172631	0.000344	2.847727
Within Groups	0.433551	14	0.030968			
Total	2.137895	20				

Table 13.8

Details of ANOVA of triglyceride content in the hepatopancreas of various male morphotypes and their transitional stages collected from the growout

SM	WOC	SOC	t-SOC	WBC	SBC	OBC
14.377	13.63	18.692	18.528	18.319	13.665	12.482
16.673	12.408	19.367	14.108	16.237	13.009	19.08
14.871	15.937	17.348	17.824	17	14.772	15.499

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	45.921	15.307	1.460476
Column 2	3	41.975	13.99167	3.211562
Column 3	3	55.407	18.469	1.056387
Column 4	3	50.46	16.82	5.640112
Column 5	3	51.556	17.18533	1.109442
Column 6	3	41.446	13.81533	0.793992
Column 7	3	47.061	15.687	10.90991

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	52.44824	6	8.741373	2.530391	0.071473	2.847727
Within Groups	48.36376	14	3.454554			
Total	100.812	20				

SUMMARY

SUMMARY

The giant fresh water prawn *Macrobrachium rosenbergii* (de Man) is emerging as a prime candidate species in fresh water aquaculture as a global basis and therefore, receiving much attention in recent years. This species has been cultured under different levels of scientific management practices in India because of its high growth rate, large size, hardiness, resistance to diseases, excellent export demand and good market value. However, during the process of larval culture and domestication, a perceptable difference in growth pattern of males became apparent due to the morphotypic differentiation. Sexually mature male populations of *M. rosenbergii* belonging to the same group has been differentiated into three distinct morphotypes such as small males (SM), orange clawed males (OC) and blue clawed males (BC). Besides, two transitional stages of OC viz. weak orange clawed males (WOC) and pre-transforming orange clawed males (t-SOC) are distinguishable and therefore, the fully differentiated OC are known as strong orange clawed males (SOC). Among the different forms of blue clawed males, the weak blue clawed males are the transitional stages of pre-transforming orange clawed male (t-SOC) and strong blue clawed males (SBC) while old blue clawed males (OBC), the terminal stage is characterised by relatively small body in terms of carapace length and body weight disproportionate to claw length. Each morphotype develops in a sequential pattern in the adult male population from small males through orange clawed males and further to dominant blue clawed males. These morphotypes are known to exhibit a complex social and organizational hierarchy comprising dominant, subdominant and subordinate groups of animals which are having distinct morphologically distinguishable characteristics. The wide disparity in size structure of the harvested stock from the grow outs which is profoundly associated with the

male morphotypes apparently appears to be the most commercial disadvantage of this species for commercial aquaculture practices. In spite of the fact that these male morphotypes and their transitional stages are well characterised morphologically from growouts as well as natural habitats in India and abroad, however, no concerted attempt has so far been made to unravel the puzzling biological phenomenon involved in the morphogenesis, to establish the role of various internal organs intrinsically associated with morphotypic differentiation and transformation and also to elucidate the structural variation of internal organs responsible for morphotypic expression and difference in somatic growth and reproductive activity expressed by the male morphotypes.

Against this background, the present work was aimed at to study the histological variations, if any, in the reproductive system viz. testes, vas deferens including androgenic gland, hepatopancreas and the neurosecretory system viz. eye stalk, brain and thoracic ganglion among the male morphotypes and their transitional stages of *M.rosenbergii* from growouts. This study was also aimed at to bring out the histochemical variations, if any, in the reproductive system comprising of testes, vas deferens including androgenic gland and the hepatopancreas among the male morphotypes and their transitional stages collected from growouts. Biochemical characterisation of various male morphotyes and their transitional stages have also been attempted in order to find out biochemical evidence, if any, in the morphotypic transformation.

Histological study of the testes of three male morphotypes viz., SM, SOC and SBC and their transitional stages viz., WOC, t-SOC, WBC and OBC have been carried out with a view to unravel the structural and functional differences of the testes, if any, of these morphotypes. Testicular lobes are composed of long cylinders

compactly held together by connective tissue. Histological study was useful in bringing out the differences in the content of the cylinders among the different morphotypes and their transitional stages. The structural properties of the testes are found fully conforming with the differential reproductive strategies exhibited by the three male morphotypes and their four transitional stages. Testes of SM, WOC and t-SOC are characterised by the presence of a spermatogenic zone and spermatozoans in their seminiferous cords and this would lend to support its reproductive potential. On the contrary, SOC and WBC are characterised by the absence of spermatozoans and presence of spermatocytes only in their testicular lobules, an observation which justifies its submissive or inactive nature of reproductive activity. Interestingly, the testicular lobules of SBC are exclusively filled by mature sperm, an observation, which fully complements to the high reproductive capability seen in SBC. Empty lobules found in the testes of OBC male morphotypes, can very well explain the reproductive in capability show by this morphotypic stage.

