STUDIES ON THE REPRODUCTIVE PHYSIOLOGY OF THE GREEN TIGER PRAWN PENAEUS *(PENAEUS)* SEMISULCATUS DE HAAN

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fulfilment of the requirements for
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by

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COCHIN - 682 014

OCTOBER 1995

DECLARATION

I hereby declare that this thesis entitled **"Studies on the reproductive physiology of the green tiger prawn *Penaeus (penaeus) semisulcatus* de Haan"** has not previously formed the basis for the award of any degree, diploma, associateship or other similar titles or recognition.

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K.SIVACHANDRABOSE

CERTIFICATE

This is to certify that the thesis entitled **"Studies on the reproductive physiology of the green tiger prawn *Penaeus (penaeus) semisulcatus* de Haan"** is the bonafide record of the research work carried out by **Shri K.Sivachandrabose,** under my guidance and supervision in Post-graduate programme in Mariculture, CMFRI, and that no part thereof has been presented for the award of any other degree.

Cochin - 682 014
10th Oct., 1995

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Senior Scientist
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PREFACE

The present global fish production through aquaculture is estimated at about 12.7 million tonnes which forms 12.5% of the total fish production of the world (Anon., 1995). A recent study of the trends in global aquaculture production (Csavas, 1995) indicates that the total fish production through farming in the world would be to the tune of 21.1 million tonnes by 2000 AD and 42.0 million tonnes by 2025 AD. Bulk of this anticipated production is expected to come from the inland waters. The share from sea farming is relatively of a low order. Though this state of affair is precisely applicable to the Indian situation, development of sea farming has been given top priority in recent years in the national developmental programmes in the country. It is noteworthy that India has made considerable advancement during the past two decades in developing technologies for commercial farming of a number of marine organisms including shell fishes, fin fishes, sea cucumbers, seaweeds etc. (Devaraj, 1995). As regards shrimp culture technology, the efforts are mainly restricted to a few species of the Genus *Penaeus* which are both marine and estuarine in occurrence. However, experimental studies so far conducted in the country on strictly marine species have shown great potential for developing aquaculture technology for a number of them which at present remain neglected for this purpose. The green tiger prawn *P. (P). semisulcatus*, which supports lucrative capture fisheries in some parts of India, is a typical example for such species showing promises for sea farming. Keeping this in view, a study of the reproductive physiology of *P. (P). semisulcatus* was undertaken as this information is an essential prerequisite for broodstock development for hatchery operations, and the results are embodied in this thesis.

The thesis is presented in seven chapters. Chapter I comprises an introduction highlighting the importance of the various aspects of study with a comprehensive review of literature on the subject. Chapter II describes the

material and methods used for the study. The results of the study on various aspects were described and discussed in detail with suitable illustrations and photographs in Chapters III to VII.

Chapter III deals with the reproductive biology of *P. (P). semisulcatus* encompassing descriptions of the external genitalia, morphology of male and female internal reproductive organs, maturation process, size frequency of maturing ova, size at first maturity, gonado- and hepatosomatic indices, fecundity, sex ratio, spawning and influence of physico-chemical parameters on spawning.

Chapter IV deals with the process and events leading to the maturation of ovary and testis. The process of oogenesis and spermatogenesis were studied by using histological and electron microscopic methods. Based on the changes evident in the cytoplasm and nucleus of the oocyte and yolk accumulation in a graded manner, the complete development process of oocyte was classified into five vitellogenic phases. Spermatogenesis was found to involve progressive reduction in cytoplasm volume and condensation of chromatin matter leading to the formation of mature sperms.

Chapter V describes the biochemical and mineral changes during ovarian maturation. The metabolic components like protein, free amino acid, lipid, carbohydrate, carotenoids and moisture in the hepatopancreas, ovary and muscle were estimated for different maturity stages. The mineral contents such as macro minerals (Na, K, Ca and Mg) and micro minerals (Cu, Mn, Fe, Zn, Ni, Pb and Cd) in hepatopancreas, ovary and muscle were estimated for different maturity stages.

Chapter VI deals with the histochemical studies in relation to ovarian maturation, testes and spermatophore. The protein, lipid and carbohydrate contents of ovary were estimated for the different vitellogenic phases. The protein, lipid, carbohydrate and cholesterol contents were estimated in testes and spermatophore.

Chapter VII presents the results of induced maturation experiments by eyestalk ablation, CNS extract injection and UV rays application on immature female prawns.

The thesis concludes with a 'Summary' highlighting the significant findings, followed by a list of literature consulted under 'References'.

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CHAPTER I

INTRODUCTION

INTRODUCTION

India is one of the leading nations in the production of marine shrimp contributing to about 5.1% of the world shrimp production which is estimated to be to the tune of 2.65 million tonnes in 1991 (Anon., 1993). With the development of an organised export industry for seafoods in the country, which has earned to the national exchequer over 32727 million rupees as foreign exchange during 1994-95 (Anon., 1995), commercial exploitation of shrimp in the inshore fishing grounds has increased considerably during the past three decades. Though this has resulted in the augmentation of shrimp production to a considerable extent (2.4 lakh tonnes in 1993) and increase of our export earnings from shrimp products which form the mainstay of the seafood export industry of the country, apprehensions are expressed as to the sustainability of such increasing trend. It is generally believed that further increase of catch from the conventional shrimp grounds is not possible to any appreciable extent as the exploitation level has reached the optimum. Realising this situation in the production of marine shrimp, top priority has been assigned at regional as well as national levels for finding out additional means of augmenting shrimp production. The two important and possible ways of achieving this objective are extending the fishing operation to areas beyond the conventional fishing grounds ie., by deep-sea fishing, and through aquaculture.

At present, over fifty countries carryout shrimp aquaculture on commercial scale and produce an average of about seven lakh tonnes per year (Anon., 1993). Considerable progress has been achieved in shrimp farming in many of the southeast Asian countries in recent years. In India, realising the imperative need for popularising shrimp farming practices to increase the production for the growing export industry, various organisations in the country have initiated efforts to develop suitable technologies for commercial shrimp farming. As a result of this, scientific shrimp farming has taken root

in the country now and it is coming up as an organised industry throughout the Indian coast (Sakthivel, 1993).

According to a recent survey, prawn farming is practised in about 80,000 ha of brackishwater areas in the country out of the total cultivable areas of 1.4 million ha (Anon., 1993). Aggressive shrimp farming programmes are underway to bring the rest of the areas also under commercial shrimp farming (Sakthivel, 1993). During the year 1993, the country has reached the third position amongst the shrimp farming nations of the World by producing about 60,000 metric tonnes of shrimp through farming as against its fourth position in the previous years.

A perusal of publications on shrimp farming would indicate that most of the culture technologies developed and practiced in India are restricted to a few species of the Genus *Penaeus* which are marine and estuarine in occurrence (Suseelan, 1983). While much efforts have gone into developing viable technologies for culture of these estuary-dependent species, quite a number of other penaeid prawn species, which are purely marine, remain neglected for any aquaculture purpose although most of them have the potentiality to form candidates for aquaculture. The green tiger prawn *Penaeus (Penaeus) semisulcatus* is a typical example of such species which has not received serious attention for the development of culture technology in India. As other members of Genus *Penaeus*, this is also a large growing species attaining maximum size of about 250 mm in total length. It is distributed throughout the Indo-west pacific region and in the Mediterranean sea, supporting major fisheries in the Gulf of Aden, Persian Gulf and in Pakistan and Indian coast (Holthuis, 1980).

In India, the species *P. semisulcatus* accounts about 2.9% of the total prawn landings, contributing an average of about 5,500 tonnes annually (Anon., 1979, 1980, 1982). Though it is caught almost throughout the Indian coast, the bulk of the fishery is from the Southeast coast region particularly

the Palk Bay and Gulf of Mannar waters (Thomas, 1974). According to Rao *et al.* (1993), this species forms about 14.8% of the penaeid landings of the east coast with an average annual production of 4,387 tonnes. Due to the increasing demand for this species for processing and export, there has been considerable fishing pressure on the stock of this species by shrimp trawlers in the Palk Bay and Gulf of Mannar during the past two decades. According to recent studies (Sampson Manickam *et al.*, 1989; Rao *et al.*, 1993), the catch of this species from this part of the country is in a state of decline. This situation warrants proper measures of conservation and management. Sea ranching using hatchery produced seed is considered as an imperative step for repopulating the species in the Palk Bay and Gulf of Mannar waters (Rao *et al.*, 1991; Neelakanda Pillai *et al.*, 1991). Experiments on breeding and seed production under controlled conditions have yielded encouraging results and the possibility of establishing a viable technology for large-scale hatchery production of seed of the species is being developed by the Central Marine Fisheries Research Institute, at Mandapam Camp (Maheswarudu *et al.*, 1990). Farming trials using juvenile *P. semisulcatus* in ponds at Mandapam have also yielded encouraging results (Maheswarudu *et al.*, 1995), thereby indicating scope for culturing this species on commercial scale.

In the hatchery production of seeds, an important problem encountered is the shortage of breeders. Often the availability of the spawners in nature is inadequate and unpredictable. The only alternative to overcome this problem is to establish broodstock in the hatchery itself through induced gonadal maturation in captivity. Broodstock management therefore becomes an integral part of shrimp culture. For evolving an efficient broodstock technology, a thorough understanding of the reproductive physiology of the species is an essential prerequisite. Since, no serious attempts have been so far made on this aspect in *Penaeus semisulcatus* (Plate 1), the present topic was taken up and the results obtained are described in the thesis.

Reproductive biology

An understanding of the reproductive biology of any species selected for aquaculture is an essential prerequisite for its successful farming. In India, scientific investigations on the biology of prawns dates back to the middle of seventeenth century. Since then, a wealth of information has been generated on the various aspects of the biology of commercially important species of prawns available along the Indian coast and the result of these studies have been consolidated and documented in a series of species synopsis published in the Proceedings of the World Scientific Conference on 'The biology and culture of shrimps and prawns' held at Mexico in 1967 (Mistakidis, Ed., 1970). Later, Silas *et al.* (1984) reviewed the biological characteristics of Indian penaeids indicating the importance of the same in the scientific management of the shrimp fishery. Many subsequent workers have also added to our knowledge on the biology of commercially important prawns, as could be seen from the works of Sukumaran (1983, 1993), Lalithadevi (1987, 1988), Muthu *et al.* (1987), Silas *et al.* (1987), Rajyalakshmi *et al.* (1988) and Rao (1989).

Various aspects of reproductive biology of penaeid prawns have been dealt with in a number of publications that came out from different parts of the world as part of the biological studies. Detailed works on reproductive biology of penaeid prawns have been documented by many workers like Hudinaga (1942), King (1948), Lindler and Anderson (1956), Cummings (1961), Hudinaga and Miyamura (1962), Tuma (1967), Brown and Patlan (1974), Wickins and Beard (1974), Badawi (1975), Penn (1975, 1980), Perez-Farfante (1975), Tirmizi and Javed (1976), Motoh (1978), O'connor (1979), Motoh and Buri (1980), Primavera (1980), Kennedy and Barber (1981), Tseng and Cheng (1981), Crocos (1987a,b), Crocos and Kerr (1983), Potter *et al.* (1986, 1989, 1991). Villegas *et al.* (1986). Hong Young Yan (1987). Courtney and Dredge (1988), Tan-Farmin and Pudadera (1989), Castille and Lawrence (1991), Yukihiro (1991), Chu *et al.* (1993), mostly from the Atlantic and Pacific waters. In India, the reproductive biology has been studied in varying details by many

workers like Rao (1967) in *P. monodon*, Menon (1957), Subrahmaniyam (1963), Rao (1968) and George and Rao (1968) in *P. indicus*, Thomas (1974, 1975) in *P. semisulcatus*, Menon (1953, 1957), Shaikhmahmud and Tembe (1958, 1961), George and Rao (1968) and Rao (1968) in *P. stylifera*, Menon (1952, 1957), George and Rao (1968), Rao (1968) and Thomas *et al.* (1974b) in *M. dobsoni*, George (1959), George and George (1964), George and Rao (1968), Nalini (1975, 1976), Rao (1989) in *M. monoceros*, Menon (1957), Rao (1968), George and Rao (1968) and Thomas *et al.* (1974a) in *M affinis*, Nagabhushanam and Kulkarni (1983) in *P. hardwickii*. These workers have furnished considerable information on the structure and development of secondary sexual characters, structure of male and female reproductive systems, maturation process, fecundity, spawning season, spawning grounds and other aspects of reproductive cycle of a number of penaeid species supporting the commercial fishery.

With the development of commercial fishery for *P. semisulcatus* from the beginning of seventies in the Arabian Gulf waters, Indian coasts, and the Australian coasts, efforts to understand the biology of the species have been initiated by various workers. Considerable work in this line has been done during the past two decades in the Gulf countries like Kuwait and Bahrain. In Kuwait waters, various biological aspects such as maturation and spawning, spawning seasons, migratory pattern between inshore and offshore grounds associated with reproduction through tagging experiments and population characteristics of exploited stocks such as size distribution, age and growth and spawning stocks of species have been undertaken as could be seen from the works of Enomoto (1971), Al-Attar and Ikenoue (1974), Badawi (1975), Farmer and Al-Attar (1981), Jones and Van Zalinga (1981) and Mohamed *et al.* (1981). Abdulqader and Naylor (1995) have delineated the bionomics and ecology of the species from Bahrain waters, while Mohamed (1978) and Browdy (1989) threw light on the reproductive aspects from the same region. Price (1976) and Coles *et al.* (1987) correlated the abundance of the species during the juvenile phase of life with seagrass beds dominated by *Halodule uninervis*. Badawi

(1975) estimated the spawning frequency of the species in the Arabian Gulf based on spawner distribution in the commercial catches. In the Israelian waters, Browdy (1989) studied the reproductive biology of this species. The observations of Liao and Huang (1972), Samocha and Lewinsohn (1977), Samocha (1980) and Tseng and Cheng (1981) in the same waters showed that *P. semisulcatus* is highly tolerant to fluctuations in environmental conditions and hence a promising species for aquaculture purpose. Much work has been carried out on the biology of the species from the Australian waters in the recent past. This species is found to contribute to substantial part of the commercial catches along with other species in the Gulf of Carpentaria (Grey *et al.*, 1983; Kirkwood and Somers, 1984; Coles and Lee Long, 1985; Staples *et al.*, 1985; Poiner *et al.*, 1987, Somers, 1987 and Somers *et al.*, 1991). Somers and Kirkwood (1984) studied the movements of the species based on tagging experiments, Crocos (1987) the reproductive dynamics, Loneragan *et al.* (1994) the juvenile ecology with reference to seagrass habitats. Various studies on the population of exploited stocks have also been undertaken by a number of workers like Kirkwood and Somers (1984), Somers *et al.* (1987) and Loneragan *et al.* (1994) who worked out the size distribution, sex ratio, asymptotic lengths and spawner abundance in the fishery from different depths covered by the commercial vessels in the Western Gulf of Carpentaria. The benthic phase of the life cycle and reproductive activity of the species *P. semisulcatus* have been studied by Tom *et al.* (1984) and Shlagman *et al.* (1986) in the Southeastern Coast of Mediterranean.

In India, when compared with other commercially important penaeid prawns, the work on *P. semisulcatus* is of limited nature. The available information from the West coast of India is restricted to only a few observations made by Rao and Kathirvel (1971) on the distribution and abundance of the species in relation to environmental parameters in Cochin backwaters and by Suseelan and Kathirvel (1983) on the occurrence of juveniles in abundance in the Ashtamudi backwaters in Kerala. These authors have shown that *P. semisulcatus* can tolerate low salinity conditions in the

juvenile stages to some extent. On the east coast of India, Thomas (1974, 1975, 1977) carried out, for the first time, a detailed study of the biology of the species encompassing reproduction, fecundity, sex ratio, age and growth, length weight relationship, relative condition factor and food and feeding habits from the Palk Bay and Gulf of Mannar in the Southeast coast. Subsequenlty, a few ecological observations relating to the juvenile stages from the nursery areas have been reported by Manissery (1983, 1986) and Sampson Manickam *et al.* (1989) from the Southeast coast. Recently, Neelakanda Pillai *et al.* (1991) reported the growth and migratory pattern of the species in the Palk Bay through tagging experiments.

Attainments of maturation of penaeid prawns in culture ponds has also been reported by a few workers during pond culture experiments in other parts of the world. Johnson and Fielding (1956) and Jhingran (1974) could propagate the white prawn *P. aztecus* in captivity using prawns raised in coastal ponds. Jhingran (1974) observed *M. brevicornis* maturing in experimental brackishwater ponds of Japara, Indonesia, Rodriguez (1981) made observations on growth and sexual maturation of *P. kerathurus* in salt ponds. Using *P. indicus* raised in brackishwater ponds, Primavera *et al.* (1982) compared the maturation, spawning, fecundity and hatching rates of ablated and unablated females. Yano (1984) observed maturation of Kuruma prawn *P. japonicus* in earthen culture ponds.

Rematuration and spawning experiments have been conducted in recent years from different parts of the world, with varying degrees of success, in many penaeid prawn species such as *P. monodon*, *P. stylirostris* and *P. vannamei* (Aquacop, 1979; Emmerson, 1980; Beard and Wickens, 1980), *P. japonicus* (Lumare, 1981; Yano, 1984), *P. semisulcatus* (Browdy and Somocha, 1985a,b) and *P. canaliculatus* (Choy, 1987). Influence of various environmental parameters such as pH, temperature, salinity, photoperiod etc. maturation and spawning of penaeid prawns have been discussed by many workers like

Wickins (1976a), Muthu *et al.* (1984), Crocos and Kerr (1986) and Primavera (1984) to mention a few.

The natural breeding of *M. dobsoni* in brackish water systems during high salinity have been reported (Menon, 1952; George, 1974; Rao and Kathirvel, 1973; Silas *et al.*, 1982). Muthu and Sampson Manickam (1973) collected matured males and females of *M. burkenroadi* from Chilka lake and presumed the possibility of breeding of the species in the lake. Krishnamurthy and Ganapati (1985) encountered *P. indicus* specimens with ovary in early maturing stage in Cochin backwaters. Kathirvel and Selvaraj (1989) observed maturing females of Kuruma prawn, *P. japonicus* in earthen ponds having brackishwater conditions at Muttukkadu near Madras. Lazarus and Nandakumaran (1985) studied the growth and survival of tiger prawn *P. monodon* in sandy beach ponds at Calicut. Lazarus and Nandakumaran (1986) conducted the experiments on the culture of *P. indicus* in polythene film lined ponds at Calicut. Muthu *et al.* (1987) studied the growth of the Indian white prawn *P. indicus* in relation to stocking density.

Johnson (1989) provided an annotated bibliography of the works so far done in India encompassing all available information on the reproductive biology of marine prawn.

Gametogenesis

The reproductive physiology of crustaceans has received considerable attention all over the world in recent years on account of its importance in broodstock management associated with aquaculture operations. In penaeid prawns, detailed studies on the structure of male and female reproductive systems have been carried out in a number of species like *P. japonicus* (Hudinaga, 1942), *P. setiferus* (King, 1948), *P. stylifera* (Shaikhmahmud and Tembe, 1958 and Rao, 1969), *P. duorarum* (Cummings, 1961), *P. indicus* (Subrahmanyam, 1965 and Mohamed, 1989), *P. merguiensis* (Tuma, 1967) and

P. monodon (Motoh, 1978 and Motoh and Buri, 1980). The general pattern of gametogenesis in decapod crustacean has been studied through histological examination by few workers like Ryan (1967), Chandran (1968) and Pillai and Nair (1971).

Among penaeid prawns, detailed works on oogenesis has been dealt histologically by many investigators like Hudinaga (1942) in *P. japonicus*, King (1948) in *P. setiferus*, Cummings (1961) in *P. duorarum*, Shaikmahamud (1961) in *P. stylifera*, Subrahmanyam (1965) in *P. indicus*, Pillai and Nair (1971) in *M. affinis*, Kennedy *et al.* (1977) in *Sicyonia brevirostris*, Motoh (1978) in *P. monodon*, Joshi *et al.* (1982) in *P. stylifera*, Anderson *et al.* (1984) in *S. ingentis*, Tan-Fermin *et al.* 1985 and Tan-Fermin and Pudadera (1985, 1989) in *P. monodon*, Yano (1985) in *M. ensis*, Ramos and Torras (1986) in *P. notialis*, Yano (1988) in *P. japonicus*, Browdy (1989) in *P. semisulcatus*, Mohamed (1989) in *P. indicus*, Tsumura and Nakagawa (1989) in *P. paucidens*, Quinitio *et al.* (1989) in *Pandalus kessleri*, Vasudevappa (1992) in *M. dobsoni* and Quinitio and Millamena (1992) in *P. indicus*. The reproductive organs and process of gametogenesis in *P. stylifera* from Bombay waters have been described briefly by Shaikhmahmud and Tembe (1958) and in detail by Joshi *et al.* (1982). Few electron microscopic study on oogenesis has been reported in penaeid prawns by Duronslet *et al.* (1975) in *P. aztecus* and *P. setiferus*, Mohamed (1989) in *P. indicus* and Chow *et al.* (1993) in *P. setiferus*. Comprehensive accounts on the oogenesis have been given by Raven (1961), Norrevang (1968), Adiyodi and Subramoniam (1983) and Papathanassiou and King (1984).

The origin of the yolk in crustaceans has been investigated using biochemical and electron microscopic methods by Beams and Kessel (1963), Hinsch and Cone (1969), Lui and O'connor (1977), Varadarajan and Subramoniam (1982) and charniaux-cotton (1985). The chemical nature of the crustacean yolk has been studied by using cytochemical methods in *Artemia salina* (Fautrez-Firlefyn, 1957), *Palaemon adspersus* (Bonina, 1974), *Balanus*

amphitrite (Fyhn and Costlow, 1977) and *Clibanarius clibanarius* (Varadarajan and Subramoniam, 1980) and *Orchestia gamarella* (Zerbib, 1980).

Recently, Quackenbush (1991) reviewed in detail about the regulation of vitellogenesis in Penaeid shrimp and pointed out the focused studies made in recent years on the basic mechanism of egg yolk protein production during shrimp maturation. Establishing the presence of two basic stages for the egg maturation process, namely, primary and secondary vitellogenesis, these authors have outlined the details of endogenous or exogenous yolk protein synthesis with particular reference to *Sicyonia ingentis*. Considerable amount of *in vitro* and *in vivo* work have been reported on different species of penaeid prawns such as *P.semisulcatus* (Fainzilber *et al.*, 1989a, Browdy *et al.*, 1990; Rosenthal and Diamant, 1990; Khayat *et al.*, 1992; Shafir *et al.*, 1992; Tom *et al.*, 1987a,b, 1992; Shenker *et al.*, 1993; Khayat *et al.*, 1994) and *P. vannamei* (Quackenbush, 1989a,b; Rankin *et al.*, 1989). The characterization of vitellin from the ovary of the prawn *P. monodon* have been studied in detail (Quinitio *et al.*, 1990; Quinitio and Millamena, 1992; Chang *et al.*, 1993, 1994). In decapods, hepatopancreas was proposed as a logical site for extraovarian Vitellogenin synthesis (Vg). The histology of the cells of the hepatopancreas demonstrates that this tissue is capable of both synthesizing proteins and storing fats as reported in *P. semisulcatus* (Al-Mohanna *et al.*, 1985 a,b and 1986; Al-Mohanna and Nott, 1987 a,b and 1989), *P. schmiti* (Menendez, 1990) and other penaeid prawn (Sarasquete *et al.*, 1985; Georgina *et al.*, 1990).