The morphology and histology of the vas deferens of the three male morphotypes and their four transitional stages of *M.rosenbergii* have been studied. The vas deferens of each side is long tube arising from the testes from slightly behind its middle and opening as a genital pore at the coxopodite of the fifth pereiopod. From its structure the vas deferens can be divided into four portions ie., a short, thin proximal portion; a long convoluted portion; a straight distal portion and a thick, enlarged distal portion. The histological study revealed that the epithelium of vas deferens is glandular and consists mostly of simple columnar cells and partly of high columnar cells. The proximal portion contains loosely dispersed spermatozoans and a comparatively small amount of gelatinous substances which is slightly stained with hemotoxylin. In the convoluted portion a strongly eosinophilic matrix is observed along

the peripheral epithelium, in addition to spermatozoa and the basophilic matrix. As the basophilic matrix increases in quantity towards the convoluted portion, spermatozoa became aggregated and form a sperm mass buried in the matrix. The distal and enlarged distal portions are similar in fundamental structure to the convoluted portion, a well developed thick muscle layer composed of transverse and longitudinal muscles surrounds the enlarged distal portion. This muscle layer seems to be used to ejaculate the contents of the vas deferens at mating. Surprisingly the lumen of vas deferns of three male morphotypes and their four transitional stages contained sperms irrespective of differences shown by the morphotypes in their reproductive capability.

The location, morphology and histology of the androgenic gland of the three male morphotypes and their four transitional stages of *M. rosenbergii* have been studied. As in other malacostracans, the androgenic gland of male morphotypes of *M. rosenbergii* appeared as a very thin elongated plate like structure, whose proximal end is broad and firmly attached to the wall of the vas deferens. Structurally it is compact with several longitudinal strands ensheathed by a membrane, with non-uniform number, the number being maximum towards the distal end. Based on the cytological characters five types of glandular tissues were identified such as type I, II, III, IV and V. A well defined localisation of a particular type of cells to a specific region of gland could be observed among these morphotypes. The androgenic gland of male morphotypes and their transitional stages showed variations in their activity. The presence of active nuclei surrounded by rich homogeneous cytoplasm throughout the entire gland indicate high activity of androgenic gland in SM and SBC. In WOC, t-SOC and WBC, the intermediary stages and in OBC, the terminal stage, regions are present especially at the basal and terminal portions and

along the sides, showing signs of degeneration like high vacuolated and disintegrated cytoplasmic area and pycnotic nuclei. The presence of young tissues in the middle portion of the androgenic gland indicate a medium activity of gland in these morphotypes. On the contrary, in SOC, the high incidence of nuclear pycnosis and cytoplasmic vacuolisation of the androgenic gland along with the presence of areas showing late phases of degeneration suggest that the activity of androgenic gland is glaringly low in this morphotype.

The morphology and histology of hepatopancreas of the three male morphotypes and their four transitional stages *M. rosenbergii* have been studied. The hepatopancreas, the main digestive gland in male morphotypes of *M. rosenbergii*, a bilateral evagination of mesenteron, is a large compact organ which occupies the greater part of the cephalothoracic cavity, posterior to the cardiac portion of the stomach. They are composed of compact arrays of blind ending tubules. The tubules of the hepatopancreas of various male morphotypes of *M. rosenbergii* are lined with an epithelium in which four cells can be recognized such as embryonic (E) cells, fibrillar (F) cells, secretory (B) cells and absorptive (R) cells. Histological study of hepatopancreas revealed that the composition of these four types of cells showed variations in the hepatopancreas of these three morphotypes and their intermediary stages. Embryonic cells and fibrillar cells are dominated in SM and WOC morphotypes, which are characterised by retarded growth rate. The most conspicuous cells observed in SOC, t-SOC and WBC are the secretory cells and are characterised by high somatic growth. The hepatopancreas of SBC and OBC, the penultimate and terminal stages respectively are constituted mainly by the absorptive cells. These morphotypes are characterised by reduced growth rate.

Histology of the neurosecretory system viz., eye stalk, brain and thoracic ganglion of three male morphotypes and their four transitional stages have been studied. The eye stalk neurosecretory system consists of four regions, lamina ganglionaris, medulla externa, medulla interna and medulla terminalis. Scattered neursecretory cells are observed in the lamina ganglionaris while groups of neurosecretory cells are present in the medulla externa, medulla interna and medulla terminalis. Three types of neurosecretory cells designated as 'C', 'D' and 'E' are present in these groups of neurosecretory cells. The brain of various morphotypes of M. rosenbergii can be subdivided into three main regions ie., the protocerebrum, deutocerebrum and tritocerebrum, with a principal portion of the protocerebrum ie., the medulla terminalis having moved into the eye stalk. The secretory neurons in the brain can be divided into four separate groups viz. B1, B2, B3 and B4. These groups of neurosecretory cells are present on the median, posterior, anterior and also on the lateral sides of the brain. Four types of neurosecretory cells designated as 'B', 'C', 'D' and 'E' are present in these groups of secretory centers. In the thoracic ganglion, the neurosecretory cells are arranged in four groups. These are, a single each of anterior, posterior, a single median and three pairs of lateral groups. The lateral groups are designated as anterio lateral, midlateral and posterio lateral groups. Five types of neurosecretory cells designated as 'A', 'B', 'C', 'D' and 'E' are present in the thoracic ganglia. As a whole, five different types of neurosecretory cells have been identified in the neurosecretory system. It can be concluded that type 'C' and 'D' cells of the eye stalk, type 'B', 'C' and 'D' cells of the brain and type 'A', 'B' and 'C' cells of the thoracic ganglion showed variations in their activity among these morphotypes and their transitional stages.

The histochemical characterisation of the reproductive system including androgenic gland and hepatopancreas of three male morphotypes and their transitional stages have been studied. Localisation of various metabolites like protein, glycogen, lipid and RNA of the reproductive system showed positive reactions to these histochemical preparations. Similarly, hepatopancreas also showed positive reaction to the histochemical localisation of protein glycogen and lipid. No distinct and clear cut demarcation cloud not be arrived at with the hotochemical analysis of various male morphotypes and their transitional stages.

Biochemical characterisation of various male morphotypes and their transitional stages was also carried out to know the possible changes in the biochemical constituents such as protein, lipid, carbohydrates, moisture and ash content in the different organ tissues such as muscle and hepatopancreas; variations in the lipid fractions such as cholesterol, phospolipids and triglyceride content in the muscle tissue and hepatopancreas and amino acid profiling of muscle tissue commensurating with morphogenesis. Biochemical composition of the muscle tissue showed that the variations are not so pronounced except in the case of protein, carbohydrate and moisture. The lipid and ash content of the muscle tissue do not show any significant variation. The muscle tissues are rich in protein and poor in carbohydrate. The highest values of both protein and glycogen are observed in SOC morphotype where as it was lowest in SBC morphotype. Faster somatic growth in SOC can well be explained with the help of higher values noticed in the protein content of muscle tissue. The lipid content was found to be higher in hepatopancreas than in muscle tissue. Carbohydrate content of hepatopancreas of SOC showed significant variation from SBC which manifests the structural and functional variations of hepatopancreas of SOC and SBC of *Macrobrachium rosenbergii* commensurate with

the respective growth and reproductive stage represented by these morphotypes.

Studies on the lipid components viz., cholesterol, phospholipid and triglyceride in the muscle tissue and hepatopancreas have been carried out. Significant differences in the cholesterol, phospholipid and triglyceride content in the muscle tissue could be observed. Significant difference was noted in the hepatopancreas phospholipid content while the cholesterol and triglyceride do not show any significant variations. In the both the cases the highest values of these components was noted in SOC morphotypes.

Amino acid profiling of the muscle tissue of various male morphotypes revealed that the lowest value was observed in SBC while the highest value recorded in SOC. Among the amino acids glutamic acid registered the highest amount followed by Aspartic acid. A clear and specific variation in biochemical composition, lipid fractions and aminoacid profile could be established in different male morphotypes of *M. rosenbergii* in the present study.

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PUBLICATIONS

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Joseph, A., Kurup, B.M. 2000. Histological variations of testes of male morphotypes of *Macrobrachium rosenbergii* (de Man) Asian Fisheries Science (communicated).

Joseph, A., Kurup, B.M., 2001. Histological characterization of Male morphotypes of *Macrobrachium rosenbergii* (de Man). In: Reverine and Reservoir Fisheries of India. (Boopendrnath, M.R., Meenakumari, B., Joseph, J., Sankar, T.V., Praveen, P., and Edwin, L., Eds.) P. 245-255. Society of Fisheries Technologists (India), Cochin.

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Histological Characterization of Male Morphotypes of *Macrobrachium rosenbergii* (de Man)

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Histological characterization of three male morphotypes of *Macrobrachium rosenbergii*, *viz*., small male (SM), strong orange clawed male (SOC), strong blue clawed male (SBC) have been carried out to bring out the structural and functional differences in the organ tissues of these morphotypes. The organ tissues studied for morphological characteristics were the reproductive system including androgenic gland, hepatopancreas and the neurosecretory system, *viz*., eye stalk, brain and thoracic ganglion. The present study indicated clear structural and functional differences in the organ tissues of male morphotypes, lending support to the morphological variations among male morphotypes of *M. rosenbergii*.

Key words : Histological characterization, male morphotypes, *Macrobrachium rosenbergii*.

Among mature males of the freshwater prawn *Macrobrachium rosenbergii*, three distinct morphological types, *viz*., small males (SM), orange-clawed males (OC) and blue-clawed males (BC) are discernible. These morphotypes represent three developmental stages of male maturation process. SM occupies the initial stage of developmental pathway. They transform to strong orange clawed males (SOC) through an intermediary stage known as weak orange clawed morphotype (WOC). The OC in turn transforms to BC through two transitional stages, *viz*., pre-transforming strong orange clawed males (t-SOC) and weak blue clawed males (WBC) before culminating in strong blue clawed males (SBC). The terminal stage of this transformation pathway is old blue clawed morphotype (OBC) which is characterised by relatively large second cheliped. (Ra'anan, 1982; Ra'anan & Cohen, 1985; Kuris *et al*., 1987; Karplus *et al*., 1992; Harikrishnan & Kurup, 1997a).

Though a lot of work has been carried out to characterize these male morphotypes morphologically, allometrically and biochemically (Cohen *et al*., 1981); Harikrishnan & Kurup, 1997a; Sureshkumar & Kurup, 1998), no attempt has so far been made to bring out the

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structural and functional differences of different body organs causing morphotype transformation. The present paper aims at histological characterization of three behaviourally distinct male morphotypes of *M. rosenbergii*, *viz*., small males (SM), strong orange clawed males (SOC) and strong blue clawed males (SBC).