The formation of sperm or spermatogenesis in animals involves specific morphological and cytological changes in the meiotic products (Cohn, 1979). General accounts on spermatogenesis of decapod crustaceans, including penaeid prawns, have been provided by many workers like Adiyodi and Subramoiam (1983), *Pochon-Masson* (1983), Adiyodi (1985), Bauer (1990), Boddeke *et al.* (1990), Felgenhauer (1990), Hinsch (1990), Subramoniam (1990, 1993) and Pandian (1994). Sperms are produced in the seminiferous tubules of testis of male animal. As a general rule, in the sexually mature male, the

primitive germ cell, or spermatogonia, undergoes repeated mitotic multiplications prior to meiosis. The products of mitotic division, primary spermatocytes, then undergo meiosis. The secondary spermatocytes results from the first meiotic division, and the final meiotic products, each of which is haploid cell, are the spermatids, Spermatogenesis, the process of spermatid maturation, results in the formation of functional spermatozoa or sperm. Detailed histological studies on spermatogenesis in penaeid prawns have been carried out on many cultivable penaeid species. Some of the notable contributions are those of King (1948) in *P. setiferus*, Subrahmanyam (1965) and Mohamed (1989) in *P.indicus*, Joshi *et al.* (1982) in *P.stylifera*, Vasudevappa (1992) in *M dobsoni* and Bauer & Min (1993) in *Trachypenaeus similis*. Very few light and ultrastructural studies have been made on spermatogenesis by Ro *et al.* (1988) in *P. setiferus*, Mohamed (1989) in *P. indicus*, Chow *et al.* (1991) in *Aristeus antennatus*. Most of the crustaceans transfer sperm from male to the female during copulation via a specialized sperm packet known as spermatophore. The processes leading to the formation of a sperm mass in the vas deferens and the deposition of the main layers of the body and of the wing have been studied by Malek and Bawab (1974 a,b) in *Penaeus kerathurus*. The structure of spermatophore of penaeid prawns has been described in varying details by Heldt (1938), Eldred (1958), Tirmizi (1958), Tirmizi and Khan (1970), Perez-Farfante (1975), Hug (1981), Champion (1987), Bell and Lightner (1988), Ro *et al.* (1988, 1990) and Talbot *et al.* (1989). Evaluation of reproductive quality in male penaeid shrimp have been attempted by Leung-Trujillo and Lawrence (1987, 1991, Alfaro and Lozano, 1993), Heitzmann *et al.* (1993) and Pratoomchat *et al.* (1993). Histochemical studies with reference to reproduction in prawns have been carried out by a number of workers like Bonina (1974), Sandifer and Lynn (1981), Sarojini *et al.* (1981, 1986), Joshi *et al.* (1982), Munuswamy and Subramoniam (1984, 1986), Gutierrez *et al.* (1985) and Sarasquette *et al.* (1985, 1986). Felgenhauer (1990) reviewed the morphological diversity and Subramoniam (1990) on chemical composition of spermatophores in decapod crustaceans. Carrying out detailed investigations, these authors have pointed out the significance of

spermatophore morphology in hybridization through artificial insemination. More recently, Subramoniam (1993) discussed in detail the spermatophores and sperm transfer in marine crustaceans. The sperm-egg interaction in natantians has been studied at both the light and electron microscopic levels by Nath (1937), Brown (1967), Pochon-Masson, (1968a,b), Koehler (1979), Yudin *et al.* (1979, 1980), Clark *et al.* (1980, 1981a), Sandifer and Lynn (1981) and Chow (1982).

Biochemical and mineral changes in relation to ovarian maturation

A gametogenic process in the female reproductive cycle of crustaceans involves the synthesis of nutritive yolk in the ooplasm to meet the basic requirements of embryonic development independent of the maternal organism (Adiyodi and Subramoniam, 1983). Ovarian maturation places enormous demands on the energy reserves of females. Biochemical analysis shows that it is having an important physiological role to an animal for normal function, maintenance and growth as it involves ingestion, digestion, absorption and transport of nutrients and waste removal (Akiyama *et al.*, 1992). A survey of the literature on the biochemistry of crustaceans would reveal that several workers have focused their attention to this very important aspect in recent years especially with reference to the maturation process of female gonads. Some of those who contributed substantially in this aspect are Rahaman (1967), Chandran (1968), Adiyodi (1968), Gilbert and O'Connor (1970), Yamaoka and Scheer (1970), Pillai and Nair (1973), Diwan and Nagabhushanam (1974), Rice and Armitage (1974), Shyamasundari and Erribabu (1979), Varadarajan and Subramoniam (1982) and Adiyodi and Subramoniam (1983).

The hepatopancreas has been identified as the primary organ responsible for the storage of organic reserves, while the haemolymph plays the role of transporting these metabolites to the different tissues (Yamaoka and Scheer, 1970). Both hepatopancreas (Quackenbush, 1989) and haemolymph

(Lui and O'Connor, 1976) have been proposed to involve as logical sites for extraovarian Vg synthesis.

Studies on biochemical changes in relation to reproductive cycle in penaeid prawns are limited comparing with those on other crustaceans. The fluctuations in the biochemical constituents such as water, nitrogen, non-protein nitrogen, protein, lipid, carbohydrate and glycogen in gonad, muscle and hepatopancreas in relation to ovarian maturation have been studied in the penaeid prawns viz. *Metapenaeus affinis* (Pillai and Nair, 1973), *Penaeus vannamei*, *P. stylirostris* and *P. setiferus* (Lawrence *et al.*, 1979), *Parapenaeopsis hardwickii* (Kulkarni and Nagabhushanam, 1979), *P. indicus* (Read and Caulton, 1980), *Metapenaeus affinis* (Sarojini *et al.*, 1986), *P. aztecus* and *P. setiferus* (Castille and Lawrence, 1989), *P. monodon* (Dy-Penaflorida and Millamena, 1990), *P. indicus* (Mohamed and Diwan, 1992) and *Metapenaeus dobsoni* (Vasudevappa, 1992), Solenocerid shrimp *Pleoticus muelleri* (Jeckel *et al.*, 1989) and the caridean prawn *Crangon crangon* (Haefner and Spaargaren, 1993; Spaargaren and Haefner, 1994). Castille and Lawrence (1991) studied the biochemical composition according to the changes in the size of gonads and digestive glands in penaeid prawns *P. aztecus* and *P. setiferus*. Biochemical variation according to the moulting cycle has been studied by Kanazawa *et al.* (1976) and Diwan and Usha (1987) in the penaeid prawns *P. japonicus* and *P. indicus*, respectively. Clarke *et al.* (1990) studied the biochemical composition in *Macrobrachium rosenbergii* in relation to embryonic development. Subsequent to this, the biochemical constituents in relation to body, head and other parts have been studied by Gopalakrishnan (1951) and Dietz (1982) in *M. rosenbergii*, Achuthankutty and Parulekar (1984) in *M. affinis, M.dobsoni, P. merguiensis* and *P. stylifera*, Sarasquete *et al.* (1986) and Yano (1988) in *P. japonicus*.

Protein requirement is a summation of individual amino acids (Akiyama *et al.*, 1992). Significant differences in amino acid content during embryonic development and/ovarian maturation in penaeid prawns have been

studied by Richard and Ceccaldi (1977) in *P. serratus,* Dy-Penaflorida and Millamena (1990) and Fang *et al.* (1992) in *P. monodon*, Marangos *et al.* (1988) in Crangon crangon studied the amino acid profile in the prawn tissues subjected to thermal stimulation.

It is an established fact that, the crustacean reproduction is the production of yolk laden eggs. The yolk is a combination of proteins, lipids, sugars and some steroid hormones (Adiyodi, 1985). Fatty acids and lipid classes are main components of lipids involving major physiological roles in various aspects, namely, maintaining the body in normal condition, growth, maturation and sequence of moulting in crustaceans. Variations of fatty acid composition and lipid classes in muscle, hepatopancreas, ovary and carapace according to the maturation and other aspects have been dealt by many authors in numerous penaeid species like *P. duorarum* (Gehring, 1974), *P. japonicus* (Teshima and Kanazawa, 1983 and 1987), *P. japonicus* (Teshima *et al.*, 1987, 1988, 1989), *Pleoticus muelleri* (Jeckel *et al.*, 1989, 1990, 1991), *P. schmitti* (Vincent *et al.*, 1989), *P. monodon* (Chuang 1990; Fairs *et al.*, 1990; Millamena and Pascual, 1990; O'Leary and Matthews, 1990), *P. kerathurus* (Mourente *et al.*, 1990; Mourente and Rodrique, 1991), *P. californiensis*, *P. occidentalis, P. setiferus, P.stylirostris* and *P. vannamei* (Araujo and Lawrence, 1993), *P. chinensis* (Li *et al.*, 1993). Teshima and Kanazawa (1976) studied the variation in lipid classes during the molting cycle of *Palaemon serratus* and *P. paucidens*, respetively. Total lipid content and fatty acid composition have been studied by Guary *et al.* (1975) in *P. japonicus* and Hopkins *et al.* (1993) in *Pandalus borealis*, according to the seasonal variation. The level of biochemical constituents such as protein, free amino acids, free sugars, total lipid and metallic ions in the haemolymph serve as useful indicators of physiological condition of the particular species. Teshima and Kanazawa (1978) and Mirajkar and Nagabhushanam (1981) studied the release and transport mechanism of lipids, and variation in the cholesterol content in the prawns *P. japonicus* and *M. kistensis* during the reproductive cycle, respectively. Kanazawa *et al.* (1976) investigated on the tissue uptake of radioactive

cholesterol in the prawn, *P. japonicus* during induced ovarian maturity. Nagabhushanam and Kulkarni (1980) studied the neuroendocrine regulation of blood glucose as well as variation in relation to ovarian maturation in *Parapenaeus hardwickii*, while Rodriquez (1981) reported on the relationship between osmoregulation and the total protein in *P. vannamei* and the protein levels during moult cycle and ovarian maturation in *P. notialis*. Smith and Dall (1982) and Natarajan (1990) estimated the blood protein, blood volume and extracellular space relationship in *P. esculentus* and *P. plebjus* and the blood sugar in *P. indicus* and *P. monodon*. Mohamed (1989) and Vijayakumaran (1990) studied the protein, lipid and carbohydrate contents in relation to ovarian maturation in *P. indicus*.

Crustaceans are well known for accumulation of carotenoids in the gonads and eggs in the form of chromoprotein (Cheeseman *et al.*, 1967; Goodwin, 1950, 1951; Wallace *et al.*, 1967). Cheeseman *et al.* (1967) and Gilchrist and Lee (1972) reported that the ovaries and eggs of fish and shellfish are almost invariably pigmented due to the presence of carotenoids and or carotenoproteins. The pigments of the eggs are known to be derived from the haemolymph as conjugates of yolk precursor protein, "vitellogenin" which is then sequestered into the growing oocyte for final deposition. The chief protein fraction of the crustacean vitellus is a high density lipoprotein or lipoglycoprotein frequently associated with a carotenoid pigment and usually referred to as lipovitellin (Wallace *et al.*, 1967). Miki *et al.* (1982) reported about the carotenoid variation in fish and shellfish (Pandalus borealis, *P. orientalis*). Among penaeids, the biosynthesis of astaxanthin pigment in the prawn *P. japonicus* has been investigated by Tanaka *et al.* (1976) and the carotenoid content in the ovaries of *P. orientalis* by Miki *et al.* (1982). Goodwin (1984) threw light on the overall picture of carotenoids which exist in three forms in the crustacea: (1) as free pigments, carotenes and unesterified xanthophylls, (2) as xanthophylls esterified to long-chain fatty acids and (3) as xanthophylls attached to protein as carotenoproteins. Carotenoproteins have been observed in ovaries, hypodermis, cuticle, retina, gut and to some extent,

the hepatopancreas. The carotenes and esterified xanthophylls appear to be storage forms and accumulate dissolved in lipids in the hepatopancreas from which they are transferred to other sites in the body via the haemolymph. Depledge and Bjerregaard (1989) reviewed the haemolymph protein composition and copper levels in decapod crustaceans. Thus, carotenoids are widely distributed in living organisms and they represent the main pigments of most aquatic species like fishes and crustaceans. Astaxanthin is considered to be the major carotenoid of crustacea, comprising about 90% of the total pigments in *P. japonicus* (Ishikawa *et al.*, 1966) and it can be recovered from the carapace and the internal organs of the prawn (Katayama *et al.*, 1972). Several studies on carotenoid metabolism (Negre-Sadargues, 1978; Goodwin, 1984) of different crustaceans have demonstrated that most decapods are able to absorb and metabolize ß-carotene. Negre-Sadarques *et al.* (1992) investigated the utilization of synthetic carotenoids by the prawn *P. japonicus* reared under laboratory conditions. Dersan Kour and Subramoniam (1992) studied carotenoid metabolism during embryonic development of the marine crab, *Emerita asiatica*. However, information is scanty regarding accumulation and nature of pigment metabolism within the developing eggs of crustacea in common and of penaeid prawns in particular.

The accumulation of minerals are low in quantity in the tissue of crustacea comparing with mollusca. Although the requirement of micronutrients is in small quantities, their presence is essential for the proper functioning of different organs. Minerals serve as essential components of enzymes, vitamins, hormones and pigments, and are metabolic co-factors, catalysts and enzyme activators (New, 1987; Akiyama *et al.*, 1992). They act as constituents of the exoskeleton and tissues, for balancing osmotic pressure. Mineral deficiencies cause sluggish movements, lack of appetite, loss of disease resistance, reduction in growth rate, abnormal shape and physiological activities (New, 1987). Some minerals, which are required in considerable quantities are termed macro-minerals, while the others, which are required in lesser amounts, are referred to as micro-minerals. White and Rainbow (1982,

1986) studied the regulation of copper, zinc and cadmium and accumulation of cadmium by *Palaemon elegans*, while Chu *et al.* (1988) traced out the changes in mineral levels in body tissues during the moult cycle of the shrimp *P. chinensis*. Nugegoda and Rainbow (1988) studied the effect of salinity changes on zinc uptake and regulation by the caridean prawns *Palaemon elegans* and *P. varians*. Ionic regulation of the estuarine prawns *P. longirostris* has been studied by Campbell *et al.* (1989). Prasad *et al.* (1989) reviewed the trace metal metabolism in marine crustaceans. Gross examination of the dietary essentiality of thirteen minerals and evaluation of the dietary zinc requirement and effect of phytic acid on zinc and phosphorus bioavailability have been studied in *P. vannamei* by Davis and Lawrence (1993). Vijayakumaran (1990) elucidated the biochemical and mineral changes during ovarian maturation in *P. indicus*.

Captive Maturation and Spawning

The first captive spawning of penaeid prawn was demonstrated by Hudinaga in 1942 using a mature *P. japonicus* collected from the sea (Hudinaga, 1942). However, successful maturation and spawning in captivity was achieved only after three and a half decades by Shokita (1970) in *P. latisulcatus*. Since then several attempts in this line were made up in different parts of the world and as many as 23 Penaeid species have been successfully matured of which 14 spawned in captivity (Primavera, 1984). A few instances of natural maturation and spawning of unablated captive *P. monodon* in seawater ponds and tanks have been reported by Chen (1976) and Liao (1977) from Taiwan, Primavera and Yap (1979) from Philippines and Aquacop (1979) from Tahiti. Ryther (1979) observed that in China *P. orientalis* routinely matured in captivity. According to Primavera (1978), the SEAFDEC laboratory succeeded in maturing and spawning of *P. merguiensis*, *P. indicus* and *Metapenaeus* spp. in running seawater system. At Conway, U.K., Beard *et al.* (1977) reared many generations of *P. merguiensis* in captivity in rectangular concrete tanks with subgravel filters.

In India, Raje and Ranade (1972 a,b) succeeded in the laboratory spawning of *M. monoceros*, *P. merguiensis*, *M.affinis* and *P. stylifera* and studied their larval development. Subsequently, similar success has been achieved by Thomas *et al.* (1974) in *P. stylifera* and Thomas *et al.* (1974 a,b,c) in *P. acclivirostris*, *M. affinis* and *M. dobsoni* under laboratory conditions. Muthu *et al.* (1974, 1986) recorded the spawning of *P. indicus* and gave a note on the eggs and larvae. The breeding and larval rearing of different species penaeid prawns were successfully made by Devarajan *et al.* (1978) in *P. semisulcatus*, Silas *et al.* (1978) in *P. monodon*, Rao (1978) in *M. brevicornis*, Gopalakrishnan *et al.* (1985) in *M. kutchensis*, Nandakumar *et al.* (1989) in *M. moyebi*.

Endocrine control of reproduction

Endocrine control of reproduction in crustaceans has been investigated in a variety of species. The first concrete evidence for hormonal activities in crustaceans was obtained in the 1920's when Koller (1925, 1927) and Perkins (1928) demonstrated that crustaceans produce hormones that effect colour changes in those animals. In decapods the sinus gland is considered as the source of gonad inhibiting hormone (Panouse, 1943, 1947). The relationship between the removal of one (or both) eyestalks of a female decapod and ensuing gonadal development was first discovered by Panouse (1943) in the shrimp *P. serratus*. This discovery of Panouse was subsequently confirmed in many other decapod crustaceans also by Brown and Jones (1949), Adiyodi and Adiyodi (1970), Idyll (1971), Caillouet (1972), Bomirski and Klek (1974), Quakenbush and Herrnkind (1981), Chim *et al.* (1983), Deecaraman and Subramoniam (1983), Radhakrishnan and Vijayakumaran (1984 b), Kulkarni *et al.* (1984) and Anilkumar and Adiyodi (1985) Panouse (1943, 1947) observed that depending upon the moult stage, eyestalk removal in *P. serratus* resulted in accelerated ovarian development and spawning. Similar observations have repeatedly been made in a number of other natantians, including *Crangon crangon* (Klek-Kawinska and Bomirski, 1975), *Metapenaeus*

dobsoni (Muthu and Laxminarayana, 1977), *Palaemon paucidens* (Kamiguchi, 1971), *P. serratus* (Meusy *et al.*, 1983), *Pandalus hypsinotus* (Aoto and Nishida, 1956), *P. stylifera* (Muthu and Laxminarayana, 1977), *P. chinensis* (Arnstein and Beard, 1975), *P. esculentus* (Crocos and Kerr, 1986), *P. indicus* (Muthu and Laxminarayana, 1977; Emmerson, 1980, 83; Mohamed and Diwan, 1991), *P. japonicus* (Yano, 1984) and *P. kerathurus* (Hasen and Hamid, 1988), *P. kerathurus* (Lumare, 1979), *P. monodon* (Muthu and Laxminarayana, 1977; Santiago, 1977; Halder, 1978; Primavera, 1978; Emmerson, 1983; Hillier, 1984), *P. plebejus* (Kelemec and Smith, 1980) and *Artemesia longinaris* (Petriella and Diaz, 1987).

Unilateral eyestalk ablation has been employed to induce both ovarian maturation and spawning in prawns with varying success in many species by many investigators like Alikunhi *et al.* (1975) in *P. monodon* and *P. merguiensis*, Aquacop (1975, 1977, 1979) in *P. aztecus, P. monodon, P. stylirostris* and *P. vannamei, Arnstein* and Beard (1975) in *P. orientalis, P. occidentalis* and *P. monodon*, Duronslet *et al.* (1975) in *P. aztecus* and *P. setiferus*, Beard *et al.* (1977) and Beard and Wickins (1980) in *P. merguiensis* and *P. monodon*, Brown, *et al.* (1980) in *P.setiferus*, chamberlain and Lawrence (1981a,b) in *P. vannamei* and *P. stylirostris*, Emmerson (1980, 1983) in *P. indicus* and *P. monodon*, Kelemec and Smith (1980) in *P. plebejus*, Lawrence *et al.* (1980) in *P. setiferus*, Lumare *et al.* (1979, 1981) in *P. kerathurus* and *P. japonicus*, Primavera *et al.* (1979, 1982, 1984) in *P. monodon* and *P. indicus*, Wear and Santiago (1976) and Santiago (1977) in *P. monodon*, Browdy and Samocha (1985 a,b) and Browdy *et al.* (1986) in *P. semisulcatus*, Murugadass *et al.* (1987) in *Macrobrachium malcolmsonii* and Tan-Fermin (1993) in *P. monodon*. In recent years, the effects of eyestalk ablation on reproductive performance of spawners and larvae, egg production and food conversion efficiency, spawning in captivity, egg and larvae quality with diet, energy balance, spawning and hatching rate and cell growth and cuticle expansion have been studied by Vogt *et al.* (1989) in *P. monodon*, Marians and Murugadass (1991) in *M. malcolmsonii*, Redon and San Feliu (1993) in *P.*

japonicus, Rosas *et al.* (1993) in *P. notialis*, Yano and Wyban (1993) in *P. vannamei* and Okazaki and Freeman (1993) in *Palaemonetes pugio*, respectively.

Bray and Lawrence (1992) reviewed about the eyestalk ablation affects the hormone balance for numerous physiological processes in addition to stimulation of gonadal hypertrophy and various opinions have been made in penaeid prawns concerning use of unieyestalk ablation and bieyestalk ablation in captive reproduction by many workers like Moore *et al.* (1974) in *P. californiensis*, Nurjana and Yang (1976) in *P. merguiensis*, Primavera and Yap (1979) in *P. monodon*, Beard and Wickens (1980) in *P.monodon*, Emmerson (1980) in *P. indicus*, Kelemec and Smith (1980) in *P. plebejus*, Chamberlain and Lawrence (1981b) in *P. stylirostris* and *P. vannamei*, Lumare (1981) in *P. Japonicus*, Primavera *et al.* (1982) in *P. indicus*, Poernomo and Hamami (1983) in *P.monodon*, Browdy and Samocha (1985 a,b) and Browdy *et al.* (1986) in *P. semisulcatus*, Choy (1987) in *P. canaliculatus*, Makinouchi and Primavera (1987) in *P. indicus* and Robertson *et al.* (1987) in *P. setiferus* and *P. stylirostris*.

Basic understanding of crustacean endocrinology has advanced rapdily in recent years since the pioneering classical endocrinological experiments involving glandular ablation and reimplantation (Quackenbush, 1986; Fingerman, 1987). In very recent year, the importance and enrollments of methyl farnesoate and neuropeptides on crustacean female reproduction have been reviewed in detail (Chang 1992; Laufer and Landau, 1991; Subramoniam and Keller, 1993). In prawn, it is apparently noted that the neuropeptide hormone having active roll in brain, mandible organ and thoracic ganglion on precocious ovarian maturation, since it is having gonad stimulating hormone. This kind of work has been studied in detail by many authors like Kulkarni *et al.* (1981) in *P. hardwickii*, Joshi *et al.* (1982) in *P. stylifera*, Nagabhushanam and Joshi (1986) and Nagabhushanam *et al.* (1993) in *P. stylifera* and *M. affinis*, Takayanagi *et al.* (1986) in *Paratya compressa*, Yano *et al.* (1988) and

Yano and Wyban (1992) in *P. vannamei*, Jayalakshmi *et al.* (1989) in *M. lamerri*.

Studies on *in vitro* fertilization and the use of hormonal injections to induce maturation in penaeid prawns have also been carried out with varying degrees of success in recent years. Clark *et al.* (1973) succeeded in achieving *in vitro* fertilization with non-motile spermatozoa of brown shrimp *P. aztecus*, while Nair (1987) obtained similar results in the banana shrimp *P. merguiensis*. Kulkarni *et al.* (1979) showed that progesterone stimulated oogenesis in *P. hardwickii*. Nagabhushanam and Kulkarni (1982) used vertebrate male steroid hormone such as testosterone acetate and androgen and found that these hormones have inhibiting effects on ovarian maturation. Yano (1985) induced ovarian maturation and spawning in the Greasy back shrimp *M. ensis* by administering progesterone. Laufer and Landau (1991) reported that the mandible organ was identified as the source of methyl farnesoate (MF) producing a "gonad stimulating hormone" in penaeid prawn (*P. semisulcatus*, *P. vannamei, P. duorarum*) using a *in vitro* methods.

In *Penaeus semisulcatus*, Browdy and Samocha (1985 a,b) succeeded in ovarian maturation through eyestalk ablation, while Radhakrishnan *et al.* (1993) obtained the same result by manipulating of environmental parameters.

CHAPTER II

MATERIAL AND METHODS

MATERIAL AND METHODS

The material for the present study was obtained entirely from the Gulf of Mannar and Palk Bay (Fig.1 & Plate 2a,b) where *P. (P). semisulcatus* is exploited commercially throughout the year. The fishing grounds of Gulf of Mannar extended from Lat. 8° 50'N to 9° 10'N and Long.78° 35'E to 79° 40'E where the depth ranged between 15 and 50 metres. The trawling grounds in the Palk Bay region was located in Lat. 9° 20'N to 10° 05'N and Long. 79° 05'E to 79° 40'E where the bottom was comparatively shallow ranging from 8 to 14 metres. The fishing ground was predominantly muddy in the Gulf of Mannar and sandy in the Palk Bay. The biological analysis of the commercial catches, histological and histochemical studies, biochemical analysis and the experimental work on live animals were all carried out at the Regional centre of Central Marine Fisheries Research Institute, Mandapam Camp. The study extended for a period of 35 months from February 1991 to December 1993.

Reproductive biology

The various aspects of reproductive biology such as sex segregation, sex ratio, anatomy of reproductive systems, maturation and spawning and fecundity were studied from the regular samples collected from the commercial catches landed at Mandapam from February 1990 to January 1993. On the observation day, a random sample of 1 to 2 kg of prawns was collected from the trawler catch (Plate 3) and analysed at the landing centre itself for sexwise length measurements and maturity condition of female assessed by visual observation of size and colour of ovary visible through the exoskeleton (Thomas, 1974, Castille and Lawrence, 1991). Small incision was made on the dorsal side of the body between carapace and first abdominal somite in order to ascertain the stage of gonadal development in doubtful cases. Other information such as developmental stage of petasma and thelycum, presence

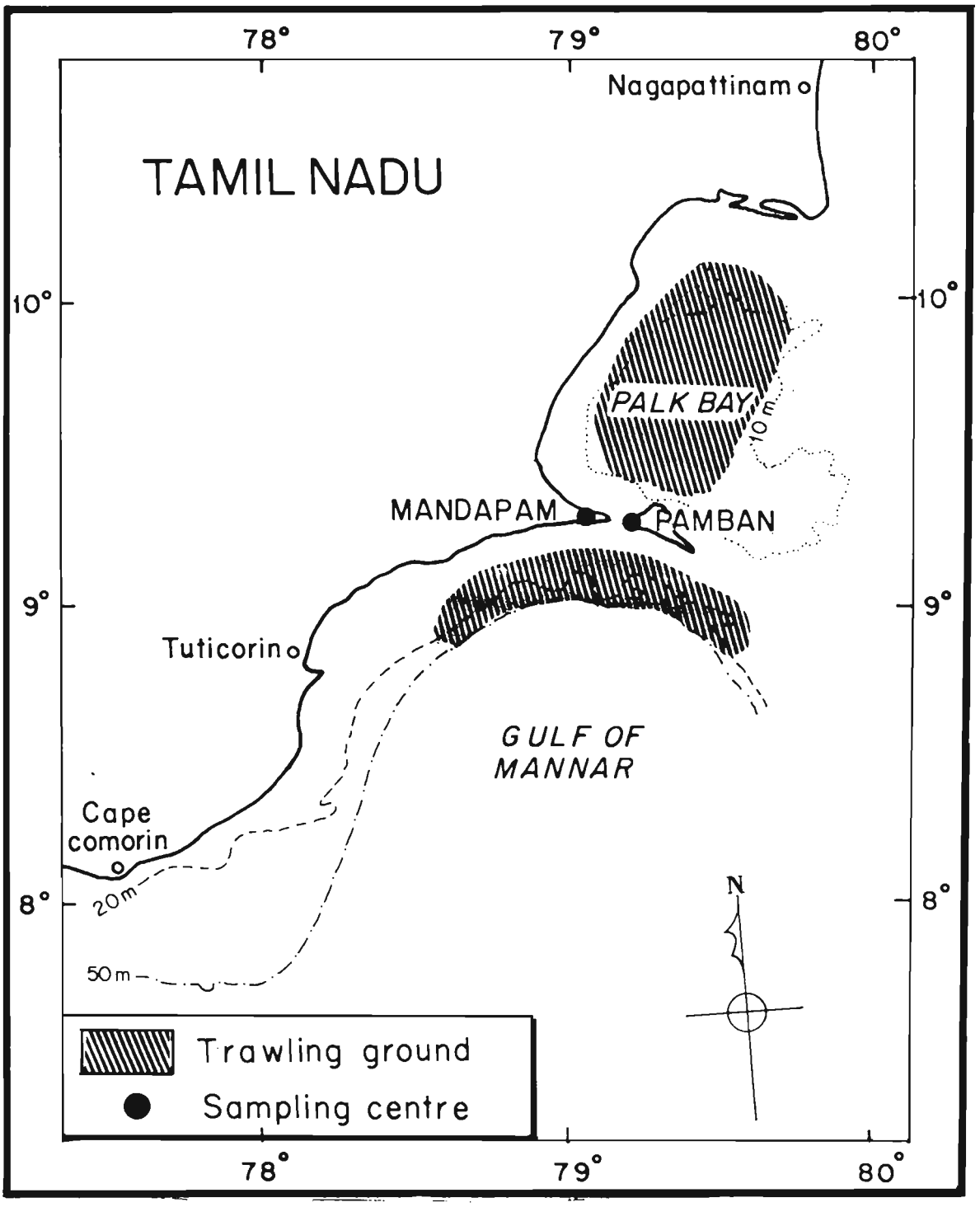


FIG. 1. Map showing the shrimp fishing grounds and sampling centres

or absence of spermatophores at the base of the fifth leg in the case of male and the impregnated (Plate 4a) or non-impregnated condition of thelycum in the case of female, were also recorded. The total length was measured to the nearest 0.5 mm from tip of rostrum to tip of telson with the body in stretched condition. In order to understand the possible influence of environmental factors such as temperature, dissolved oxygen, salinity and pH and the nutrients like phosphate, silicate, nitrate and nitrite on the reproduction of *P. semisulcatus*, monthly samples of seawater from the surface and bottom of the fishing ground were collected and analysed for the above parameters and their mean values used. The temperature was measured using a normal mercury thermometer. Dissolved oxygen was estimated by modified Winklers method as followed by strickland and Parsons (1968). Salinity was estimated as followed by Stickland and Parsons (1968) and calculated by using Knudsen's equation. Phosphate was estimated using the method of Murphy and Riley (1962) which is a modification of earlier methods. Silicate was estimated by using the method of Chow & Robinson (1953). Nitrate and nitrite were estimated by the methods of Mullin & Riley (1955) and Bendschneider & Robinson (1952), respectively. The values of phosphate, nitrate, nitrite and silicate are expressed as µg-atm/1. All the biological data collected at fortnightly intervals were pooled monthwise and statistically analysed. Details of the procedure followed for the various aspects of the study are described below.

The morphology of male and female reproductive systems was studied based on fully mature specimens in the fresh condition. The reproductive organs were carefully dissected out and drawn using camera lucida.

The length at maturity has been estimated for female based on 614 prawns in the size range 100 mm to 240 mm TL caught from the Gulf of Mannar during the year 1991. These measurements were grouped into 10 mm length intervals and the percentage of individuals in fourth stage of maturity was calculated against each of the class intervals to estimate the minimum

size of maturity at 50% level. The percentage frequencies were plotted against the class intervals and the size at cumulative 50% maturity was estimated using probit analysis and regression.

The fecundity was estimated by counting mature ova of gravid ovaries by the method of Choy (1985). The individual prawn and ovary were weighed to the nearest milligram and subsamples of ovary were taken from the middle lobe, weighed separately and preserved in 4% formalin or 10-30% sodium hypochlorite solution for 24 hrs. After teasing out the eggs, they were counted using plankton counter under binocular microscope. From the egg count data thus collected, the fecundity was estimated using the formula:

$$F = \frac{nG}{g}$$

where, F = Fecundity
n = number of eggs in the subsample
G = total weight of ovary in g
g = weight of the subsample in g

The relationship between the fecundity (F) and the total length (L), fecundity and total body weight (W) and fecundity and total gonad weight of the female prawn are determined using regression equations. In order to measure the sizes of the developing ova at different stages of maturation and to construct the ova diameter size frequency profiles, a small portion of ovary was dissected out from the middle lobe and posterior lobe and fixed in 4% formalin or 10-30% sodium hypochlorite solution for one to two days. Thereafter, the sample of ovary was teased out and the diameter of the ova was measured along the vertical and long axes by using an ocular micrometer (ERMA, Japan) which was calibrated with a stage micrometer under monocular microscope as given by Rao (1968). The average of these two measurements was taken as the actual diameter of the ovum. From each sample representing a maturity stage,

at least 300 ova were measured. In the same way, the measurements of ova diameter were also recorded from prepared histological slides.

For determining the gonadosomatic index (GSI), the female prawns at different maturity stages were weighed individually to the nearest milligram after blotting the animal. The ovaries were carefully dissected out and weighed to the nearest milligram. The gonadosomatic index (GSI) was calculated using the method described by Giese and Pearse (1974).

$$GSI = \frac{\text{Wet weight of ovary (g)}}{\text{Wet weight of animal (g)}} \times 100$$

The hepatosomatic index (HSI) was calculated using the following method described by Giese and Pearse (1974).

$$HSI = \frac{\text{Wet weight of hepatopancreas (g)}}{\text{Wet weight of animal (g)}} \times 100$$

Histological and histochemical studies

2.1 Collection of animals

Live female and male prawns of different maturity stages were collected for histology and histochemical examinations from the Palk Bay and Gulf of Mannar sea off Mandapam from trawl operations with the help of fishermen. After hauling the net, *P. semisulcatus* were immediately segregated sexwise from the catch and kept in a fibre glass tank containing water which was frequently changed. The tissues dissected from live prawns were fixed in appropriate fixative for the future study.

PLATE 2

A. A view of Mandapam landing centre showing shrimp trawlers returning after fishing in the Palk Bay.

B. An areal view of Pamban landing centre showing shrimp trawlers returning after fishing in the Gulf of Mannar.

PLATE 2

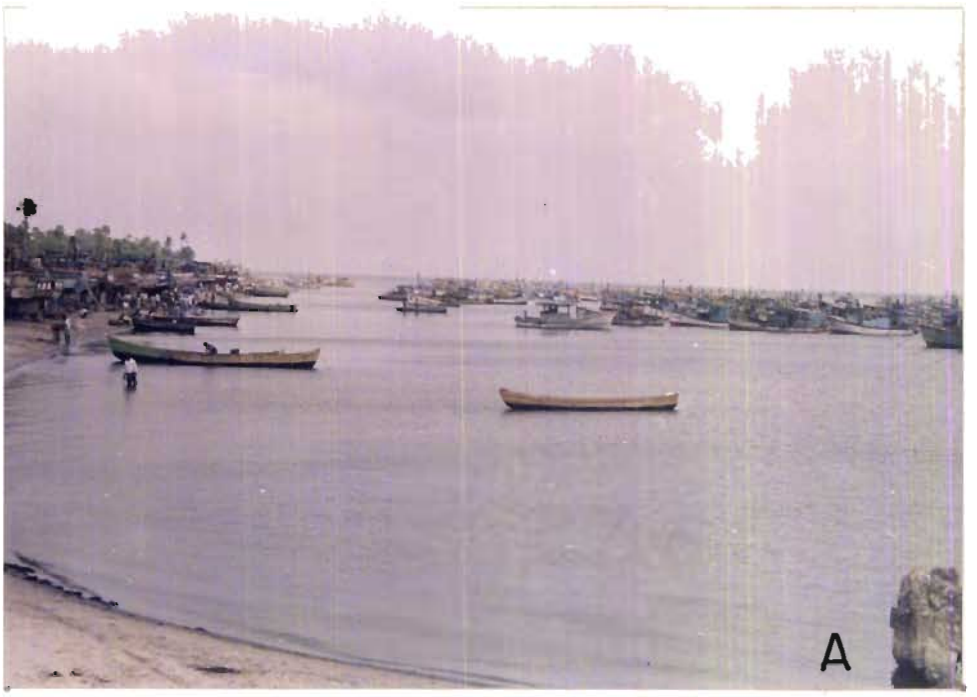


PLATE 3 A commercial catch of *P. (P.).semisulcatus* landed at Mandapam.

PLATE 3

2.2 Dissection, fixation and spermatophore extrusion

The middle lobe of the ovary of various maturity stages, hepatopancreas of mature female prawn and male gonad parts such as testicular lobes, proximal vas deferens, mid vas deferens, distal vas deferens and terminal ampoule were dissected out and preserved in Bouin's solution for histological examination. For histochemical examination, they were preserved in 10% neutral buffered formalin, carnoy's fluid and Baker's formol-calcium according to the nature of tests for 24 hrs respectively. Generally, the volume of the fixation used was 20 times to that of the tissue. Fresh materials of both male and female gonads were collected for lipid histochemical analyses and the cryostat sections used for the analyses were taken using hitostat™ cryostat Microtome (Ao® model 975c and 976c).

To study the structural details of sperm and spermatophore, the live male prawns were collected and transported to the laboratory. The spermatophore extrusion was made by delivering 12 V current at the base of fifth walking leg through an electrocautery apparatus. As soon as the extruded spermatophore contacted the water, it spreads like an umbrella. Thereafter, it was subjected to gross morphological observation under a dissection microscope and its structure was drawn and photographed with the appropriate measurements. The spermatophore was preserved for histological and histochemical analysis as described above. For ultrastructural studies, it was fixed in 4% Buffered Glutaraldehyde.

2.3 Processing and sectioning

All tissues fixed in Bouin's fluid were washed overnight in running tape water to remove the excess of picric acid. Both Bouin's and formalin fixed tissues were dehydrated using an alcohol series (30% to 100% propanol) and cleared in xylol. The tissues were further cold impregnated overnight with wax using xylene and wax shavings in a 1:1 ratio. Subsequently the solvent was

evaporated by placing the tissues in an oven at 58°C. The tissues were then transferred through two changes of fresh molten wax (paraffin wax MP 58-60°C). Tissue blocks were prepared by using proper orientation of wood.

Serial sections of the blocks were cut at approximately 6-8 µm thickness using a rotary microtome. Sections were affixed on clear glass slides using fresh Mayer's egg albumin and flattened by placing on a slide warmer with a drop of distilled water. Subsequently the water was drained off and the slides were allowed to dry. These slides were then used for histological and histochemical staining. Frozen sections of fresh unfixed tissues or those fixed in Baker's formol-calcium and cold neutral buffered formalin were cut using an American opticals cryostat microtome at 8-10 µm thickness and used for lipid histochemical studies. Formol-calcium and cold neutral buffered formalin fixed tissues were washed in tap water for 5 to 10 minutes and blotted repeatedly with filter paper until the blocks were as dry as possible. Fixed and unfixed fresh tissues were placed on the block holder and freezed with a few drops of distilled water. As indicated above, 8-10 µm thickness sections were cut and an antirol guide was used to prevent curling of the individual sections. After this, sections were transferred to the slide by using a fine camel hair brush and then processed for staining with lipid histochemical techniques.

2.4 Staining

Routine staining for gross morphological observations was carried out using Harris haematoxylin stain with 1% alcoholic eosin as the counter stain. Sections to be stained were first deparaffinized in two changes of xylene and then hydrated through a descending series of propanol grades. Sections were blued using tap water or ammonia solution. Eosin stained sections were repeatedly washed in an ascending series of propanol grades to remove excess eosin and cleared in xylene. In the same way as described above, Mallory's Triple stain was used for histological examination using mordanting solution, 5% sodium thiosulphate for clearing and Acid fuchsin and orange G as counter

stains. Sections were mounted with DPX or Canada Balsam of neutral pH and examined under a monocular microscope.

2.5 Histochemical tests

Histochemical tests with appropriate controls were carried out to elucidate the chemical nature of the ova and yolk and also to find out the pattern of the yolk accumulation. Broadly, the histochemical tests were performed to detect proteins, nucleic acids, carbohydrates and lipids in the oocytes and sperm cells. The intensity of the staining was recorded as '+++' (intense positive reaction), '++' (strong positive reaction), '+' (positive reaction), '±' (very mild positive reaction of doubtful nature) and '-' (negative reaction).

2.5.1 Tests for detection of proteins

For the detection of general proteins and different amino acid and groups the following detailed histochemical tests were carried out.

a) Mercuric Bromophenol Blue test for general proteins (Mazia *et al.* , 1953)

The mercuric ions of bromophenol blue solution react with acidic, sulphydryl and aromatic residues of protein to give a blue colour.

b) Aqueous Bromophenol blue test for basic proteins (Pearse, 1968)

The acidic groups of bromophenol blue react with basic groups of protein to give a blue colour. Deamination with nitrous acid was carried out as control.

c) Toluidine blue test for acidic groups (Pearse, 1968)

Toluidine blue which is a basic dye in aqueous medium reacts with acidic groups of protein to give a blue colour. Mild methylation using Methanol-Hcl mixture was used to block acidic end groups.

d) Ninhydrin Schiff test for amino groups (Pearse, 1968)

The stable tissue aldehydes produced in the course of oxidative deamination with ninhydrin are coloured with Schiff's reagent. The reaction was blocked by deamination before the stage of oxidative deamination.

e) Ferric ferricyanide method for -SH groups (Pearse, 1968)

This method depends on the reduction of a fresh solution of ferricyanide in acid solution at pH 2.4 by sulphydryl groups in the tissues. The resulting ferrocyanide combines with ferric ion (in ferric sulphate) to give an insoluble prussian blue precipitate. Mercaptide block with saturated mercuric chloride at 30°C for 1 hour was performed as control.

f) Thioglycollate Ferric-Ferricyanide Method for SS Groups (Pearse, 1968)

Unreactive disulphide groups are reduced to reactive sulphydryl groups by thioglycollate. The sulphydryl groups reduce the ferricyanide and give the prussian blue for the presence of disulphide groups. Slides treated with 0.5 M thioglycollate for 4 hours at 37°C as the control.

g) Million's test for tyrosine (Pearse, 1968)

Million's reagent, a mixture of mercurous and mercuric nitrates and excess of nitric acid, reacts with phenolic groups (tyrosine) to give a reddish or

yellowish red colour. Slides iodinated with Gram's iodine solution and 3% ammonia were used as the control.

h) DMAB - Nitrite method for tryptophan (Pearse, 1968)

The aldehyde component of p-dimethylamino-benzaldehyde (DMAB) solution reacts with tryptophanyl reactive sites and forms a blue coloured compound called B-carboline pigment. Sodium nitrate solution was used to intensify the colour of the pigment. Slides pretreated with 40% formaldehyde were used as control.

2.5.2 Tests for detection of Carbohydrates

The following histochemical tests were performed to detect the different types of carbohydrate substances.

a) Periodic Acid Schiff (PAS) reaction (McManus and Mowry, 1960)

This reaction is based on the fact that aqueous periodic acid will oxidize 1, 2 glycol groups in tissues, largely in materials that comprise of carbohydrates, to produce aldehydes that are coloured by Schiff's reagent. Stained sections were placed under running tap water briefly to enhance the colour.

A number of control slides were run to confirm the presence of the variety of PAS positive materials present. In order to detect the interference of free aldehydes in the reaction a slide was stained with Schiff alone without prior oxidation. Malt diastase digestion at 37°C was used to confirm the presence of glycogen. Absence of PAS positivity after acetylation in a acetic anhydride pyridine mixture for 12 hours served to confirm the presence of 1, 2 glycol groups. Further deacetylation by treatment with alkali restored the

PAS reactivity. Deamination prior to the PAS test removed the Schiff reactive aldehyde in tissue proteins. To affirm the presence of glycolipids slides were pretreated with chloroform/methanol and then subjected to the PAS test.

b) Best's Carmine test for glycogen (Pearse, 1968)

The active principle of the natural dye carmine is carminic acid which at a pH on the alkaline side of its isoelectric point (4.0-4.5) is negatively charged and behaves like an acid dye staining 1, 2 glycol groups. Malt diastase treated slides were used as the control.

c) Toluidine blue at different pH test for Acid Mucopolysaccharides (AMP) (Pearse, 1968)

Toluidine blue, a basic dye reacts with acid mucopolysaccharides (AMP) at different pH. At lower pH the dye colours the sulphated AMP whereas in higher pH it stains the phosphated AMP. The metachromasia at lower and higher pH indicates the presence of sulphated and carboxylated AMP, respectively. Slides hydrated with 1N hydrochloric acid for 96 hrs at 37°C as the control.

d) Alcian CEC Method for Acid Mucopolysaccharides (AMP) (Pearse, 1968)

Both sulphated and carboxylated mucins bind with alcian blue *in situ* in the presence of low concentrations of electrolytes (below 0.3 M magnesium chloride), whereas only sulphated mucosubstances do so with higher concentration (above 0.8 M).

e) Bracco-Curti's test for sulphated AMP (Pearse, 1968)

This method was specific for sulphated AMPs. Benzidine in 2% boric acid reacts with the sulphate groups of AMP to form benzidine sulphate. Potassium dichromate oxidizes the benzidine sulphate to give a blue colour indicating the presence of sulphated AMP.