Materials and Methods

Live specimens of male morphotypes of *M. rosenbergii*, *viz*., SM, SOC and SBC, belonging to a single age group were collected from a growout adjacent to Vembanad Lake (Kerala), during 1998-1999. They were identified into three distinct morphotypes (Kuris *et al*., 1987; Sagi & Ra'anan, 1988) and brought to the laboratory in live condition. The organ tissues selected for the study consisted of the reproductive system including androgenic gland, hepatopancreas and the neurosecretory system, *viz*., eyestalk, brain and thoracic ganglion. Samples of these tissues, collected from five specimens, displaying unequivocal morphotypic characteristics were selected for histological study. For microscopical studies, paraffin sections of these organ tissues were taken at 7 μ, after fixation in Bouin's fluid, and then stained with Haemotoxylin-eosin, Heidenhain's Azan and Mallory's Triple stains for the reproductive system, hepatopancreas and neurosecretory systems, respectively (Humason, 1982). The slides were examined by light microscopy and photographed using a binocular microscope and Nippon camera combination at high power.

Results and Discussion

Testes

The testicular lobes are composed of long cylinders, compactly held together by connective tissue. Light microscopy revealed differences in the content of the cylinders among the different male morphotypes. The testes of small males contained cylinders, most of which were enveloped by a single layer of epithelium. Part of the epithelium is multilayered and included cells of variable size, forming a spermatogenic zone containing germinal cells and sustentacular cells. Mature spermatozoans were seen in the lumen of a few cylinders (Fig. 1).

The testicular cylinder of strong orange clawed male is filled with spermatocytes only, which appeared similar in size, shape and cytological features. It is characterized by a complete absence of spermatozoans. (Fig. 2). The testicular lobules of blue clawed males contained mature spermatozoans almost to the exclusion of other cell types. The spermatogenic zone was barely observable (Fig. 3).

Histological examination of the testes of these three morphotypes revealed that the testes show difference in structure and function. The available reports suggest that the male morphotypes of *M. rosenbergii* such as SM, SOC and SBC, show perceptible difference in reproductive activity and somatic growth among them (Sagi & Ra'anan, 1988; Sagi *et al*., 1988; Sureshkumar & Kurup, 1998; Joseph & Kurup, 2000). SM and SBC are sexually active participating in mating