2.5.3 Tests for the detection of Lipids

The following histochemical tests were carried out to detect the different classes of lipids.

a) Sudan black B test for lipids (Pearse, 1968)

Sudan black B is a diazo dye and being slightly basic because of its amino groups combines with the acidic groups of compound lipids such as phospholipids to give a black or blue colour. Delipidized control slides were prepared by treatment with a chloroform: methanol (1:1) mixture for 18 hours at 60°C.

b) Nile blue method for neutral and acidic lipids (Pearse, 1968)

Nile blue method stained neutral fats red, acidic lipids dark blue cytoplasm pale blue. Chloroform methanol extraction was carried out for use the control.

c) Nile blue sulphate Method for phospholipids (Pearse, 1968)

Nile blue principle stained phospholipids are weakly acid to give a blue colour. Chloroform : Methanol extraction was performed to delipidize control slides.

d) Oil Red 'O' method for neutral lipids (Pearse, 1968)

Oil Red 'O' in isopropanol stains the neutral lipids red without forming dye precipitates. Chloroform:methanol extraction was performed to delipidize control slides.

e) UV Schiff reaction for unsaturated lipids (Pearse, 1968)

Frozen sections were subjected to long and short wave (254 nm) irradiation for 3-4 hours and then treated with Schiff's reagent. Control slides were not exposed to UV. The difference in staining intensity demonstrated the number of double bonds saturated by oxidation.

2.5.4 Photomicrography

Histology and histochemical preparations of the ovary and testis were photographed using a Nikon optiphot research microscope equipped with an automatic exposure system. Black and white 35 mm film (ORWO, 100 ASA) and colour 35 mm (KODAK COLOUR, 100 ASA) film were used.

3 Ultrastructural studies

3.1 Transmission electron microscopic study

Techniques for ultrastructural studies, recommended by Hawkes and Stehr (1980) for marine organisms, were adopted for processing the tissues for Transmission electron microscopic study. Female gonads of various maturity stages and matured male gonad were dissected out from the live prawn and small pieces (3-4 mm size cube) of gonad tissues were fixed in ice cold 3 percent buffered glutaraldehyde solution for 24 hours at 4°C. Fixed tissues were then washed three times each for 15 minutes in sodium cacodylate buffer, trimmed approximately 1-2 mm size cube and post-fixed in 1% osmium tetraoxide for about one and half hours until fully osmicated. Afterwards

tissues were again washed with sodium cacodylate buffer for three times each of 15 minutes to remove excess fixatives and dehydrated through an ascending series of acetone grades each of 15 minutes duration. Dehydrated tissues were impregnated with spurr DMAE by passing it through spurr-acetone medium mixture in ratios 1:1, 1:2, 2:1 each of 1 hour duration and finally embedded in pure spurr-medium using plastic moulds by keeping for eight hours at 60°C in BOD incubator. The polymerized blocks were removed from moulds and stored till sectioning in labelled polyethene bags. The blocks were trimmed and sectioned with freshly made glass knife fitted on ultramicrotome. Semithin sections of 1 µm size were stained with methylene blue-Azure 11/Basic Fuchsin or toluidine blue and observed under a compound microscope to select the desired areas. The ultrathin sections of 600-700°A thickness were taken over the copper grids (3 mm diameter) and dried. The sections were stained with saturated alcoholic uranyl acetate and Reynold's lead citrate. The dried sections were observed under Hitachi 600/philips cm 10 electron microscope and desired areas photographed.

3.2 Scanning electron microscopic study

The technique recommended by Felgenhauer (1987) for preparing crustacean tissues for scanning electron microscopic studies was used. The tissues of terminal ampoule and vas deferens of male were prefixed in buffered glutaraldehyde, dehydrated in an ethanol series (35% to 100%) critically point dried on CO_2 and sputter-coated with gold palladium, mounted and observed.

4 Biochemical studies

4.1 Collection of animals

Live female prawns *P. semisulcatus* were collected from Palk Bay and Gulf of Mannar off Mandapam from last two hauls operated during night. As soon as the nets were hauled, the prawns at different maturity stages were segregated and kept immediately in fibreglass tanks containing sea water

which was changed frequently and later on it was changed into polyethene breeder can and transported to the laboratory where it was kept in 1 ton capacity fibreglass tanks providing fresh filtered seawater with good aeration.

4.2 Collection of haemolymph and tissue samples

Haemolymph samples from individual live prawns were drawn by direct cardiac puncture using a hypodermic syringe fitted with a No.22 needle and rinsed in an anticoagulant (10% trisodium citrate) prior to each collection. The collected haemolymph samples were stored in sterilized glass vials at -20°C until analysis. Thereafter, the prawns of different maturity stages were immediately dissected and the ovary, hepatopancreas and muscle tissues were quickly excised out. The tissues were dried to a constant weight at 60°C and then macerated using an agate mortar and pestle. They were then stored in a desiccator with silicagel until analysis.

4.3 Biochemical analysis

Samples of haemolymph from prawns of all reproductive stages were analysed for total protein, amino acid, lipid, carbohydrate and carotenoids. Dried and powdered tissue samples of gonad, hepatopancreas and muscle belonging to different maturity stages were analysed for total proteins, amino acid, lipid, carbohydrate contents. For estimation of moisture and carotenoids, fresh tissues were used. Five replicates were carried out for each estimation. The various analytical methods used for biochemical estimation are described below.

a) Estimation of moisture content

The moisture content of ovary, hepatopancreas and muscle were determined by keeping pre-weighed wet samples at 60°C in a hot air oven till

constant weights were obtained. The loss in weight was taken as the water content and expressed as percentage.

b) Estimation of total proteins

Total proteins was estimated by the Biuret method of Gornall *et al.* (1949) using crystalline bovine serum albumin (Sigma) as standard. Pre-weighed dried tissue or a known aliquot of haemolymph was taken and deproteinized using 80% ethanol. The protein precipitate was dissolved in 2 ml 1 N NaOH and 8 ml of Biuret was added. The colour developed was read at 540 nm using a ECIL UV spectrophotometer against a reagent blank.

c) Estimation of free amino acids

Free amino acid was estimated by using the method of Yemm and Cocking, 1955). Preweighed tissue or known aliquot of haemolymph was taken and deproteinized using ethanol 0.5 ml of citrate buffer pH 5.0 (0.2 M) was added into sample supernatant to which 1.2 ml of solution was added. The colour developed was read at 570 nm using a ECIL UV spectrophotometer against standard and blank.

d) Estimation of total lipids

Total lipid content in the tissues and haemolymph was estimated using the method of Folch *et al.*, (1957). Pre-weighed sample or a known aliquot of haemolymph was homogenized in a chloroform: methanol mixture (2:1 V/V) and placed in an amber coloured separating funnel. The phases were separated by the addition of 0.9% NaCl solution. The lower phase was separated into a clean tube and made up the volume of the lower phase to the original quantity of chloroform added before. To estimate the lipid quantity (Barnes & Blockstock, 1973), 0.5 ml of extract was measured into a clean test tube and dried in a water bath. After dissolving with 0.5 ml of concentration

H_2SO_4, they were placed in boiling water bath for 10 minutes and cooled to room temperature. The colour developed by adding 5 ml of Vanillin reagent to 0.2 ml of this acid digest was read at 520 nm using a ECIL UV spectrophotometer against standard and reagent blanks.

e) Estimation of total carbohydrates

Total simple sugars, oligosaccharides and polysaccharides were estimated using the phenol - sulphuric acid method of Dubois *et al.* (1956). Haemolymph and tissue samples were deproteinized using 80% ethanol. To a known aliquot of the supernatant 0.05 ml of 80% phenol was added. Then 5 ml of concentrate sulphuric acid was added directly against the liquid surface to obtain good mixing. The stable orange-yellow colour developed was read at 490 nm in a ECIL UV spectrophotometer along with D-glucose standard and reagent blanks.

f) Estimation of carotenoids

The total carotenoid content was estimated by following the extraction procedure of Olson (1979). Pre-weighed fresh tissue or a known aliquot of haemolymph was taken in a clean 10 ml screw cap glass vial and 2.5 g anhydrous sodium sulphate was added. The sample was gently mashed with a glass rod until it was well mixed with sodium sulphate. The caked residue was covered with 5 ml of chloroform and placed at 0°C for 8-24 hours. An aliquot of the chloroform extract was diluted with ethanol and the absorption was read in a ECIL UV spectrophotometer at 450 nm.

g) Estimation of micronutrients

Tissues such as ovary, hepatopancreas and muscle of various maturity stages of female prawn were collected as described above and dried samples were used for the analyses of magnesium, calcium, copper, manganese, zinc,

nickle, lead, cromium, iron, potassium and sodium by using the triple acid extraction method (Wet Digestion Technique) as proposed by (Jackson, 1973). In this method, a known weight of the tissue sample is digested with triple acid to destroy the organic fractions and to bring the mineral constituents into solution. Triple acid mixture was prepared as 9:2:1 ratio of concentrated nitric, sulphuric and perchloric acids. Preweighed samples were taken into a 100 ml conical flask where 15 ml of the triple acid mixture was added by covering the flask mouth with a small funnel and digested the flask contents over a sand bath till a clear solution was obtained. Afterwards it was diluted with distilled water and transferred to a 100 ml volumetric flask. The conical flask was again washed with small increments of water to add to the volumetric flask. In final the volume of the solution was made up to 100 ml with distilled water and taken for further analysis employing the techniques of Atomic Absorption Spectrophotometry and Flame photometry.

Atomic absorption spectrophotometer (Perkin Elmer/model-320, USA) was used for the estimation of trace minerals such as calcium, magnesium, copper, manganese, zinc, nickel, lead, cadmium and iron present in the test tissues. The accuracy of the equipment was high being less than one part per million. The prepared ash solution was aspirated directly into the flame unit of the AAS. For all elements, air-acetylene (A-AC) gas mixture was used to produce a flame of about 2400°C. For each case, the absorbance was measured and compared with respective working standard solution.

The application of emission flame photometry has made the chemical analysis of certain substances in biological fluid both rapid and fairly accurate. The direct system of analysis measures the intensity of the emitted light relating the intensity to the concentration of the material being analysed. The flame photometry was used for the estimation of sodium and potassium present in the tissues from the above prepared ash solution. Three replicates were carried out for each estimation. The values obtained from the method are expressed as PPM and was calculated as follows.

$$PPM = \frac{\text{Standard PPM}}{\text{Corresponding OD}} \times \text{sample reading} \times \frac{\text{dilution factor}}{\text{weight of sample in gram}}$$

5 Induced maturation experiments

Thirty live female prawns of *P. semisulcatus* above 140 mm TL caught from Palk Bay and Gulf of Mannar were used for induced maturation experiments. Ten prawns were maintained in a 1 ton capacity fibreglass tank provided with good aeration at the Mandapam Regional centre of CMFRI. One-third of the water in the tank was exchanged daily with fresh sea water filtered through a phytoplankton net cloth having mesh size of 50-60 micron, in order to ensure healthy condition for the animals.

5.1 Eyestalk ablation and Eyestalk extract injection

In order to study the role of eyestalk neurosecretory hormones on ovarian maturation, eyestalk ablation and eyestalk extract injection experiments were conducted. Thirty immature prawns in intermoult and premoult stages were selected from the above stock and divided into 5 groups (I, II, III, IV and V) of 5 animals each for experimentation in maturation tanks. In group I, prawns were maintained without any treatment for a period of 10 days and used as the intact control. In group II and III animals were subjected to unilateral eyestalk ablation and bilateral eyestalk ablation respectively using an electrocautery apparatus. In the case of group III, after removal of one eye, the prawns were left in maturation tank to remove the remaining eye after 24 h. In group IV, unieyestalk ablation was performed with the supernatant prepared from an aqueous extract of fresh eyestalk in the ratio of 2 eyestalk/0.2 ml by macerating fresh eyestalks (after removing the ommatidia and cuticle) in cold crustacean saline. The extracts were centrifuged

at 3000 rpm for 10 minutes for the injections. Each of the animals was injected once with 0.2 ml of prepared supernatant using a hypodermic syringe. In group V, the prawns were injected with CNS extract.

While the prawns were maintained in the maturation tanks, 50 percent of seawater was replaced with fresh filtered seawater daily providing good aeration. The animals were fed with fresh clam meat and the uneaten food and faecal matter were siphoned out twice daily. The salinity of the seawater ranged from 28 to 38 ppt, temperature from 28-30° C and pH 8.2. The pH was constantly regulated by adding anhydrous sodium carbonate (Muthu *et al.*, 1964; Browdy *et al.*, 1986) as and when required. The maturation tanks, each one of 1 ton capacity, were shaded and partly covered with blue clothes to reduce illuminations and thus prevent algal growth on the wall and at the bottom of maturation tanks. Once in ten days, the nitrofurazone bath treatment (5 ppm over 1 h) was given to prevent possible bacterial infection.

The degree of ovarian maturation in the experimental animal was checked daily morning by external macroscopic examination of the colour and size of the developing ovaries. The triangle shaped gonad discernible through the first abdominal somite was taken as an indication of attainment of full sexual maturity. After ascertaining full maturity in the maturation tank, the animals were transferred to spawning tanks maintained in the Regional Centre. When a spawn was detected, the viable eggs were counted using aliquot samples (Browdy and Samocha, 1985 b). The period taken for attaining ovarian maturation by each prawn was recorded as the latency period. Animals were sacrificed on reaching full maturation or after 10 days of experiment whichever was earlier. The moult stage was recorded and the gonad of each prawn was weighed for determination of the gonadosomatic index (GSI).

5.2 Effect of CNS extract administration

In order to assess the impact of neurosecretory activity present in the brain, the extract of suboesophageal and thoracic ganglia of *P. semisulcatus*, was administered to immature females. Experimental conditions and source of animals were similar as described in the above experiments. Ten immature females in intermoult or early premoult stages were collected and divided into 2 experimental groups of 5 animals each.

Prawns in group Va were injected with 0.2 ml of crustacean saline and was treated as the control. Each animal in group Vb was administered 0.2 ml of the ganglionic extract (ICNS equivalent) through the first abdominal somite using a hypodermic syringe. Prawns were injected only once to avoid excessive handling stress. An aqueous extract of the brain, suboesophageal and thoracic ganglia of one prawn was taken as ICNS equivalent. Freshly dissected ganglia from mature female prawn were macerated in cold crustacean saline and centrifuged at 3000 rpm for 10 minutes. The supernatant thus obtained was used for injections. Prawns were monitored daily for signs of gonadal development. After an experimental period of 10 days, all animals were sacrificed and their GSI was recorded.

5.3 Effect of UV radiation

With a view to understand the effect of ultraviolet radiation on ovarian maturation in *P. semisulcatus*, experimental work was attempted in the laboratory. Immature prawns collected and maintained alive in the laboratory tank in same manner as already explained, were subjected to the UV experiment. For this purpose, the ultraviolet radiation was set up in a closed rectangular wooden box (Fig. 43b). The front side of the wooden box was provided with a sliding shutter. In the middle of this shutter, a rectangular small glass viewer was fitted to see the condition of the experimental material inside the box which the UV rays were passed through prawns. After opening

the wooden shutter with the UV lamp in switched off condition, a 100 l capacity fibreglass rectangular tank was inserted into the UV box and fresh filtered sea water was filled up. After ascertaining that all the conditions were suitably set to conduct the experiment, one female prawn in the intermoult stage was introduced into the rectangular fibreglass tank provided with good aeration. The UV rays exposed for 12 hours in a day time on to the experimental prawn. Daily 50% of water was replaced with filtered fresh sea water. The prawn was fed with fresh meat and the uneaten food and faecal matter were siphoned out twice daily. Temperature, pH and salinity were maintained at the same level as described earlier. A total of 4 prawns were subjected to UV radiation experiment.

CHAPTER III

REPRODUCTIVE BIOLOGY

RESULTS

Study of the reproductive biology of penaeid prawns assumes considerable importance not only to understand their reproduction potential in capture fisheries but for the development and maintenance of broodstock in hatchery operations as well. A knowledge on various aspects such as maturation process, environmental influences on maturation, egg production potential, endocrinological control of reproduction etc. is an essential prerequisite for successful broodstock development. This information is also essential for microlevel understanding of the physiological events taking place in the gonads as well as other related body structures during the different phases of gonadal maturation. Several authors have focused attention on these aspects on species of cultivable penaeids in the Indo-Pacific region, as elaborated in an earlier chapter, and it could be seen that most of these investigations pertain to species of Genus *Penaeus*. Noteworthy feature generally observed in the reproductive biology of penaeid prawns is the formation of spermatophore to function as a store house of spermatozoa produced by the mature male, which during mating is implanted onto the thelycum of female in order to facilitate fertilization. The formation of this sperm packet in the vas deferens and its insemination morphology have received greater interest in recent years in view of its importance in artificial insemination and hybridization programme (Subramoniam 1993). The spermatophore shows remarkable variability in morphology and their complexity is closely related to the nature of thelycum (Bauer, 1986). Current research on shrimp maturation has focused on understanding the basic mechanism of egg yolk protein synthesis or vitellogenesis. Within the family Penaeidae, there is significant variation in the pathways of vitellogenesis. Characteristic of the quality and quantity of egg yolk proteins that the females incorporate into the eggs may have a direct and measurable effect on larval survival (Quackenbush, 1991).

In India, studies on reproductive biology are of only general nature in most of the commercial species of penaeids. Among those who have contributed substantially to this aspect on *Penaeid* group are Menon (1957), Subrahmanyam (1963), Rao (1967), Rao (1968), George and Rao (1968) and Thomas (1974, 1975). In *P. semisulcatus*, only limited information is available, mainly from the works of Thomas (1974, 1975) about two decades back. In the present study, attempts have been made to delineate the structure of male and female reproductive systems, maturation process, gonadosomatic and hepatosomatic indices at different maturity stages, fecundity and spawning.

Description of reproductive system

In general, all the members of Natantia exhibit a common structural pattern of the reproductive system although minor variations occur among the different generic groups (Dall *et al.*, 1990). As a rule, sexes are separate, and instances of complete hermaphroditism (Kagwade, 1981) or protandric hermaphroditism (Rasmussen, 1953; Horsted and Smidt, 1956; Butler, 1964) have been reported in some of the caridean shrimps. In those animals which are diocious, the male genital ducts open on the inner surfaces of the coxae of the fifth pair of pereiopods, and the oviducts open on the third pairs of pereiopods. In each case, the genital ducts descend vertically from the gonads, which are situated in the dorsum, between the heart and major arteries and the gut (Dall *et al.*, 1990). A characteristic feature in penaeid prawn is the presence of secondary sexual characters (external genitalia) such as petasma and appendix masculina in male and thelycum in female besides the usual internal reproductive organs. The petasma is the modified endopods of the first abdominal appendage or pleopods fused together medially on attainment of maturity. Generally, in species of genus *Penaeus*, this is a simple, open and pod-like structure which is used for the transference of spermatophores during copulation to the female (Dall *et al.*, 1990). The appendix masculina is a small structure found on the endopod of the second pair of pleopods and is believed to assist the petasma in the transference of spermatophore to the female. In

PLATE 4

A. A fully developed thelycum in impregnated (→) condition.

B. A male prawn with fully formed petasma in ventral view.

PLATE 4

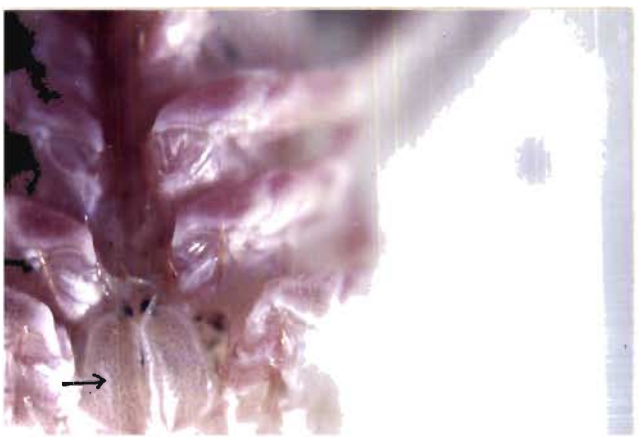
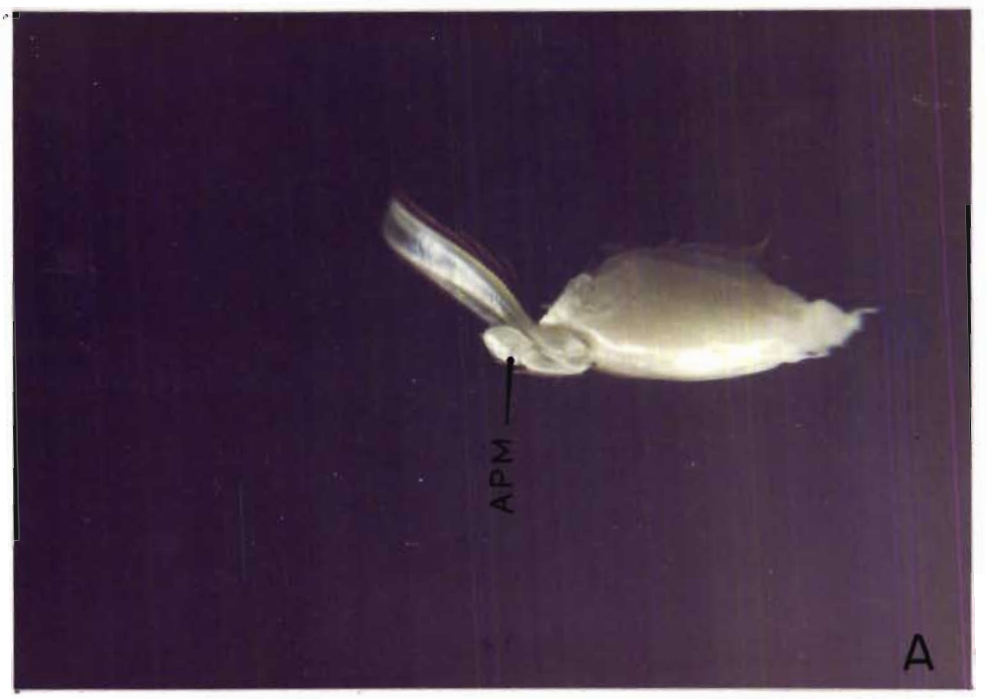


PLATE 5

A. Second pleopod of adult male showing appendix maculina (APM) and the endopod.

B. An extruded spermatophore showing different parts (Photographed without putting in water).

PLATE 5



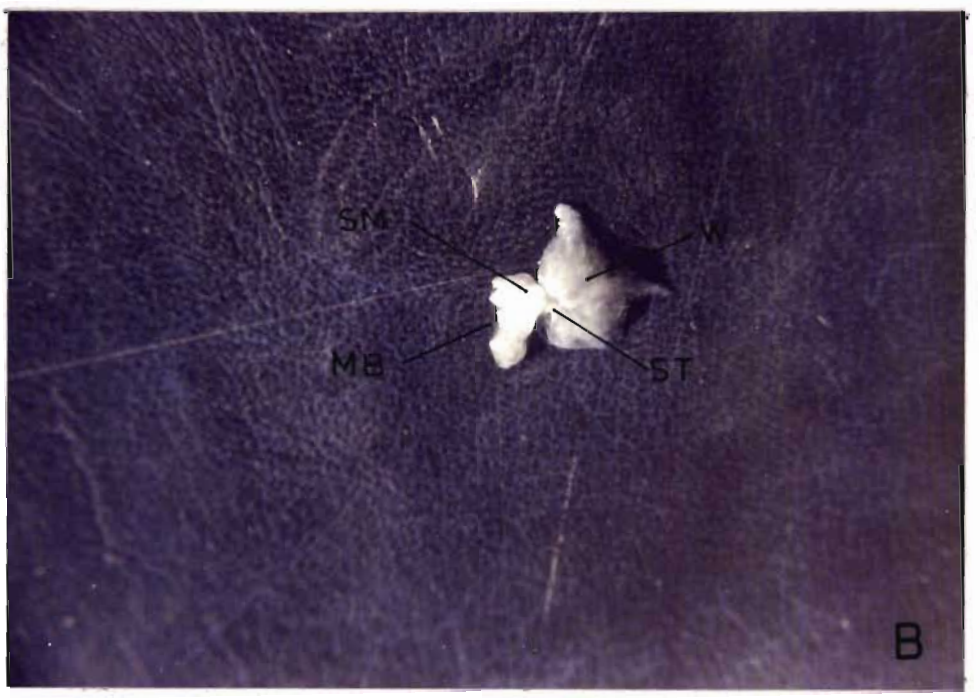
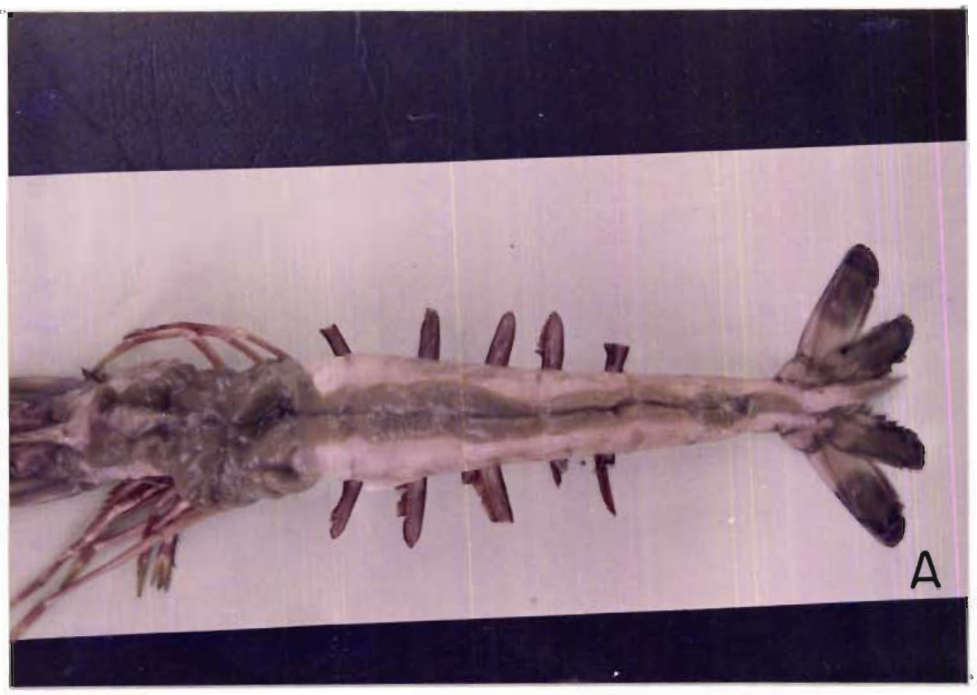


PLATE 6

A. A dissected out fully mature ovary.

B. Lateral view of anterior part of mature ovary showing the oviduct (OVD).

PLATE 6



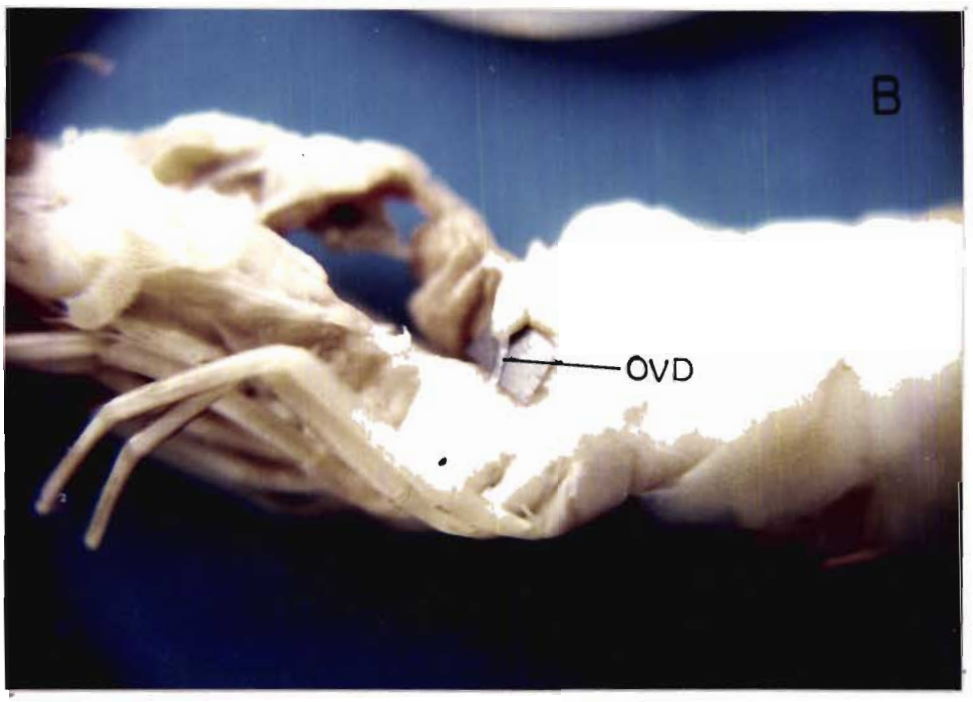
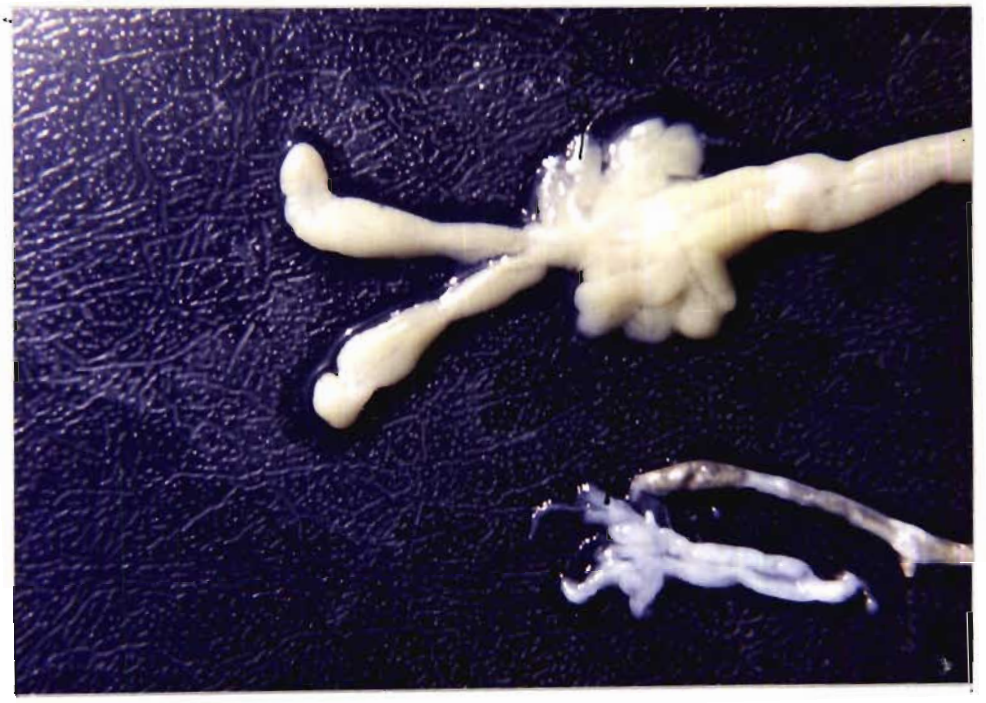


PLATE 7

A. Dissected out immature (IMO) and early maturing (EMO) ovaries.

B. Late maturing ovary, dorsal view.

PLATE 7



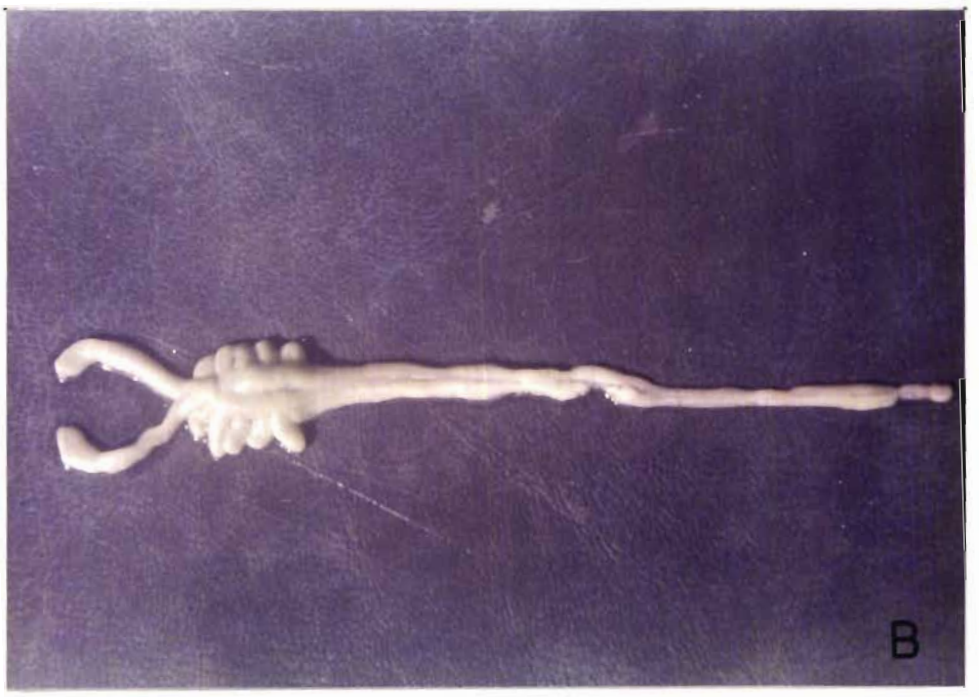
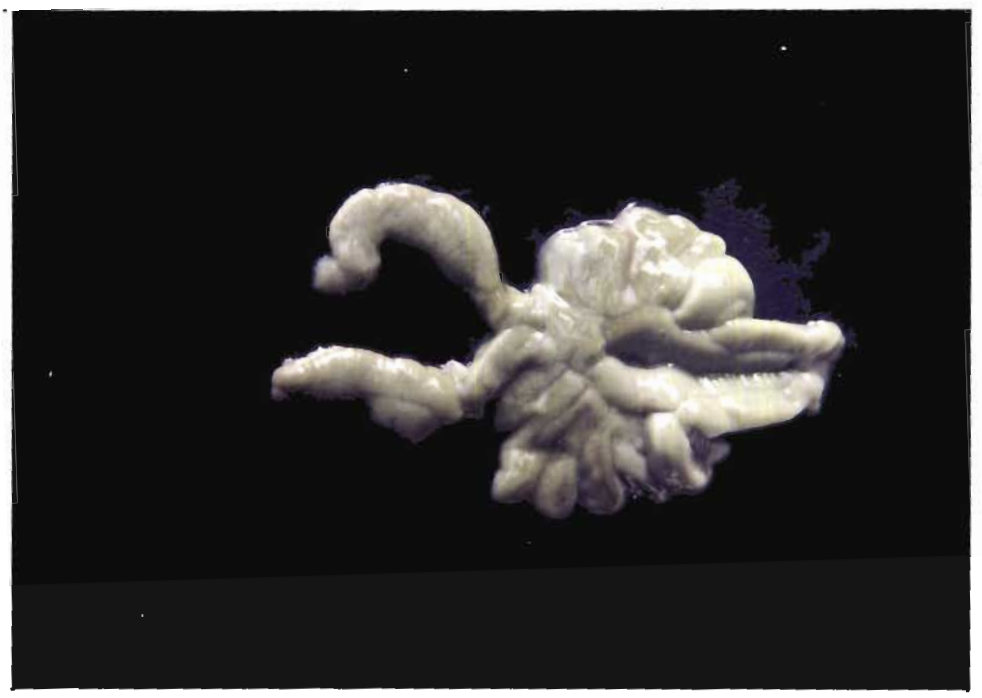


PLATE 8

A. Dorsal view of anterior part of fully mature ovary.

B. Ventral view of the anterior part of fully mature ovary.

PLATE 8



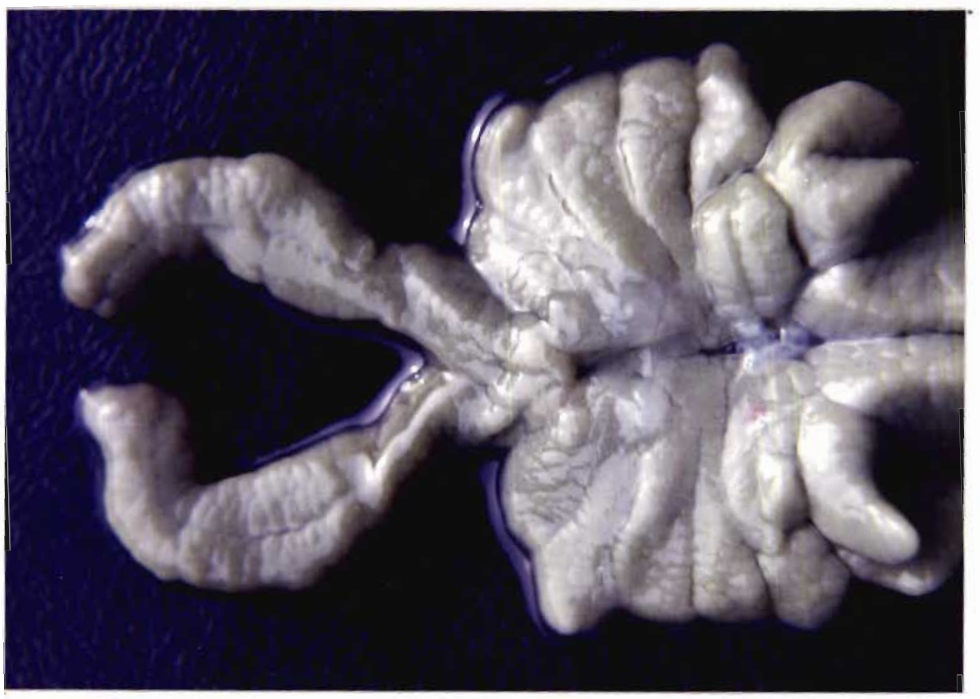
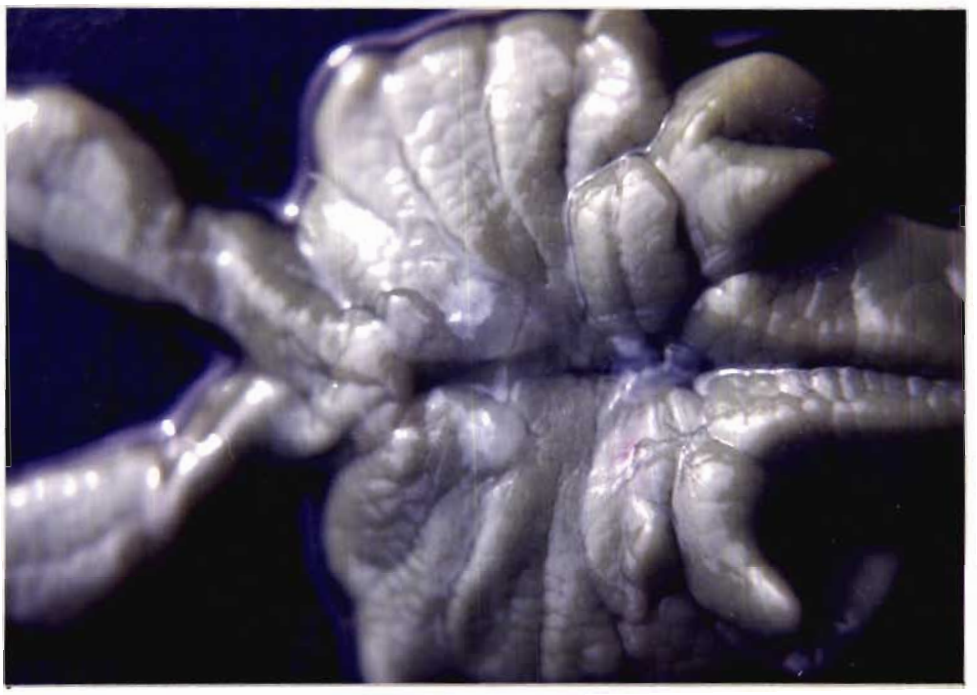


PLATE 9

A. Highly enlarged view of anterior part of gravid ovary showing the hexagonal/oval shaped blocks of mature ova.

B. Partially spent ovary, dorsal view.

PLATE 9



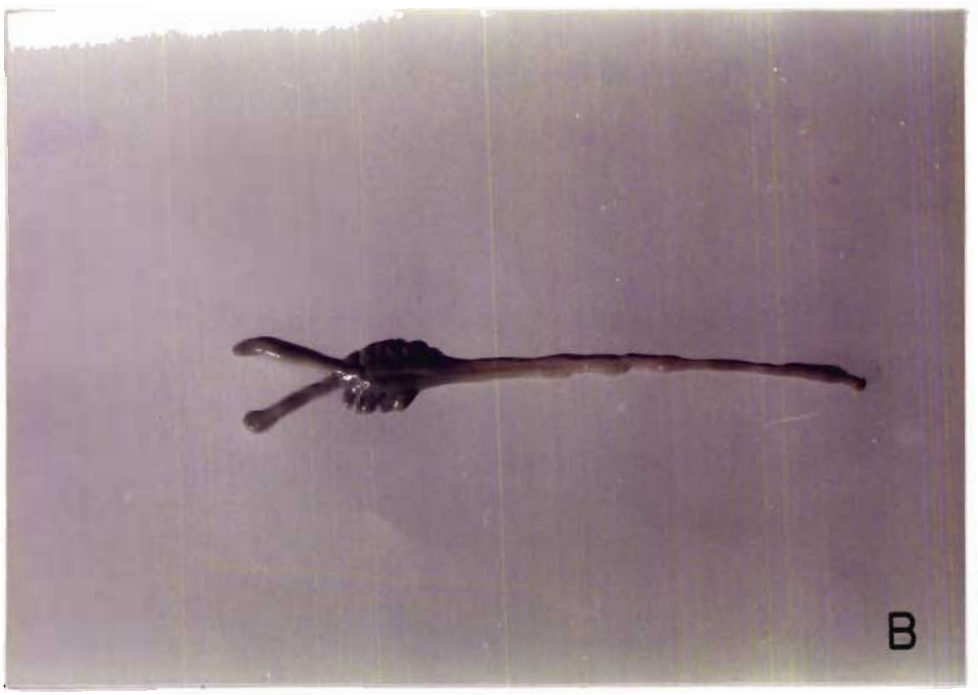
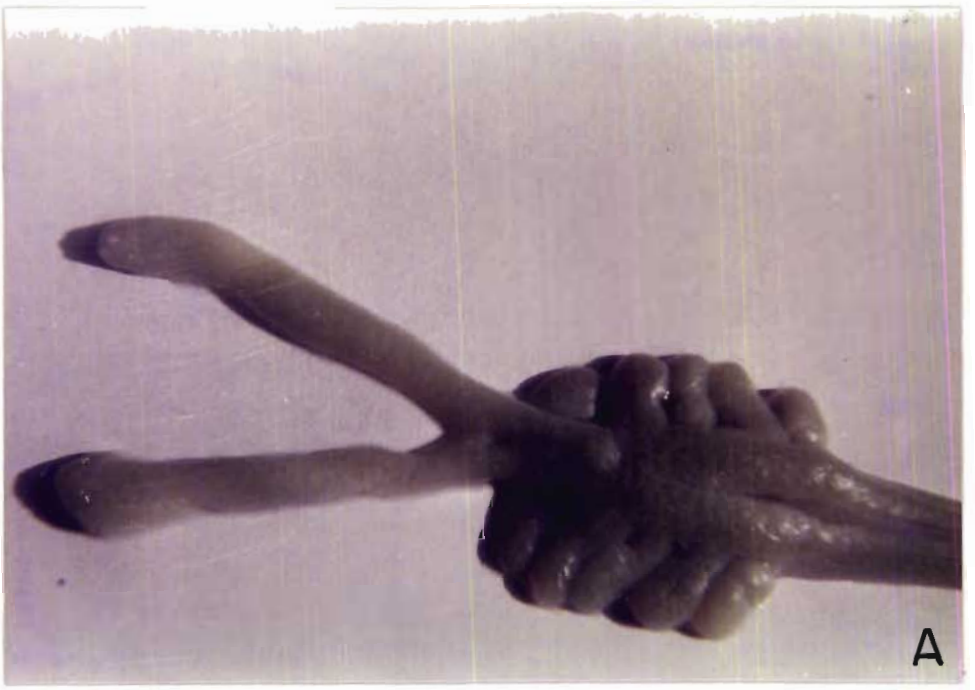