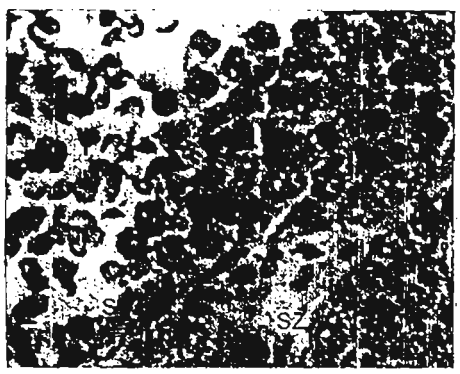

Fig 1 : CS of testes of SM (x100) SZ-Spermatogenic zone, S Sperm

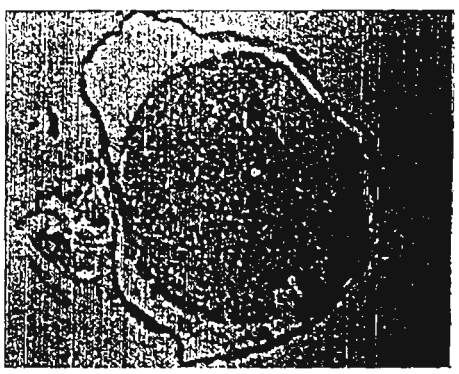

Fig 4 : CS of vas deferens of SM (x40) showing sperm

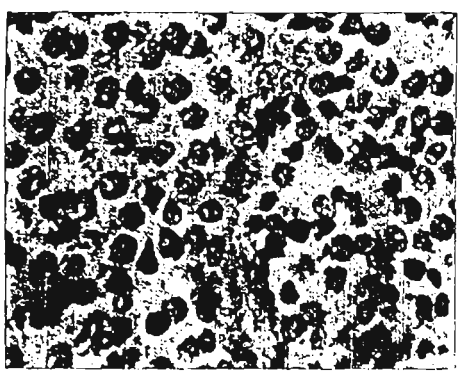

Fig 2 : CS of testes of SOC (x100) showing spermatocytes

Fig 5 : CS of vas deferens of SOC (x40) showing sperm

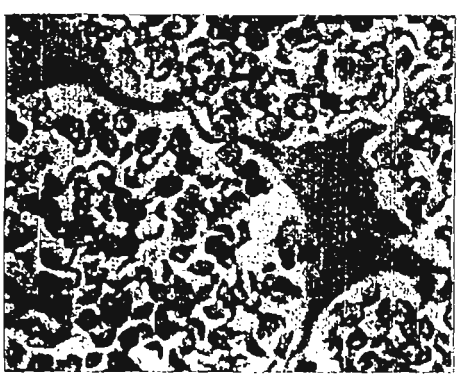

Fig 3 : CS of testes of SBC (x100) showing sperm

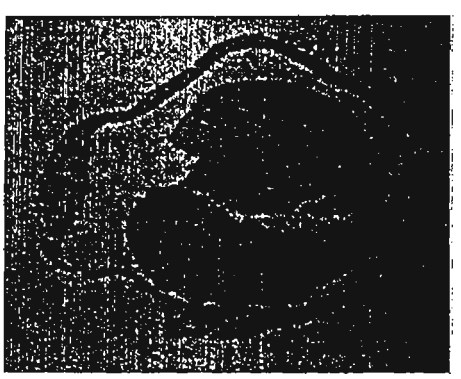

Fig 6 : CS of vas deferens of SBC (x40) showing sperm

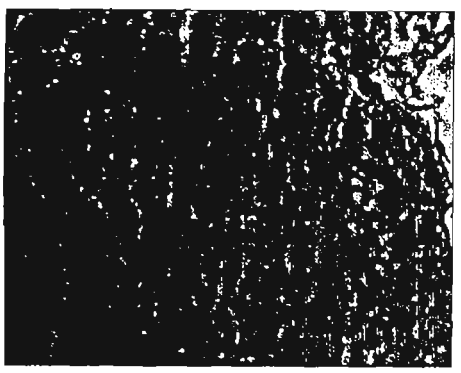

Fig 7 : CS of androgenic gland of SM (x100) showing type I androgenic gland tissue

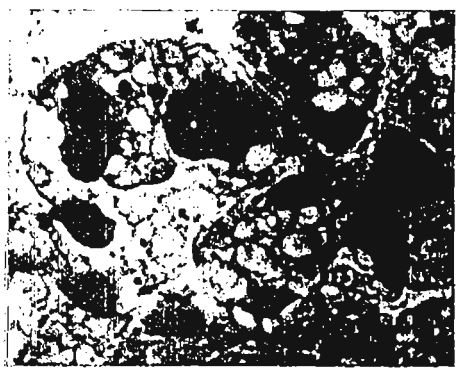

Fig 10 : CS of hepatopancreas of SM with fibrillar cells (x100)

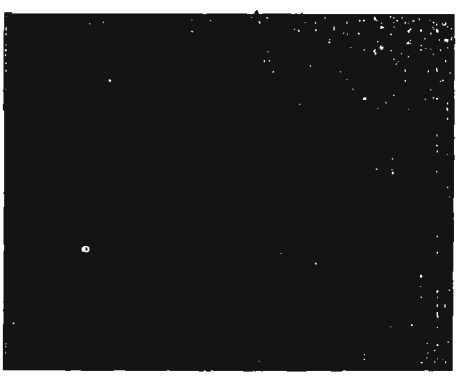

Fig 8 : CS of androgenic gland of SOC (x100) showing type II androgenic gland tissue

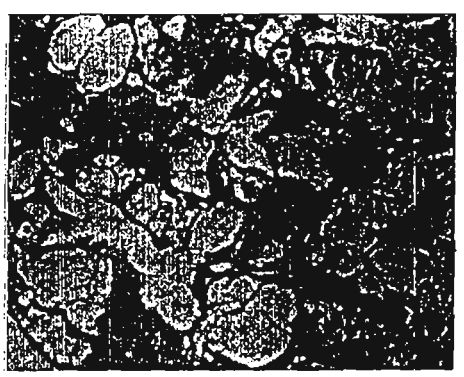

Fig 11 : CS of hepatopancreas of SOC with secretory cells (x100)

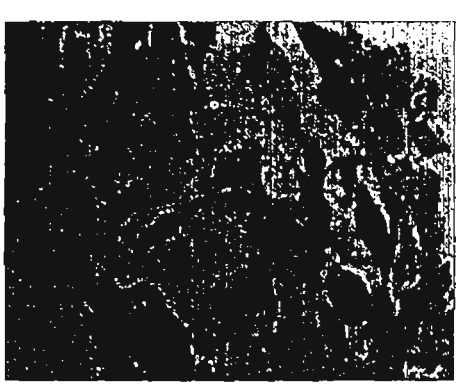

Fig 9 : CS of androgenic gland of SBC (x100) showing type III androgenic gland tissue

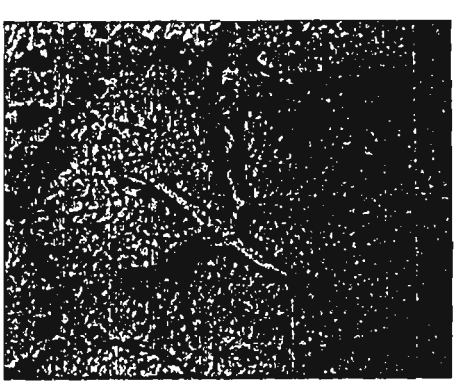

Fig 12 : CS of hepatopancreas of SBC with absorptive cells (x100)