PLATE 10

A. Anterior part of partially spent ovary, dorsal view enlarged.

B. Spent ovary, dorsal view.

PLATE 10



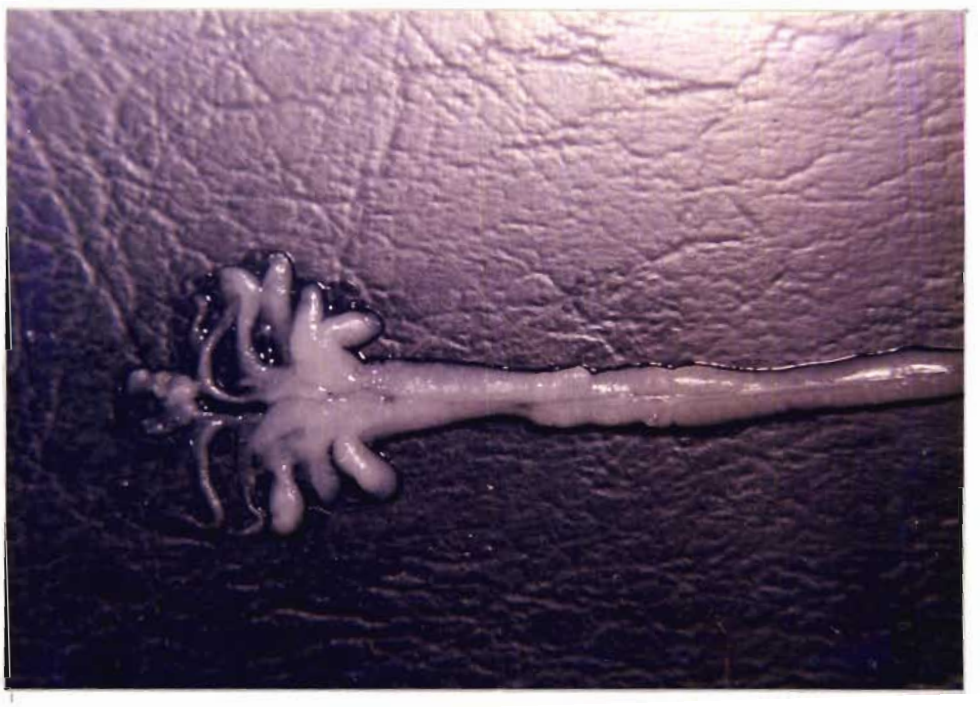
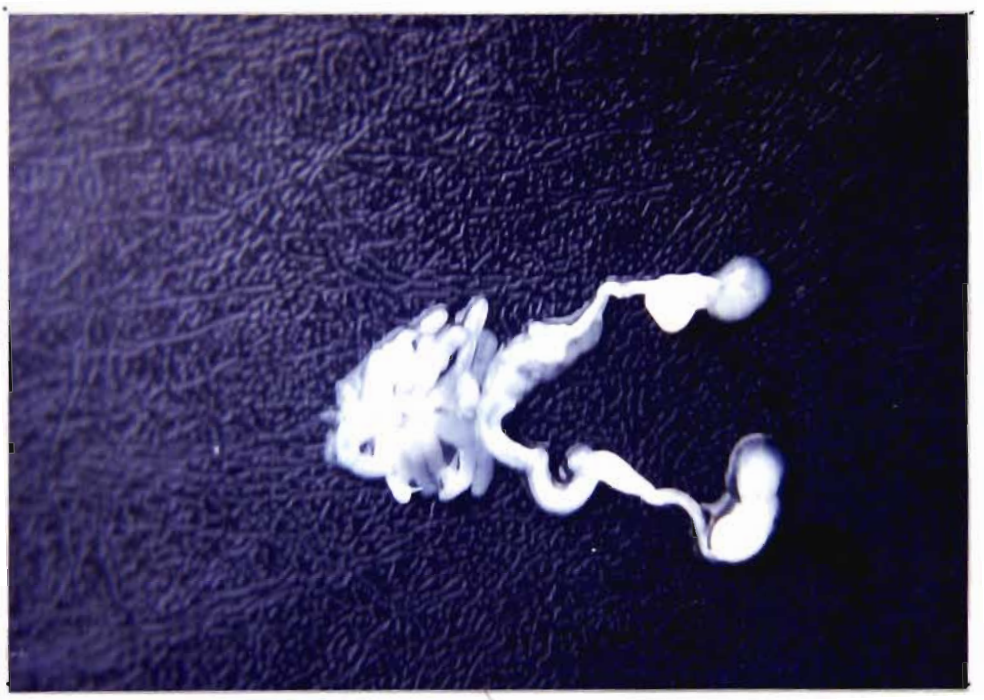


PLATE 11

A-B. A dissected out internal male reproductive organ in fresh condition.

PLATE 11



P. monodon, Motoh (1981) reports that appendix masculina probably acts as an unzipping trigger to separate the petasmal halves during copulation. The thelycum (which is the seminal receptacle) of the female consists of modified sternal plates of the seventh and eighth thoracic somites. The thelyca range from simple to complex open depressions ("open" thelyca), to paired pouches, often with single or double covers ("closed" thelyca). In *Penaeus*, the thelycum is often with median protuberance consisting of an anterior process and a posterior process on the seventh thoracic sternite and an open or usually with two lateral plates covering or almost covering the eighth thoracic sternites (Perez-Farfante, 1969). Thelycum is associated with spermatophore attachment or storage.

The reproductive system of penaeid prawns have been described by a number of workers like King (1948), Cummings (1961), Subrahmanyam (1965), Tuma (1967), Motoh (1978), Motoh and Buri (1980) and Mohamed (1989) for species of *Penaeus*, Tirmizi and Javed (1976) and Vasudevappa (1992) for species of *Metapenaeus*.

The description of the various components of male and female reproductive systems of *P. semisulcatus* noted during the present investigation is given below.

External Genitalia

Petasma

The adult petasma (Plate 4b) is pod-like and formed of the characteristic two lateral lobes and one thin median lobe. The distomedian projection of median lobe reaching as far as or slightly overhanging costae of lateral lobes. Ventral costae of lateral lobes curved distally, their free borders minutely serrated near apex; outer surface of lateral lobes minutely tuberculate without forming distinct rows or areas of spines. In a prawn of 165 mm total length, it is about 7 mm in length and 6 mm in width.

Appendix masculina

The appendix masculina (Plate 5a) is almost oval in shape with the outer margin fringed with numerous spines.

Thelycum

In adult female, the thelycum (Plate 4a) is closed type with large lateral plates, the median margin of which forming tumid lips; anterior process raised and obtusely angled apically; posterior process convex, and posteriorly lying hidden between lateral plates.

Morphology of male reproductive system

The internal male reproductive organ (Fig.2) consists of paired testes, vas deferens and terminal ampoules. Each testis has an anterior lobe and seven lateral lobes located in the cardiac region dorsal to hepatopancreas, which is unpigmented and translucent in mature condition. The right and left testes are connected anteriorly at the base of the anterior lobe. On each side, the lateral lobes of testis are connected to each other at their inner aspect and this leads to the vas deferens. The anterior lobe is comparatively larger in relation to most of the lateral lobes.

The vas deferens has three portions: a short and narrow proximal vas deferens (PVD), a thick and large glandular mid vas deferens (MVD) and a long and narrow distal vas deferens (DVD). The PVD is non-glandular and it serves to transport the mature sperm from testis to MVD through peristaltic movement as judged from histological details described in Chapter III. The MVD has an ascending limb and a descending limb which continues as the DVD. The descending limb of MVD gets constricted and becomes narrow to continue as the DVD. Histologically, it has been observed that the inner walls of the MVD and DVD are lined with glandular epithelial cells.

The terminal ampoule or ejaculatory duct is a bulbous structure at the end of the vas deferens. Embedded in the coxal muscle, it opens through the genital pore at the base of fifth pereiopod. The walls of the terminal ampoule are highly muscular, which helps in easy discharge of the spermatophore.

Morphology of female reproductive system

The internal female reproductive organ (Fig.4) consists of a pair of ovaries and a pair of oviducts. In the mature prawn, the ovary extends from the base of rostrum to the end of sixth abdominal segment occupying almost the entire cephalothoracic cavity and abdomen dorsally. Each ovary consists of three regions, a long and thick anterior lobe that reaches almost to the base of rostrum, eight stout middle or lateral lobules lying above the hepatopancreas and ventral to the pericardiac chamber, and a long posterior lobe that runs the length of the abdomen and lies dorso-lateral to the intestine (Plate 7a). Among the eight middle lobes, six are dorso-laterally placed (Plate 8a & Fig.4) and the other two directed ventrally on the posterior region (Plate 8b & 9a). The left and right posterior lobes lie jointly throughout their length. The ovarian halves are connected together at the base of the anterior lobes and at the distal end of the posterior lobes. The oviduct is a narrow tube originating from the tip of the sixth lateral lobules (Plate 6b) (counted from anterior to posterior) and terminating at the genital pores on the coxa of the third pereiopod.

Maturation process

For identical ages, male prawns are generally smaller than females as in most other penaeid species. Attainment of maturity also takes place comparatively earlier than in females. The attainment of maturation could be judged externally to some extent from the degree of petasmal development. In the early juvenile stages (90 mm), the petasma is represented by only a pair

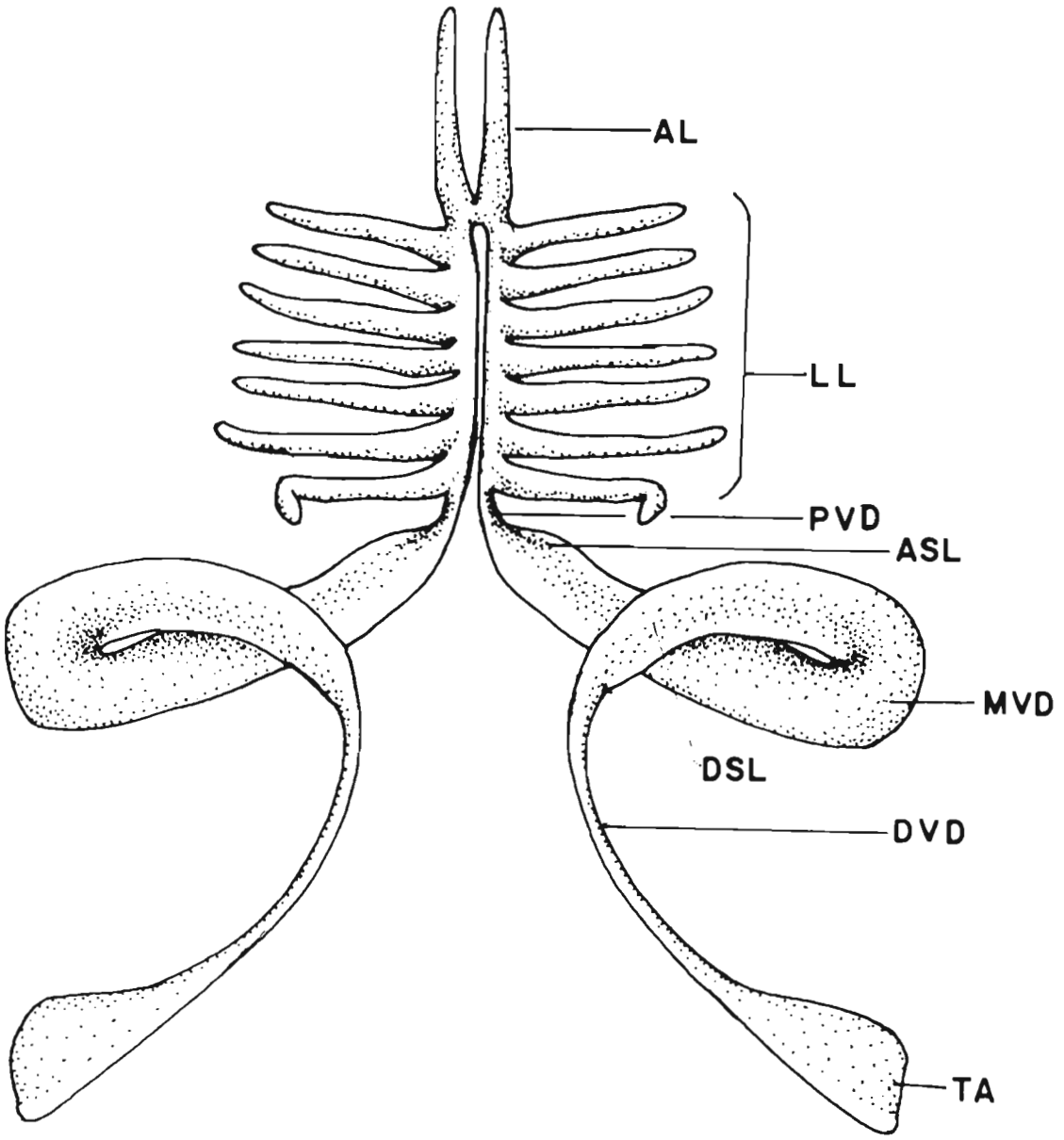


FIG. 2. Male reproductive system of *P.semisulcatus* de Haan. AL-anterior lobe, LL-lateral lobes, PVD-proximal vas deferens, ASL-ascending limb, MVD-middle vas deferens, DSL-descending limb, DVD-distal vas deferens, TA-terminal ampoule.

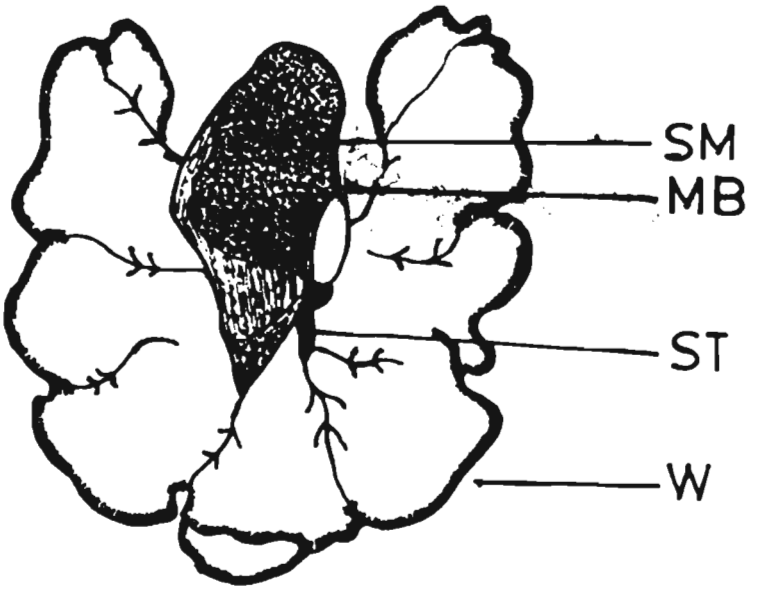


FIG. 3. An extruded spermatophore. SM-sperm mass, MB-main body, ST-stalk, W-wing.

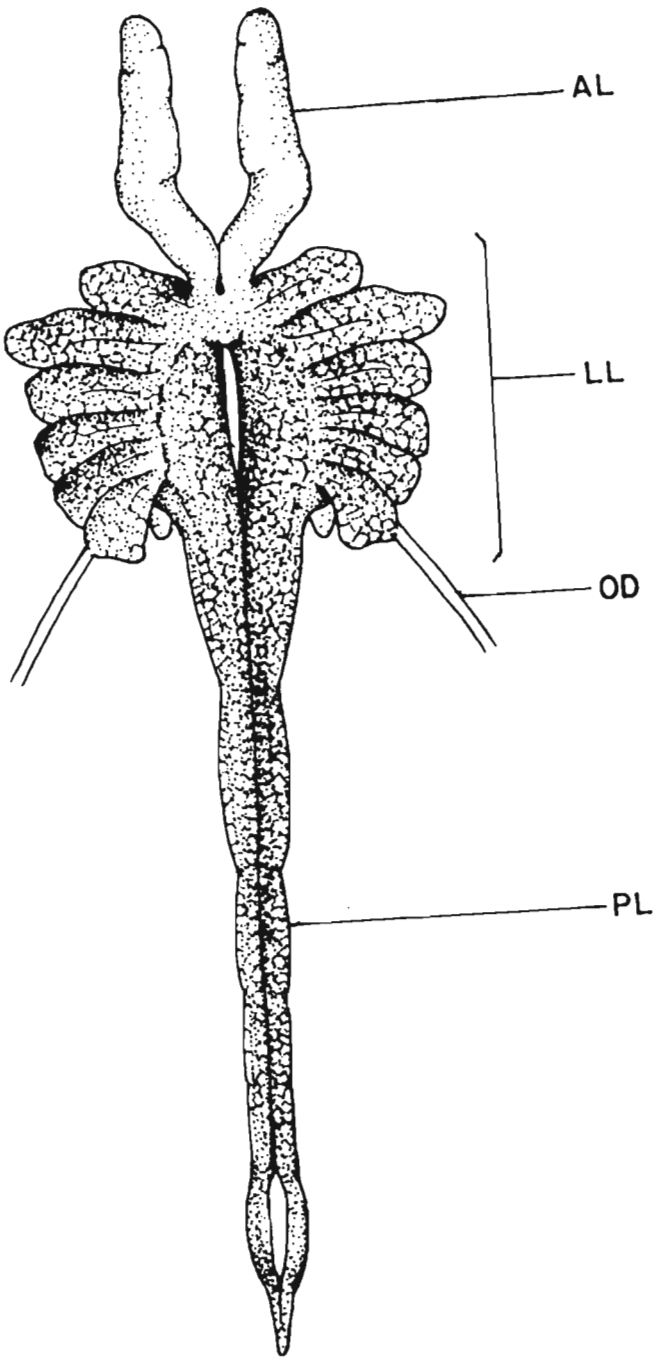


FIG. 4. Female reproductive system of *P.semisulcatus*. AL-anterior lobe, LL-lateral lobules of middle lobe, OD-oviduct, PL-posterior lobe.

of endopodal buds on the first pair of pleiopods which remain independent. As the prawn grows, these buds enlarge and join together along the dorsomedian margin to form the single structure. Initially, after fusion, the petasma is simple and membranous. Later on, it hardens and takes the specific shape. The initiation of gonadal maturation coincides with the petasmal fusion. As the hardening of petasma progresses, the gonad also undergoes a series of structural and functional changes leading to the production of spermatozoa and the formation of spermatophores. Once the petasma takes the final form and is fully hardened, spermatogenesis and spermatophore formation are very active. Changes also take place in the growth of vas deferens and terminal ampoule. The gradual size increase and the visibility of terminal ampoule to the naked eye at the base of the fifth walking leg could be taken as an external indication of the advancement of maturation of testis.

Based on the nature of petasma, structural changes of the internal reproductive organs, size of the terminal ampoule and the presence of spermatophore at the base of the 5th walking leg, the following four maturity stages have been distinguished in male.

Stage I - Immature

Testis is seen as a thin thread-like body, transparent, four lobes discernible in fused manner on either side; vas deferens uncoiled, long and almost uniformly narrow except for the proximal half which is slightly enlarged; terminal ampoule simple, transparent and oval in shape; petasmal halves separate.

Stage II - Early maturing

Testis transparent, the four lobes fairly developed and separate; vas deferens slightly coiled, differentiated into three thicker regions, middle vas deferens moderately dilated; distal vas deferens long and narrow; terminal

ampoule fairly enlarged and muscular; petasmal endopodites fused, membraneous and easily separable.

Stage III - Late maturing

All testis lobes well developed, but narrow and translucent; vas deferens more coiled and clearly differentiated into proximal, medial and distal regions, ascending and descending limbs of middle vas deferens fully differentiated; terminal ampoule dilated into a bulbous structure, more muscular and visible to naked eye at the base of fifth walking leg; petasma firmly fused and hardened. Spermatophore formation initiated in vas deferens and sometimes in terminal ampoules.

Stage IV - Mature or ripe

Testes fully developed, lobes thicker and opaque; vas deferens in typical adult form; terminal ampoule with fully formed spermatophore, the same clearly visible through exoskeleton; petasma fully formed in all respects.

At the mature or ripe stage, the prawn is ready to transfer the spermatophores onto the thelycum of the female. The animal remains in this stage even after mating, as evidenced by the presence of spermatophore in the vas deferens and as such, there is no spent stage in males after attaining maturity.

The smallest prawn with the endopodites of first pair of pleopods in united and hardened condition together with visible spermatophore at the base of fifth walking leg measured 115 mm.

Table 1. Variations in gonadosomatic index during different maturity stages of *P. semisulcatus*

Stages	TL	CL	GSI
I	117.80 ± 10.96	44.60 ± 3.78	0.6534 ± 0.275
II	118.04 ± 14.78	43.50 ± 7.53	1.5411 ± 1.317
III	144.43 ± 11.44	52.71 ± 5.38	2.0453 ± 0.890
IV	156.71 ± 17.67	57.45 ± 8.07	8.3838 ± 1.761
V	152.42 ± 10.51	55.33 ± 4.23	1.9401 ± 0.636

Table 1a. Variations in hepatosomatic index during different maturity stages of *P. semisulcatus*

Stages	TL	CL	GSI
I	117.80 ± 10.96	44.60 ± 3.78	1.9416 ± 0.545
II	115.64 ± 10.33	42.13 ± 4.21	2.1574 ± 0.653
III	143.60 ± 11.76	51.20 ± 5.21	2.6644 ± 0.774
IV	160.33 ± 15.40	61.00 ± 5.59	2.4983 ± 0.601
V	143.00 ± 17.39	51.67 ± 11.5	2.0621 ± 1.000

Table 2. Variations in fecundity of *P. semisulcatus* at fully mature stage

Total length (mm)	Carapace length (mm)	Total weight (g)	Total gonad weight (g)	Fecundity
135	45	20.0	1.995	1,29,675
136	48	26.2	3.802	2,09,110
136	51	29.0	8.365	5,43,725
143	55	26.2	1.332	90,000
143	55	26.2	1.332	86,580
150	51	31.3	6.907	4,48,955
152	58	30.8	4.867	3,16,355
154	55	36.5	7.460	4,10,300
154	55	36.5	7.460	4,84,900
156	60	38.6	9.792	6,35,180
156	60	38.6	9.792	6,36,480
157	57	32.0	7.401	4,81,065
159	60	36.3	9.534	6,19,710
159	60	38.6	9.792	6,35,180
160	63	34.0	10.700	6,95,500
169	48	50.3	3.347	1,50,615
177	66	51.0	9.792	6,36,480
179	48	51.3	8.524	5,54,060
179	48	51.3	8.524	5,54,060
192	62	58.3	10.963	7,12,595

Table 3. **Monthly sex ratio of *P. semisulcatus* in the trawling grounds of Palk Bay during 1991 and 1992.**

Months	1991			1992		
	Total No.of Prawns	Females %	Males %	Total No.of Prawns	Females %	Males %
January	-	-	-	118	58.5	41.5
February	112	51.8	48.2	91	73.6	26.4
March	108	60.2	39.8	85	51.8	48.2
April	118	57.6	42.4	91	47.2	52.7
May	255	70.2	29.8	63	57.1	42.9
June	218	60.6	39.4	68	58.8	41.2
July	159	54.7	45.3	92	46.8	53.2
August	147	65.3	34.7	100	57.0	43.0
September	093	59.1	40.9	63	63.5	36.5
October	136	51.5	48.5	78	73.0	26.7
November	55	89.1	10.9	-	-	-
December	110	70.0	30.0	43	41.9	58.1

Table 4. Monthly sex ratio of *P. semisulcatus* in the trawling grounds of Gulf of Mannar during 1991 and 1992.

Months	1991			1992		
	Total No.of Prawns	Females %	Males %	Total No.of Prawns	Females %	Males %
January	-	-	-	132	62.1	37.9
February	235	66.0	34.0	106	49.1	50.9
March	139	82.7	17.3	092	50.0	50.0
April	206	69.4	30.6	121	62.8	37.2
May	341	66.0	34.0	58	46.5	53.5
June	121	49.6	50.4	NF	-	-
July	148	70.9	29.1	NF	-	-
August	169	55.6	44.4	NF	-	-
September	74	68.9	31.1	NF	-	-
October	77	62.3	37.7	-	-	-
November	107	50.5	49.5	103	52.4	47.6
December	138	54.8	45.2	146	65.1	34.9

NF denotes no fishing.

Table 5. Monthly percentage distribution of maturity stages of *P. semisulcatus* females in Palk Bay during 1991 and 1992.

Months	Total Nos.	1991					Total Nos.	1992				
		I	II	III	IV	V		I	II	III	IV	V
January	-	-	-	-	-	-	69	43.5	14.5	4.3	15.9	21.7
February	58	72.4	8.6	12.1	6.9	-	67	34.3	16.4	3.0	20.9	25.4
March	65	7.7	26.1	6.1	60.0	-	44	4.5	4.5	4.5	59.1	27.3
April	68	8.8	8.8	2.9	47.0	32.4	43	11.6	20.9	6.98	44.2	16.3
May	179	18.4	10.6	2.2	44.7	24.0	36	11.1	5.5	8.3	36.1	38.9
June	132	31.8	22.7	1.5	21.2	22.7	40	27.5	-	2.5	50.0	20.0
July	87	26.4	9.2	2.3	35.6	26.4	43	20.9	11.6	16.3	25.6	25.6
August	96	13.5	15.6	6.3	38.5	26.0	57	28.1	5.2	10.5	28.1	28.1
September	55	18.2	23.6	5.4	25.4	27.3	40	5.0	5.0	27.5	40.0	22.5
October	70	62.8	24.3	1.4	4.3	7.1	57	29.8	21.1	12.3	19.3	17.5
November	49	-	2.0	44.9	44.9	-	-	-	-	-	-	-
December	77	24.7	37.6	6.5	19.5	11.7	18	5.5	16.7	22.2	38.9	16.7

Table 6. **Monthly percentage distribution of maturity stages of *P. semisulcatus* females in Gulf of Mannar during 1991 and 1992.**

Months	Total Nos.	1991					Total Nos.	1992				
		I	II	III	IV	V		I	II	III	IV	V
January	-	-	-	-	-	-	82	50.0	18.3	3.7	10.9	17.1
February	155	9.7	20.0	7.7	56.8	5.8	52	13.4	23.1	7.7	40.4	15.4
March	115	13.0	29.6	6.1	51.3	-	46	26.1	6.5	10.9	36.9	19.6
April	143	12.6	20.3	2.1	44.7	20.3	76	25.0	40.8	13.2	7.9	13.1
May	225	2.7	4.4	1.3	72.4	19.1	27	29.6	22.2	14.8	22.2	11.1
June	60	1.7	13.3	1.7	60.0	23.3	NF	-	-	-	-	-
July	105	1.0	3.8	1.9	77.1	16.2	NF	-	-	-	-	-
August	94	1.1	13.8	3.2	58.5	23.4	MF	-	-	-	-	-
September	51	-	-	3.9	64.7	31.4	NF	-	-	-	-	-
October	48	39.6	16.7	14.6	25.0	4.1	NF	-	-	-	-	-
November	54	53.7	7.4	11.1	14.8	13.0	54	74.1	11.1	1.8	3.7	9.3
December	74	51.3	31.1	2.7	4.1	10.8	95	64.2	22.1	4.2	-	9.5

NF denotes no fishing

Table 7. Monthly variations in water temperature, salinity and dissolved oxygen at Palk Bay during the period of study

Months	Mean water temperature °C	Mean salinity (‰)	Mean dissolved oxygen (ml/l)	Mean pH
1991				
February	28.65	29.71	3.67	8.2
March	30.68	30.48	3.21	8.3
April	32.29	32.93	3.24	8.4
May	31.72	33.12	2.75	8.4
June	30.10	35.78	2.98	8.3
July	-	-	-	-
August	-	-	-	-
September	-	-	-	-
October	27.80	35.6	3.38	8.3
November	26.50	33.60	3.07	8.2
December	26.40	30.32	2.75	8.2
1992				
January	-	-	-	-
February	-	-	-	-
March	-	-	-	-
April	29.10	34.05	2.48	8.2
May	28.60	34.15	2.65	8.2
June	27.80	34.45	3.15	8.3
July	27.40	34.56	4.06	8.3
August	27.80	35.72	3.52	8.3
September	27.20	35.62	5.43	8.3
October	27.60	35.55	4.40	8.3
November	26.80	33.60	3.93	8.2
December	25.00	28.10	4.73	8.0

Table 8. Monthly variations in phosphate, silicate, nitrate, and nitrite at Palk Bay during the period of study

Month	Mean Phosphate µg-atm/l	Mean Silicate µg-atm/l	Mean Nitrate µg-atm/l	Mean Nitrite µg-atm/l
1991				
February	0.29	6.91	0.39	0.02
March	0.04	8.29	0.53	0.03
April	0.10	6.23	0.85	0.01
May	0.07	5.77	0.43	0.01
June	0.08	6.16	0.57	0.02
July	-	-	-	-
August	-	-	-	-
September	-	-	-	-
October	0.25	9.0	0.75	0.05
November	0.05	7.0	1.12	0.06
December	0.12	11.25	1.68	0.15
1992				
January	-	-	-	-
February	-	-	-	-
March	-	-	-	-
April	0.20	18.00	1.25	0.05
May	0.23	21.00	1.81	0.08
June	0.33	10.50	1.58	0.03
July	0.08	14.02	2.02	0.06
August	0.09	13.17	2.50	0.07
September	0.10	11.00	1.50	0.07
October	0.05	6.50	1.50	0.02
November	0.07	6.50	0.75	0.02
December	0.17	12.00	2.37	0.01

Table 9. Monthly variations in water temperature, salinity dissolved oxygen and pH at Gulf of Mannar during the period of study

Month	Mean water temperature °C	Mean salinity (‰)	Mean dissolved oxygen (ml/l)	Mean pH
1991				
February	28.22	29.85	3.60	8.2
March	30.35	32.53	3.43	8.3
April	32.88	33.65	3.68	8.4
May	32.77	33.69	2.49	8.4
June	30.13	35.69	3.95	8.4
July	29.53	35.68	2.87	8.3
August	30.43	35.67	3.54	8.4
September	30.90	36.21	3.42	8.4
October	29.87	35.56	2.98	8.3
November	26.90	35.20	2.15	8.2
December	27.00	32.00	4.31	8.2
1992				
January	25.60	32.63	3.79	8.2
February	25.30	33.02	3.16	8.2
March	27.20	33.12	2.89	8.3
April	32.00	34.15	3.51	8.2
May	31.00	34.43	3.30	8.2
June	30.50	35.22	3.17	8.3
July	30.80	35.25	3.82	8.3
August	31.00	35.72	4.93	8.4
September	30.20	35.84	5.10	8.4
October	30.70	36.42	5.11	8.4
November	28.10	34.60	3.86	8.3
December	26.45	28.63	4.96	8.2

Table 10. Monthly variations in phosphate, silicate, nitrate and nitrite at Gulf of Mannar during the period of study

Months	Mean phosphate (µg-atm/l)	Mean salinity (µg-atm/l)	Mean nitrate (µg-atm/l)	Mean nitrite (µg-atm/l)
1991				
February	0.28	5.66	0.49	0.03
March	0.04	8.72	0.57	0.01
April	0.05	5.05	0.64	0.01
May	0.07	5.20	0.60	0.07
June	0.09	5.68	0.44	0.02
July	0.08	7.43	1.27	0.03
August	0.18	6.33	0.52	0.03
September	0.14	8.12	1.12	0.09
October	0.10	5.00	1.02	0.30
November	0.12	8.00	2.25	0.08
December	0.05	9.00	2.25	0.06
1992				
January	0.11	14.00	1.50	0.08
February	0.06	18.50	1.29	0.08
March	0.06	14.00	1.00	0.08
April	0.20	15.00	1.50	0.06
May	0.23	14.73	1.12	0.04
June	0.22	22.28	2.06	0.10
July	0.06	23.03	1.37	0.23
August	0.07	13.74	1.39	0.02
September	0.07	14.00	1.13	0.07
October	0.07	6.00	1.39	0.04
November	0.07	6.75	1.28	0.03
December	0.06	5.61	0.73	0.02

Female

Generally prawns of about 110 mm in total length and above exhibit indication of ovarian maturation. These prawns immediately after moulting, get impregnated with spermatophore by a mature male. In the impregnated condition, the sternal plates of thelycum are soft and the sperm mass deposited in the thelycum is visible externally as white patch on the lateral plates (Plate 4a). The commencement of maturation can be recognised externally by careful observation of the ovarian changes through the arthropodial membrane between cephalothoracic and abdomen. In the initial stage of maturation, the small anterior lobes and the lateral lobules of the middle lobe begin to enlarge in size. The anterior lobes grow almost upto the base of the rostral crest, while the lateral lobules occupy the available space in the cephalothoracic cavity. The posterior lobe increases in thickness.

Based on the size, shape and colour of the ovary, microscopic details of ova and gonado-somatic indices, the following five maturity stages are recognised.

Stage I - Immature

Ovary (Plate 7a)is smaller than adjacent gut; thin, translucent or white in colour, unpigmented. Ova uniformly small and transparent with clear nuclei. Ova diameter 32-56 µm; gonado-somatic index 0.65 ± 0.27.

Stage II - Early maturing

Diameter of ovarian lobes (Plate 7a) almost same to or slightly larger than that of the adjacent gut; anterior and middle lobes are not visible through exoskeleton, first three pairs of middle lobes joined together and remaining two separated, ovary colour light yellow, nucleus faintly visible through yolk

granules in the ovum; ova diameter 96-120 µm; gonadosomatic index 1.54 ± 1.32.

Stage III - Late maturing

Ovary (Plate 7b) visible through exoskeleton; diameter of lobe is much larger than that of gut; anterior and middle lobes completely formed; all middle lobes independent; ovary colour varies from light to olive green; ovum opaque, packed with yolk granules and nucleus not visible, ova diameter 144-288 µm; gonadosomatic index 2.04 ± 0.89.

Stage IV - Mature

Ovary (Plates 8a,b & 9a) clearly visible through exoskeleton; diameter of lobe is very much larger than that of gut; anterior, middle and posterior lobes fully developed and filling almost the entire space inside the body cavity; ovary colour dark green or greenish brown; surface of ovum appears corrugated due to cortical rods; ova diameter 288-416 µm; gonadosomatic index 8.38 ± 1.76.

Stage V - Spent

Ovarian lobes (Plates 9b & 10a,b) flaccid; ovary colour varying from cream to light yellow and diameter of lobe larger than gut and majority of ova same as in stage I, mature reabsorbing ova present in varying numbers; gonado-somatic index 1.94 ± 0.69.

Size frequency distribution of ova

Information on the size profile of ova in different stages of development in the ovary is an important indicator of the animal's spawning

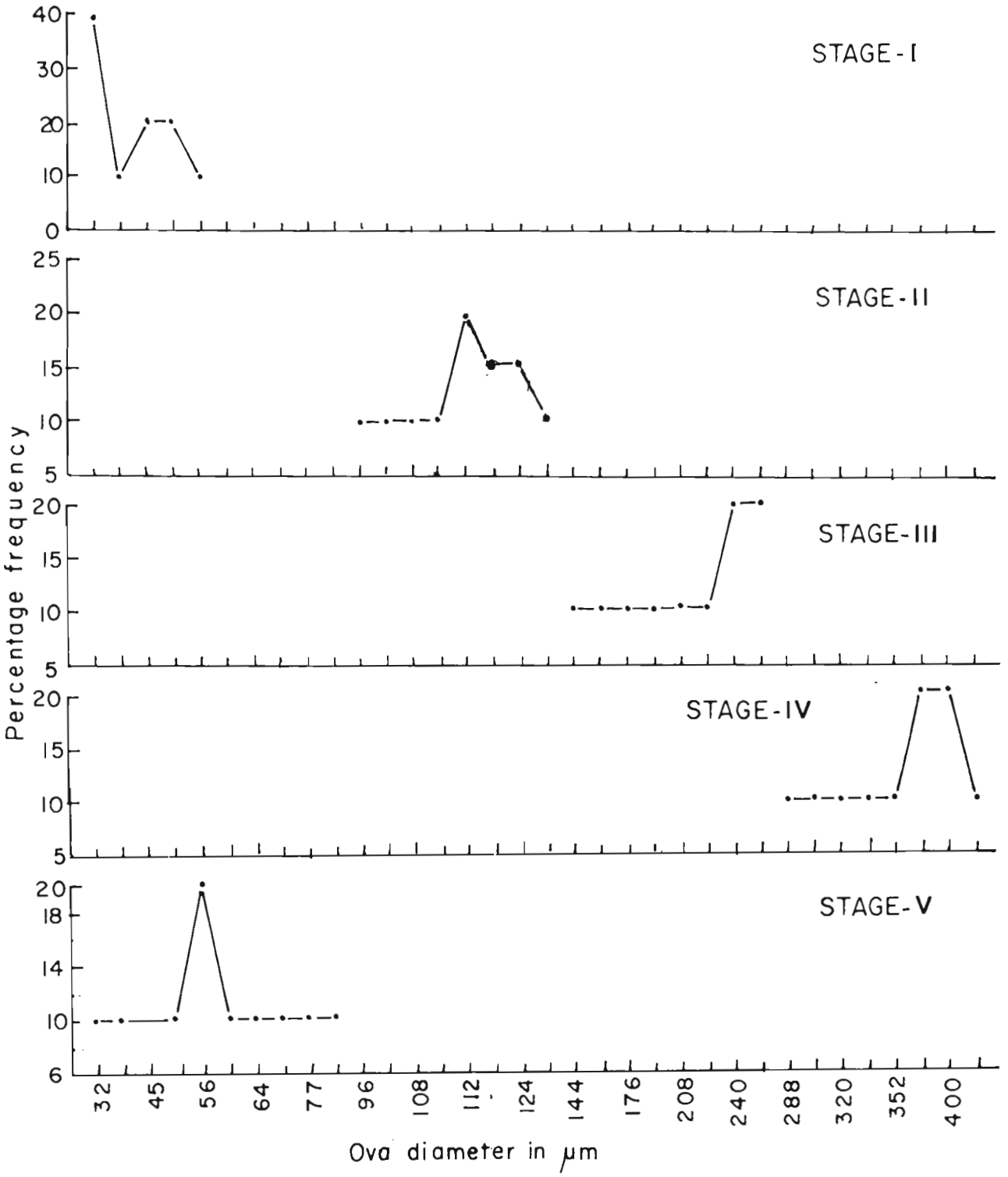


FIG. 5. Size frequency distribution of maturing ova in *P.semisulcatus*

habits. The mean sizes of ova from ten prawns in each stage of maturity and their frequency distribution are depicted in Fig. 5.

In immature stage, the developing ova had a size range of 32-56 µm with a mode at 32 µm (40%). The early maturing stage of ova was found to be in the size range of 96-128 µm with a mode at 112 (20%), while the late maturing stage of ova had a size range of 144-256 µm with a mode at 240-256 µm (40%). In the ripe or mature stage (stage IV), the ovary was found to be entirely filled with fully mature ova ranging in size 288-416 µm with a mode at 368-400 µm (40%). In the spent stage, the group of mature ova totally disappeared and majority of the existing ova had a size range of 32-80 µm, a condition more or less similar to immature stage.

The variation in the range of ova diameter at different maturity stages are summarised as follows:

Immature stage	32-56 µm
Early maturing stage	96-128 µm
Late maturing stage	144-256 µm
Mature stage	288-416 µm
Spent stage	32-80 µm

Size at first maturity

The length at first maturity has been estimated for female based on 614 prawns in the size range 100-240 mm TL caught from the Gulf of Mannar during the year 1991. These measurements were grouped into 10 mm length intervals and the percentage of individuals in fourth stage of maturity was calculated against each of the class intervals to estimate the minimum size of maturity at 50% level. The size at cumulative 50% maturity was found to be 145.07 mm total length (Fig.6). The smallest observed size of female in fully mature condition of gonad measured 110 mm total length.

Gonado-somatic index (GSI)

Study of changes in the ratio of gonad size to body weight known as the gonadosomatic index is considered as an alternate method of assessing gonadal development. In female prawn, the GSI can be closely correlated to the visual evaluation of maturity stages and it provides a quantitative measurement of gonadal development. The present study was conducted on female prawn using 5 immature (117.8 ± 10.96 mm), 44 early maturing (118.045 ± 14.58 mm), 7 late maturing (144.43 ± 11.44 mm), 22 mature (156.77 ± 17.67 mm) and 12 spent (152.42 ± 10.51 mm) stages. The mean values of GSI along with the standard deviations are given in Table 1a and the trend depicted in Fig.7.

The mean GSI was found to be 0.65 in stage I which showed a gradual increase to 1.54 in stage II and 2.04 in stage III. The highest value of GSI, 8.38 ± 1.76 was observed in stage IV followed by a steep decline in the spent stage (stage V) with a mean value of 1.94.

Hepatosomatic index (HSI)

Generally, biochemical synthesis in gonads is preceded by storage of organic reserves in other tissues, hence sizes of the storage organ would increase prior to gonadal development then decrease during gonadal development as nutrients are mobilized from storage organ to gonads (Castille and Lawrence, 1991). A study of hepatosomatic index would throw light on the extent of interrelationship between the main storage organ and the gonad during the different phases of maturation. Keeping this in view, an attempt has been made to study the hepatosomatic index in *P. semisulcatus* using 5 immature (117.8 ± 10.96 mm), 39 early maturing (115.64 ± 10.33 mm), 5 late maturing (143.6 ± 11.76 mm), 6 mature (160.33 ± 15.40 mm) and 6 spent (143 ± 17.39 mm) stages. The mean values of HSI along with the standard deviations are given in Table 1b and the trend depicted in Fig.7. The HSI

recorded for immature prawn was 1.94 ± 0.545. A gradual increase for the same could be noticed for in the early maturing and late maturing stages with the values as 2.16 ± 0.653 and 2.66 ± 0.774, respectively. In the mature and spent stages, a successive drop in the values to 2.50 ± 0.601 and 2.06 ± 1.000, respectively was observed.

Fecundity

In egg laying animals, the fecundity or the total number of eggs that could be produced in a single spawn indicates its reproductive potential. In the present study, the ovaries of 18 prawns in fully mature stage (IV) ranging from 135-192 mm TL were examined for fecundity study. The ovary of each animal was dissected out and its weight was recorded to the nearest milligram. A piece of ovary from the middle lobes was separated and weighed. The entire ova in this sample were teased out and taken into a plankton counting chamber and all the mature ova counted using Handling tally counter. The total number of ova in the entire ovary was estimated following the method described in Chapter II. The data obtained are presented in Table 2. The fecundity of individual prawn varied from 86,580 in a prawn of 143 mm TL size to 7,12,595 prawn of 192 mm TL.

Fecundity-total length relationship

The relationship between fecundity and total length of prawn was calculated statistically and the same is expressed as follows;

$$F = 0.0004392 \quad L^{4.07}$$

$$r^2 = 28\%$$

The correlation co-efficient (r^2) was found to be 28%. As indicated in fig.8, the curvilinear relationship considered does not seem to fit the data adequately as indicated by lower r^2 value.

Fecundity-total weight relationship

$$F = 1472.726 \quad W^{1.5451}$$
$$r^2 = 33.5\%$$

The relationship between fecundity and total weight of prawn was calculated statistically as shown above and the correlation coefficient of r^2 was found to be 33.5%. This curvi linear relationship as indicated in the Fig.8 shows that there is not much relationship between fecundity and weight of the prawn.

Fecundity-total gonad weight relationship

$$F = -28691.92 + 67576.48 \text{ TGW}$$
$$r^2 = 99\%$$

The relationship between fecundity and total gonad weight was found to be linear and r^2 was 99%. As evident from Fig.8, there is good relationship between fecundity and total gonad weight of the prawn.

Sex ratio

The distribution of male and female prawns in the exploited population of Palk Bay and Gulf of Mannar was studied from a total of 3266 prawns in 1991 and 1650 prawns in 1992. The monthly distribution of sex ratio in Palk Bay is shown in Table 3 and in the Gulf of Mannar in Table 4. A closer examination of the monthwise distribution of sex ratios in Table 3 would reveal that in most of the months except in April, July and December of 1992, the females predominated over males in Palk Bay, while in 1991 the females dominated to the extent of about 58% in April, 55% in July and 70% in December. The representation of female during the corresponding months of 1992 ranged from 42 to 47% only when compared to male. Similarly, in the Gulf of Mannar, the females showed distinct preponderance over males except

during June 1991 (49.6%) and February (49.1%) and May (46.5%) 1992 when the sex disparity was only marginal. This would indicate that the female dominance in the population of trawling grounds of these two waters is not consistent over different period of the any particular year.