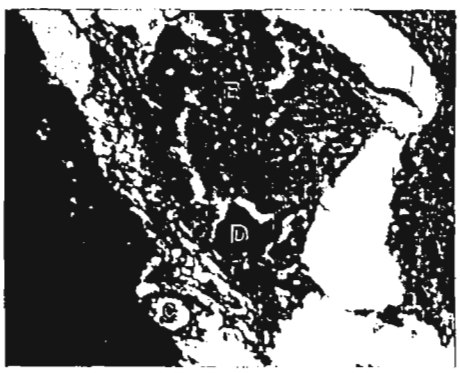

Fig 13 : LS of eyestalk of SM showing groups of neurosecretory cell types of C, D and E (x100)

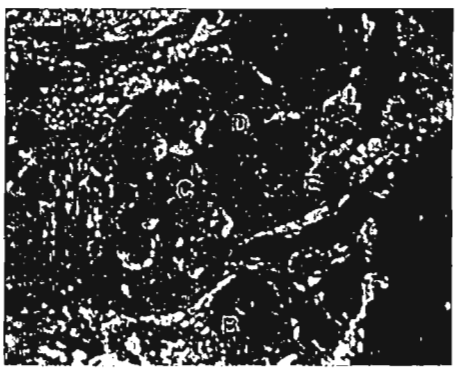

Fig 16 : TS of brain of SM showing the localisation of neurosecretory cell types of B, C, D and E (x100)

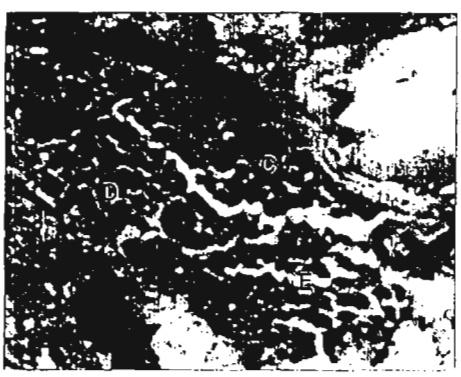

Fig 14 : LS of eyestalk of SOC showing groups of neurosecretory cell types of C, D and E (x100)

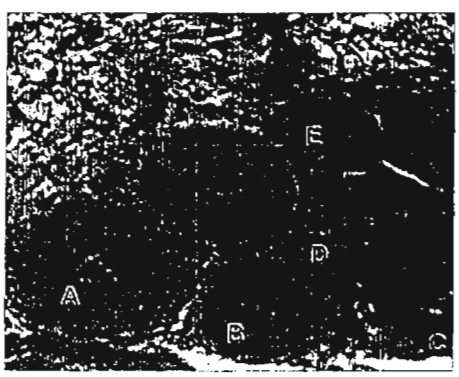

Fig 17 : TS of brain of SOC showing the localisation of neurosecretory cell types of B, C, D and E (x100)

Fig 15 : LS of eyestalk of SBC showing groups of neurosecretory cell types of C, D and E (x100)

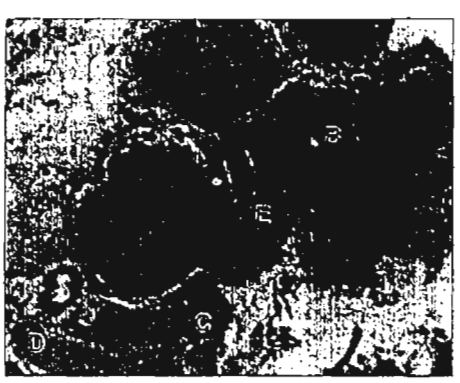

Fig 18 : TS of brain of SBC showing the localisation of neurosecretory cell types of B, C, D and E (x100)

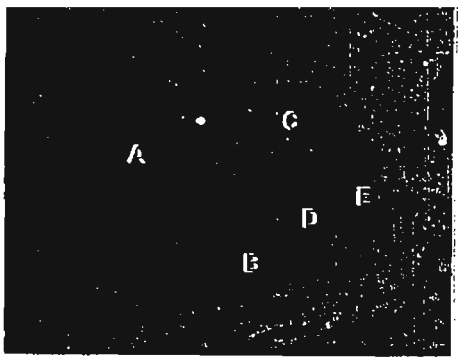

Fig 19 : TS of thoracic ganglion of SM showing neurosecretory cell types of A, B, C, D and E (x100)

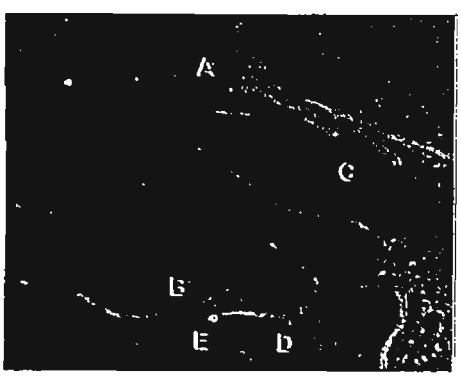

Fig 20 : TS of thoracic ganglion of SOC showing neurosecretory cell types of A, B, C, D and E (x100)

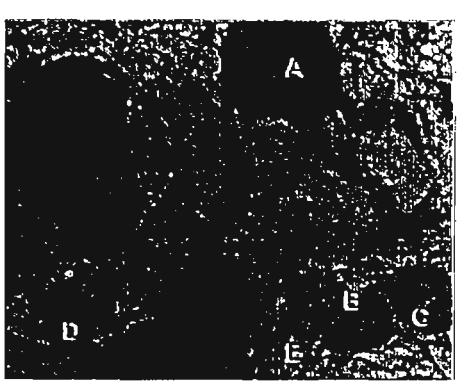

Fig 21 : TS of thoracic ganglion of SBC showing neurosecretory cell types of A, B, C, D and E (x100)

and fertilization and thereby utilizing little energy in somatic growth. On the contrary, orange-clawed males are characterized by a fast growth rate and reduced reproductive activity. (Sagi & Ra'anan, 1988; Sagi *et al.* 1988; Sureshkumar & Kurup, 1998).

The results of the present study revealed that there exists a very strong correlation between the structural properties of the testes with the reproductive activity of these morphotypes.

Vas deferens

The vas deferens of male morphotypes of *M. rosenbergii* contains four distinct regions, *viz.*, proximal vas deferens, medial vas deferens, distal vas deferens and seminal vesicles and this is comparable with that of *P. setiferus*, (King, 1948; Ro *et al.*, 1990). The present study revealed that mature spermatozoans are observed in the vas deferens of all these male morphotypes (Fig. 4, 5 & 6). However, histological study of the testes of SOC morphotype revealed that they are devoid of spermatozoans in their testicular lobules. It was also reported that SOC male morphotype has a low reproductive activity during breeding processes (Sagi *et al.*, 1988; Joseph & Kurup, 2000; Telecky, 1984; Sureshkumar & Kurup, 1998). Therefore, it can be inferred that SOC male morphotypes which are characterized by the total absence of mature spermatozoans in their testes are capable of performing low rate of reproductive activity during breeding processes, like other sexually active male morphotypes and this may be due to the

presence of spermatozoans retained in the seminiferous tubules (i.e., vas deferens and seminal vesicle) by the SM morphotypes.

Androgenic gland

The androgenic gland of male morphotypes of *M. rosenbergii* consists of strands of cells forming a pyramidal cluster, loosely associated with the posterior portion of the ejaculatory duct. The strands consist of three principal cell types. Cell of type-I are small with dense cytoplasm, often containing two nuclei. Cells of type-II are slightly larger cells and vacuolated. Cells of type- III are large cells in which most of the inter cellular space consists, of vacuoles. The androgenic gland of these male moprhotypes showed variations in their activity (Thampy & John, 1972; 1973; Sagi, 1988; Sreekumar *et al.*, 1982). In small males the androgenic gland cells are constituted mainly by type-I cells and in SBC, type-II cells constitute the gland cells (Fig. 7&8). On the contrary type-III cells forms the major component of SOC androgenic gland (Fig. 9). The presence of active nuclei surrounded by rich homogenous cytoplasm through out the gland, indicate high activity of the gland in SM and SBC. In SOC, the high incidence of nuclear pycnosis and cytoplasmic vacuolization of the gland cells along with the presence of areas showing late phases of degeneration suggest that the activity of the gland is low in this morphotype. Therefore, there is a positive correlation between the activity of the androgenic gland and the reproductive activity of these morphotypes, as the SM and SBC morphotypes exhibits high reproductive activity while the SOC morphotypes show low reproductive activity, in the presence of BC morphotypes.

Eyestalk neuropeptides such as GSH and GIH apparently act directly on the female ovaries (Cotton & Payen, 1988; Quackenbush, 1991; Fingerman, 1995) whereas, in males, their action on testes appears to be indirect, via a direct action on the androgenic gland as suggested by Adiyodi (1984) and Gupta *et al.* (1989) and Hasagawa *et al.* (1993). According to Thampy & John (1973) and Sreekumar *et al.* (1982), the androgenic gland shows signs of increased secretory activity as evidenced by increase in the size of the gland as well as the type of cells. The changes in the primary and secondary sexual characteristics of the male morphotypes of *M. rosenbergii* along with the changes in the activity of androgenic gland, inideates that there is a hormone produced in the eyestalk, which has got an inhibitory effect on the androgenic gland, in male primary and secondary sex characters as well as on the growth of these morphotypes.

It may be possible that androgenic gland which controls the primary and secondary sex characters of male crustaceans has a role in the development of male morphotypes and growth variation among them, as growth rate of *M. rosenbergii* is closely associated with morpohtypic status (Ra'anan, 1982; Ra'anan & Cohen, 1985).

Hepatopancreas

Hepatopancreas of *M. rosenbergii* is a large compact organ which occupies the greater part of the cephalothoraic cavity, posterior to the cardiac foregut. They are composed of compact arrays of blind ending tubules and are held ventrally with the gut at the junction of the pyloric foregut and anterior end of the midgut. The bulk of the tissue comprises of simple tubules, whose wall consists of a single layer of secretory epithelium. In between the individual tubules are found interstitial cells and small blood spaces. The lobules of the hepatopancreas are lined with an epithelium in which four cells can be recognized. They are the Embryonic (E-) cells, Absorptive (R-) cells, Secretory (B-) cells, and the Fibrillar (F-) cells.

i. Embryonic (E-) cells: These are small undifferentiated columnar cells seen at the distal end of the tubules, the only part where the epithelium is more than one cell thick. These cells are continuous with a short region where they undergo differentiation to become other cells of the tubules. All cells in this region appear to be morphologically similar.

ii. Absorptive (R-) cells: These cells are found lining the lumen of the hepatopancreatic tubules. These cells have a dense granular cytoplasm with a large round nucleus. Their nuclei lie medioproximally within the cells, and have a prominent nucleolus.

iii. Secretory (B-) cells: The secretory cells are mainly limited to the proximal region of the tubules. These cells have a large vacuole occupying 80-90% of the total cell volume. The nucleus of the B- cell lie proximal to the large vacuole and appears to be compressed as the latter enlarges.

iv. Fibrillar (F-) cells: These cells are in frequently seen among the R- cells and B- cells. Their nuclei are located at the basal region of the cell (Travis, 1955; Weel, 1955; Davis & Burnett, 1964; Al-Mohanna *et al.*, 1985a).

The absorptive (R-) cells functioning as a storage site for lipid and glycogen was reported by Travis (1955) and Davis & Burnett (1964). The secretory (B-) cells are suggested to function in the synthesis and release of digestive enzymes. The Fibrillar (F-) cells can be considered to be immature (B-) cells. The hepatopancreas play an important role in food assimilation, storage and secretion of digestive enzymes. The present study revealed that there exists a distinct difference in the cell types of hepatopancreas of SM, SOC and SBC. In SM, the hepatopancreas mainly constituted by embryonic cells and fibrillar cells, indicated low growth rate (Fig. 10). In SOC stage during which active feeding takes place, secretory cells are dominated in the hepatopancreas, as evidenced by the high somatic growth (Fig. 11). While in SBC, characterized by reduced growth rate, majority of cells are constituted by the absorptive cell type (Fig. 12). From the

high content of lipid droplets and glycogen present in these cells. It may be concluded that they function also as a storage site. In the inter moult storage, the main food reserve is fat and glycogen, which form the energy store in prawns.

Neurosecretory system

The neurosecretory system located in the eyestalk, brain and the thoracic ganglion produce neurohormones apparently responsible for growth, moulting, metabolic rate, water balance, dispersion of retinal pigments and sexual activity (Lockwood, 1968). Neurosecretory cells can be defined as neurons with axonal terminals that show specialization and localization for release of substances to the haemolymph (Cook & Sullivan, 1982). With the view to investigate the structure of neurosecretory cells of these systems and the role of the neurohormones produced by them, the same have been studied in SM, SOC and SBC male morphotypes of *M. rosenbergii*.

Detailed examination of the serial sections of the eyestalk, brain and the thoracic ganglion revealed that they contain certain specialized cells. These cells differ from the ordinary nerve cells in having neurosecretory material in them and hence are taken to be the neurosecretory cells. Depending on the cytological characteristics, such as the shape, size, with or without axons, condition of cytoplasm, the shape and size of their nuclei and the staining properties, the neurosecretory cells of the eyestalk, brain and thoracic ganglion of these morphotypes can be classified and designated as type A, B, C, D & E. The eyestalk neurosecretory system possesses only three types (C, D and E) of the neurosecretory cells, the brain four types (B, C, D & E) and the thoracic ganglion possesses all the five types (A, B, C, D & E) of neurosecretory cells. The neurosecretory cells are found either in scattered condition or in the form of specific groups. It has been observed that, the type C cells in the eyestalk exhibit decrease in the nuclear activity in SM and SOC than SBC, while the type D and E cells do not show any variation in these morphotypes (Fig. 13, 14, 15).

The neurosecretory cells of the brain also exhibit variations in their cytological characteristics in these morphotypes. Type B, C, and D cells become more active in SBC when compared to SM and SOC. The E cells do not show any response in these morphotypes (Fig. 16,17 &18). Type A, B and C cells of thoracic ganglion exhibits marked changes in its cytological characteristics, in these morphotypes. It shows an increase in activity in SBC than SM and SOC. Type D and E cells do not appear to play any apparent role in these morphotypes (Fig. 19, 20 & 21).

In short, it can be concluded that the type C cells of the eyestalk, type B, C and D cells of the brain and type A, B, C and D cells of the thoracic ganglion play active roles in these morphotypes. The present work establishes that type A, B and C cells of the thoracic ganglion and the B, C and D cells of the brain releases the gonad stimulating hormones in SM and SBC, accelerating the reproductive activity. The C cells of the eyestalk

produce the neurohormones, which inhibit the testicular development and sexual inactiveness in SOC morphotypes.

Gomez (1965), Estman-Reks & Fingerman (1984) and Yano (1993) showed that the brain and thoracic ganglion are the source of a gonad-stimulating hormone. The two antagonistic factors control gonadal maturation is evident from knowledge of crustacean reproductive endocrinology. One factor (inhibitory) is produced by the X-organ neurosecretory cells, the other factor(s) (stimulatory) is produced by the thoracic ganglia and brain. However, the interaction mechanism of those two antagonistic factors is not completely understood.

Hence, it can be postulated that small male possesses relatively slow growth rate, having the potential to develop, when environmental and social situations permit, through the intermediate phase of the orange claw morphotype into a dominant blue claw male morphotype.

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