Spawning

Female prawns in all maturity stages described above were encountered in both Palk Bay and Gulf of Mannar. Monthly observations were made on the distribution of different maturity stages of females in the commercial catches at these two fishing grounds during the years 1991 and 1992. In each month, random samples of the female prawns from the trawl catches were examined for the composition of individuals in different maturity stages. A total 936 prawns were studied during 1991 and 514 prawns during 1992 from the Palk Bay, and 1124 prawns during 1991 and 432 prawns during 1992 from the Gulf of Mannar. The monthly distribution of sample size and percentage composition of the different maturity stages monthwise are given in Table 5 for Palk Bay and Table 6 for Gulf of Mannar. It may be seen that females in spawning condition occurred throughout the year in both Palk Bay and Gulf of Mannar. Examination of monthly percentages of fully mature females (stage IV) and the spent ones (stage V) would reveal that the spawning is active in both Palk Bay and Gulf of Mannar with distinct peak spawning periods. In the Palk Bay, peak spawning has been observed during March to May with a minor peak in November during 1991, whereas in 1992, the peak spawning was noticed during March to June with two minor peaks, one in September and the other in December. In the Gulf of Mannar, very high percentage of mature females (58-77%) were recorded during May to September preceded by a minor peak for the abundance of mature prawns during February and March in 1991. In the subsequent year, the mature females were comparatively less represented in the fishery in most of the months when fishing was conducted. As observed in the previous year, a

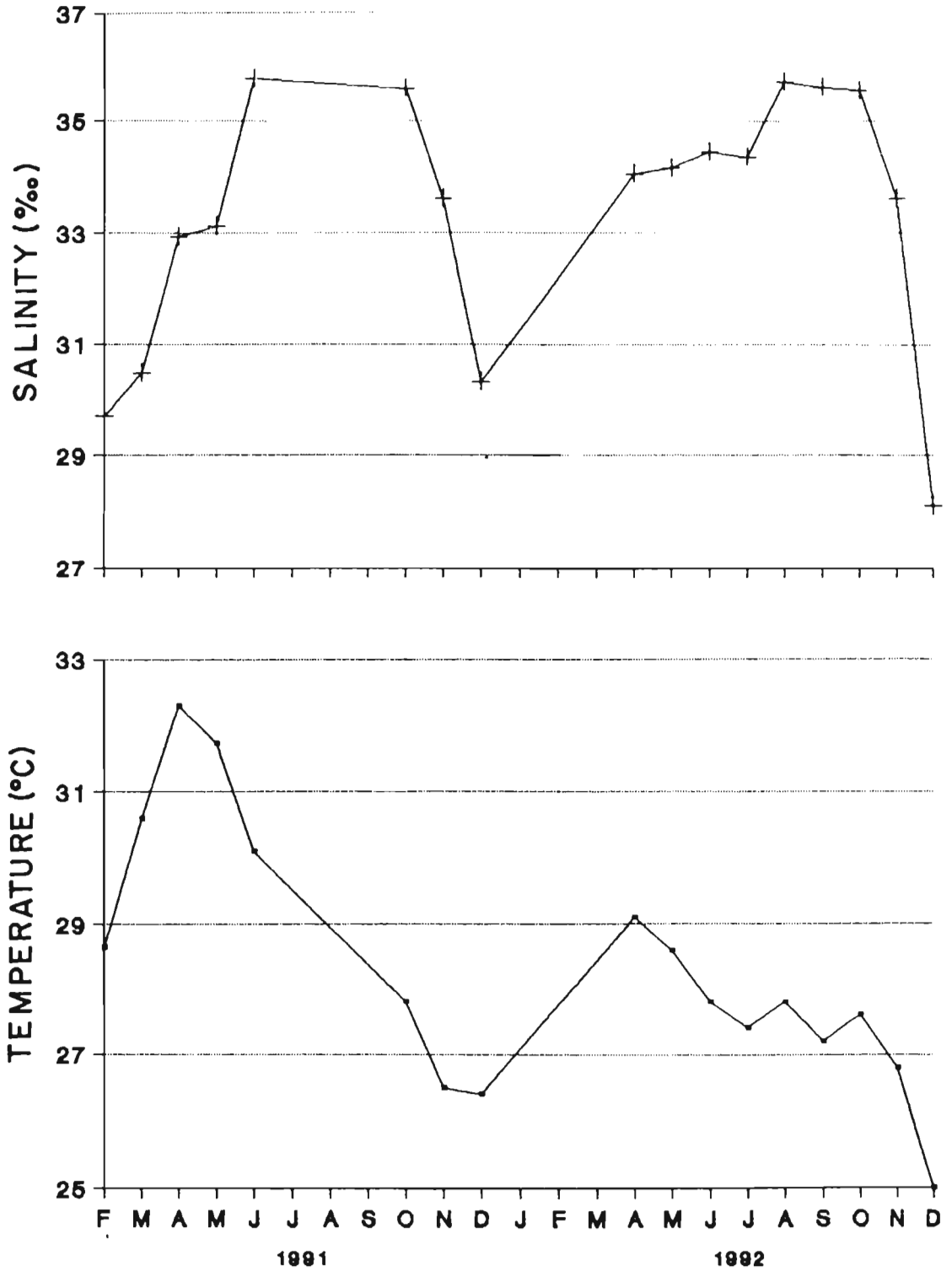


Fig. 9: **Monthly variations in sea water temperature and salinity in the fishing ground of Palk Bay during 1991 to 1992**

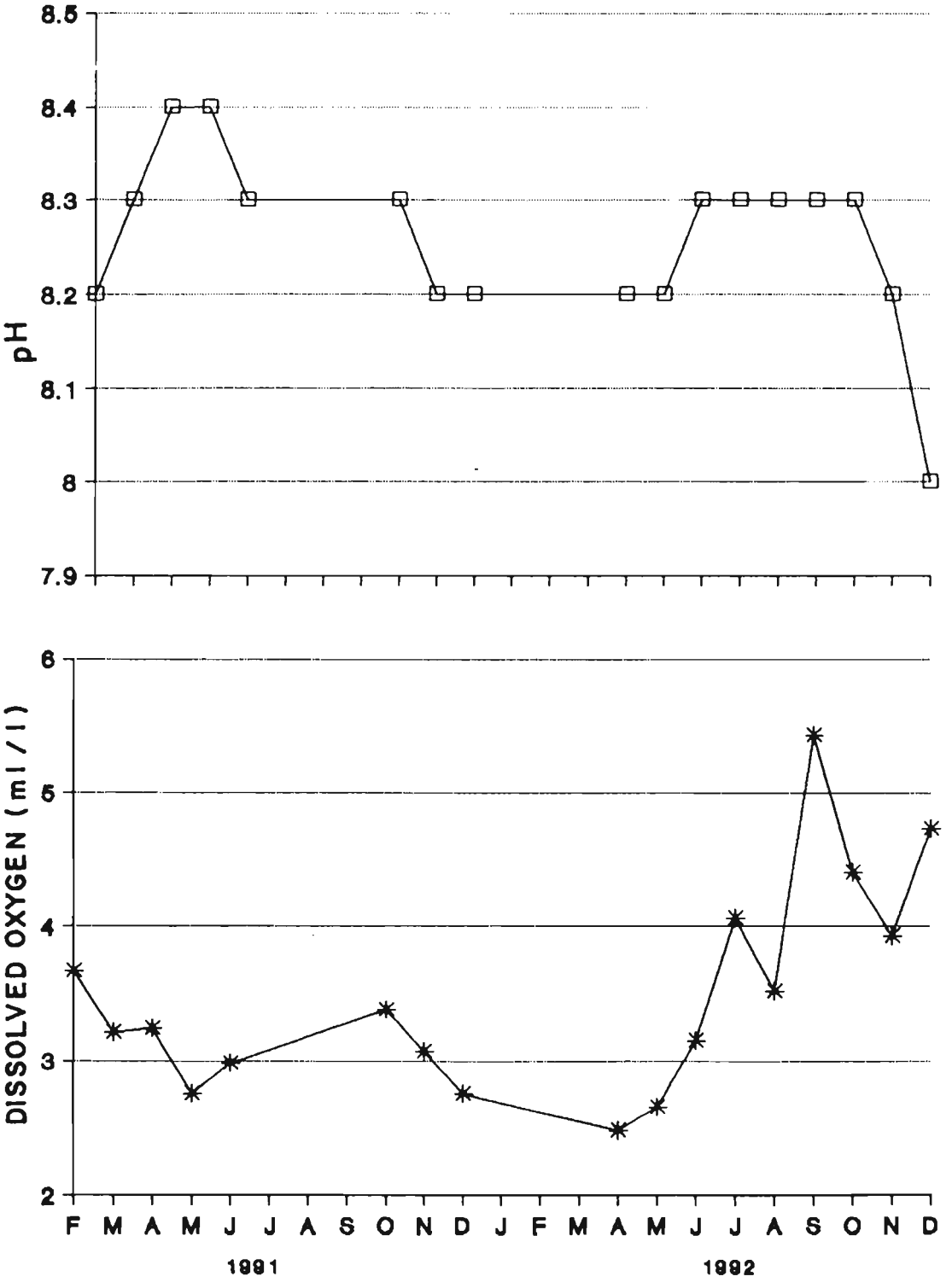


Fig.10: **Monthly variations in Dissolved oxygen and pH in the fishing ground of Palk Bay during 1991 to 1992**

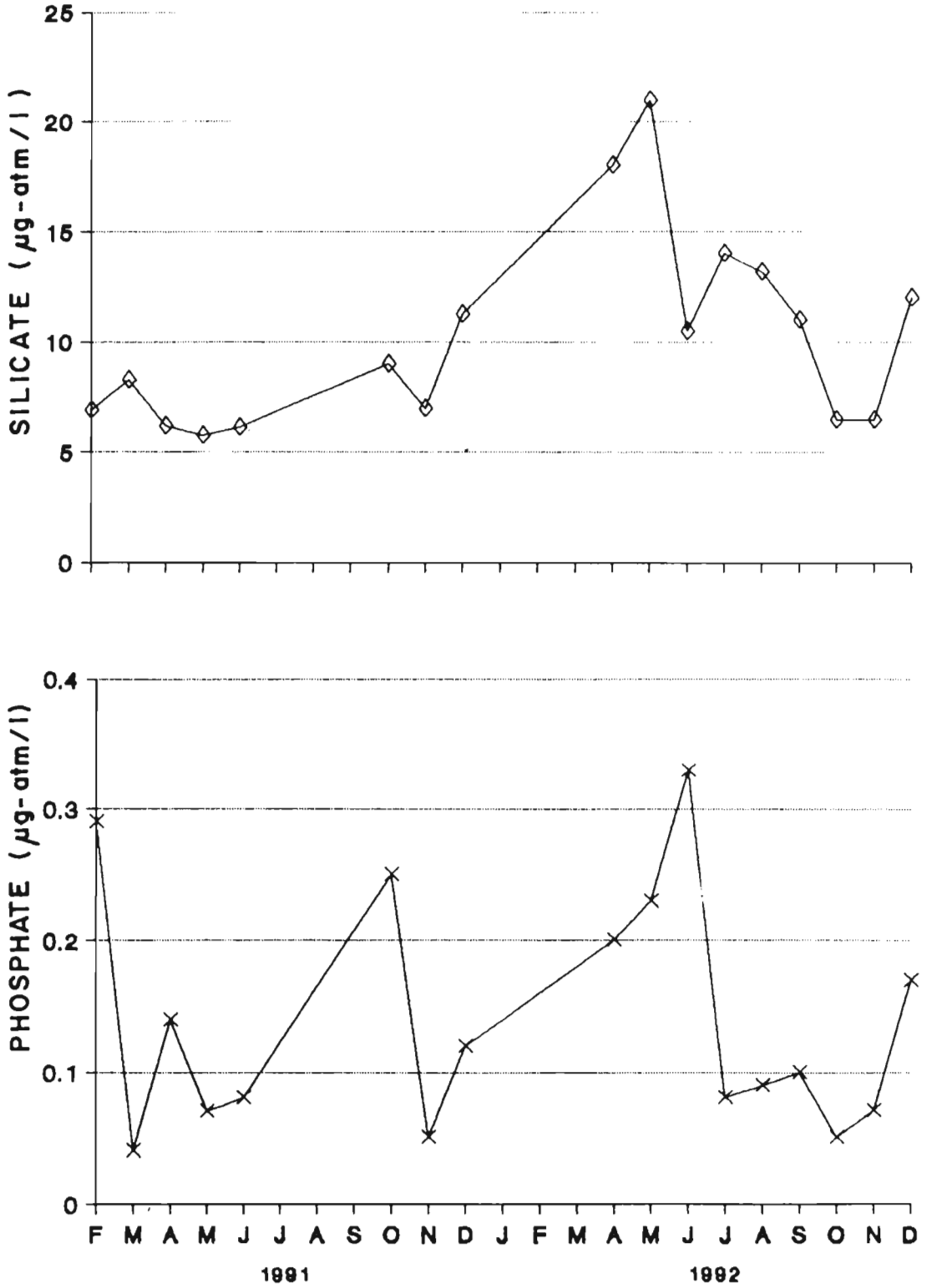


Fig.11: **Monthly variations in phosphate and silicate content of the sea water in the fishing ground of Palk Bay during 1991 to 1992**

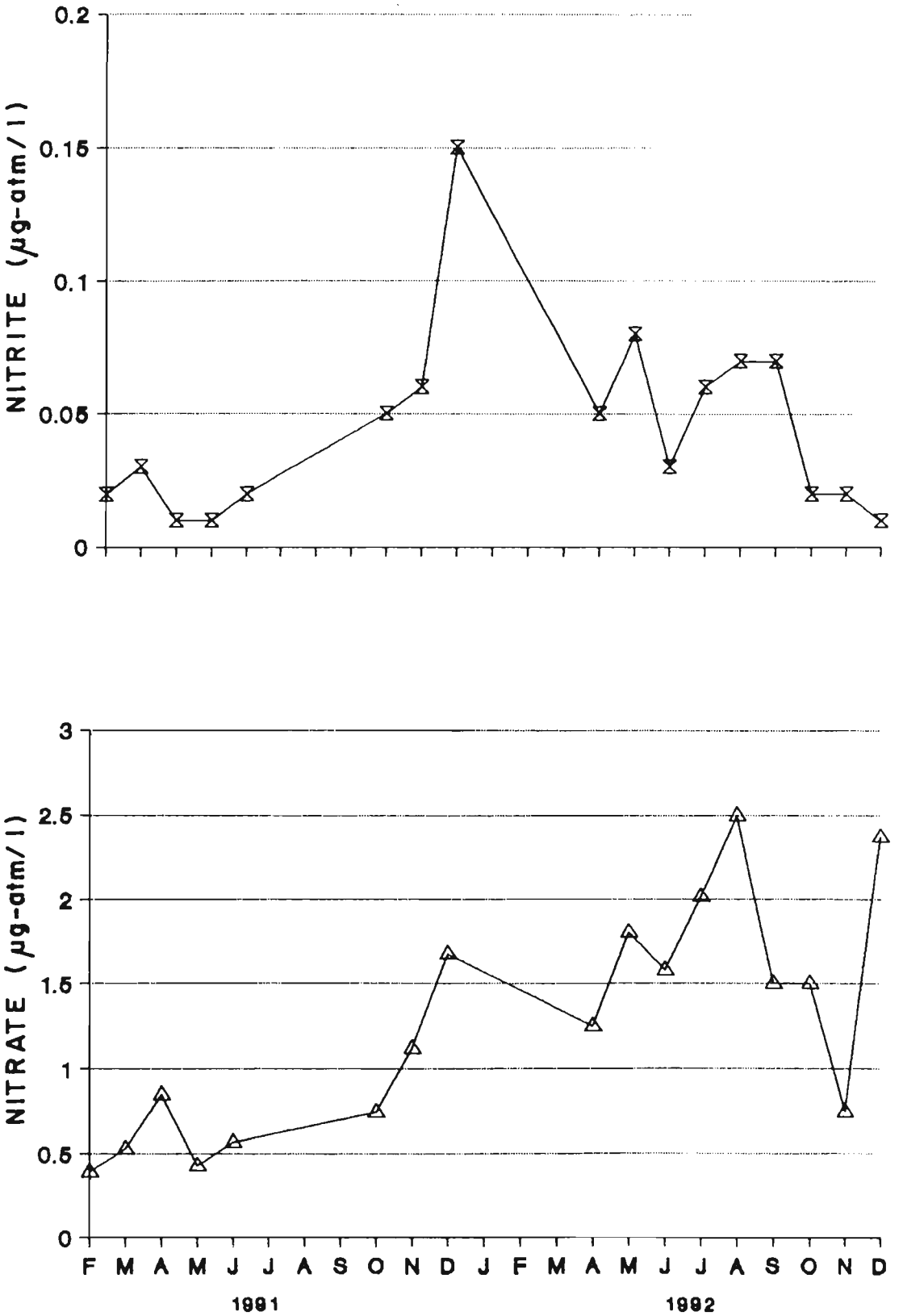


Fig. 12: **Monthly variations in nitrate and nitrite content of the sea water in the fishing ground of Palk Bay during 1991 to 1992**

comparatively higher percentage (37-40%) of gravid females was noticed during February-March.

Influence of physico-chemical parameters of fishing ground on spawning

A close relationship has been observed between the reproductive behaviour of penaeid prawns and the hydrological factors prevailing in the shrimp grounds by a number of workers like Anderson (1956). Eldred *et al.* (1961), Cummings (1961) and Rao (1968). In order to findout any possible relationship between these two in *Penaeus semisulcatus*, hydrological parameters such as water temperature, salinity, dissolved oxygen, pH, phosphate, silicate, nitrate and nitrite of the fishing grounds of Palk Bay and Gulf of Mannar were studied between February 1991 and December 1992 and the mean values recorded for the various parameters are given in Tables 7-10 and their trends shown in Figs.9-16. The mean temperature varied between 25°C in December and 32.9°C in April. A comparison of the temperature values for the two fishing grounds would reveal that relatively higher temperature prevailed in the Gulf of Mannar (25.3-32.9°C) than in the Palk Bay (25.0-32.3°C). In the Palk Bay, higher temperature regime was recorded during March-June (30.1-32.3°C) in 1991 and April-May (28.6-29.1°C) in 1992. In the Gulf of Mannar during 1991, however, the temperature was highest (32.8-32.9°C) during April-May and after a gradual decline in the subsequent period slightly increased and maintained at 29.8-30.9°C during August-October and thereafter gradually declined. In 1992, the highest temperature was recorded in April (32°C) and the lowest (25.3-26.5°C) during January-February and December. In most of the other months, more or less uniform temperature in the range 30-31°C was observed.

The salinity varied between 28.10‰ in December to 35.78‰ in June in the Palk Bay and 28.63‰ in December to 36.42‰ in October in the Gulf of Mannar. In both the fishing grounds, relatively low salinity was observed

during December-January periods. Fairly, higher salinity regimes were noticed during June-October in Palk Bay as well as in the Gulf of Mannar.

The dissolved oxygen showed a range between 2.48 ml/l in April and 5.43 ml/l in September in the Palk Bay and 2.15 ml/l in November and 5.11 ml/l in October in the Gulf of Mannar. In general, the dissolved oxygen values remained to be comparatively high during September-October period.

The mean pH ranged only narrowly in both the fishing grounds, the minimum and maximum values recorded being 8.2 and 8.4, respectively.

The range of mean values of phosphate was 0.04-0.33 µg-atm/l in the Palk Bay and 0.04-0.28 µg-atm/l in the Gulf of Mannar. Comparatively, higher values of phosphate was observed during April-June and August-September.

Mean silicate content ranged between 5.77 and 21.00 µg-atm/l in the Palk Bay and 5.00 and 23.03 µg-atm/l in the Gulf of Mannar. Comparatively, higher silicate values were noticed during April-May and December in the Palk Bay and during June-July and November-December in the Gulf of Mannar.

The nitrate contents ranged between 0.39 and 2.50 µg-atm/l in the Palk Bay and 0.44 and 2.25 µg-atm/l in the Gulf of Mannar. The highest values were recorded in the months of August (2.50 µg-atm/l) in the Palk Bay and November and December (2.25 µg-atm/l) in the Gulf of Mannar.

The nitrite values ranged from 0.01-0.15 µg-atm/l in the Palk Bay and 0.01-0.30 µg-atm/l in the Gulf of Mannar. The highest values were recorded in the month of December (0.15 µg-atm/l) in the Palk Bay and October (0.30 µg-atm/l) in the Gulf of Mannar.

An examination of the monthly trends in the mean values of the different physico-chemical parameters of the two fishing grounds would reveal

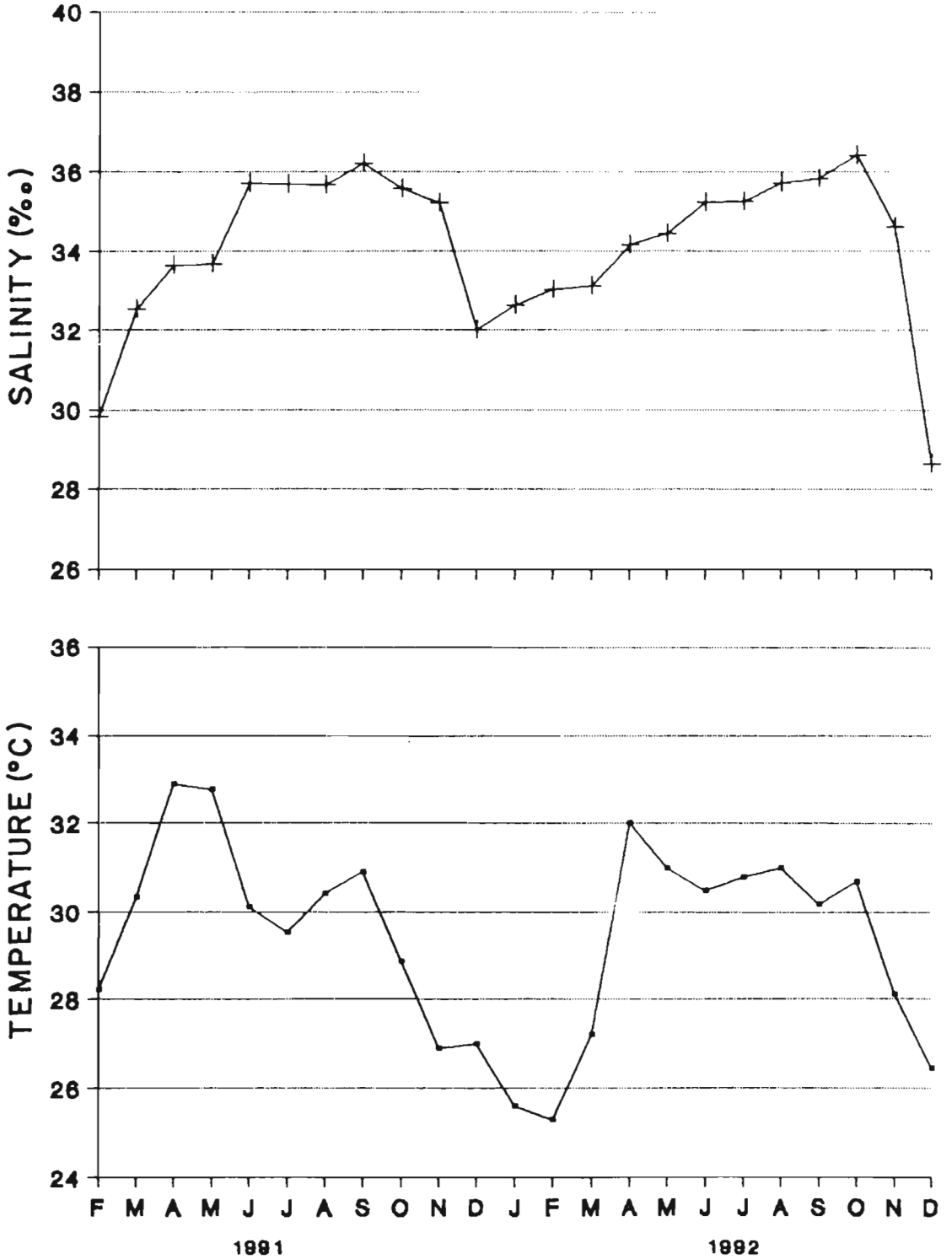


Fig.13: **Monthly variations in sea water temperature and salinity in the fishing ground of Gulf of Mannar during 1991 to 1992**

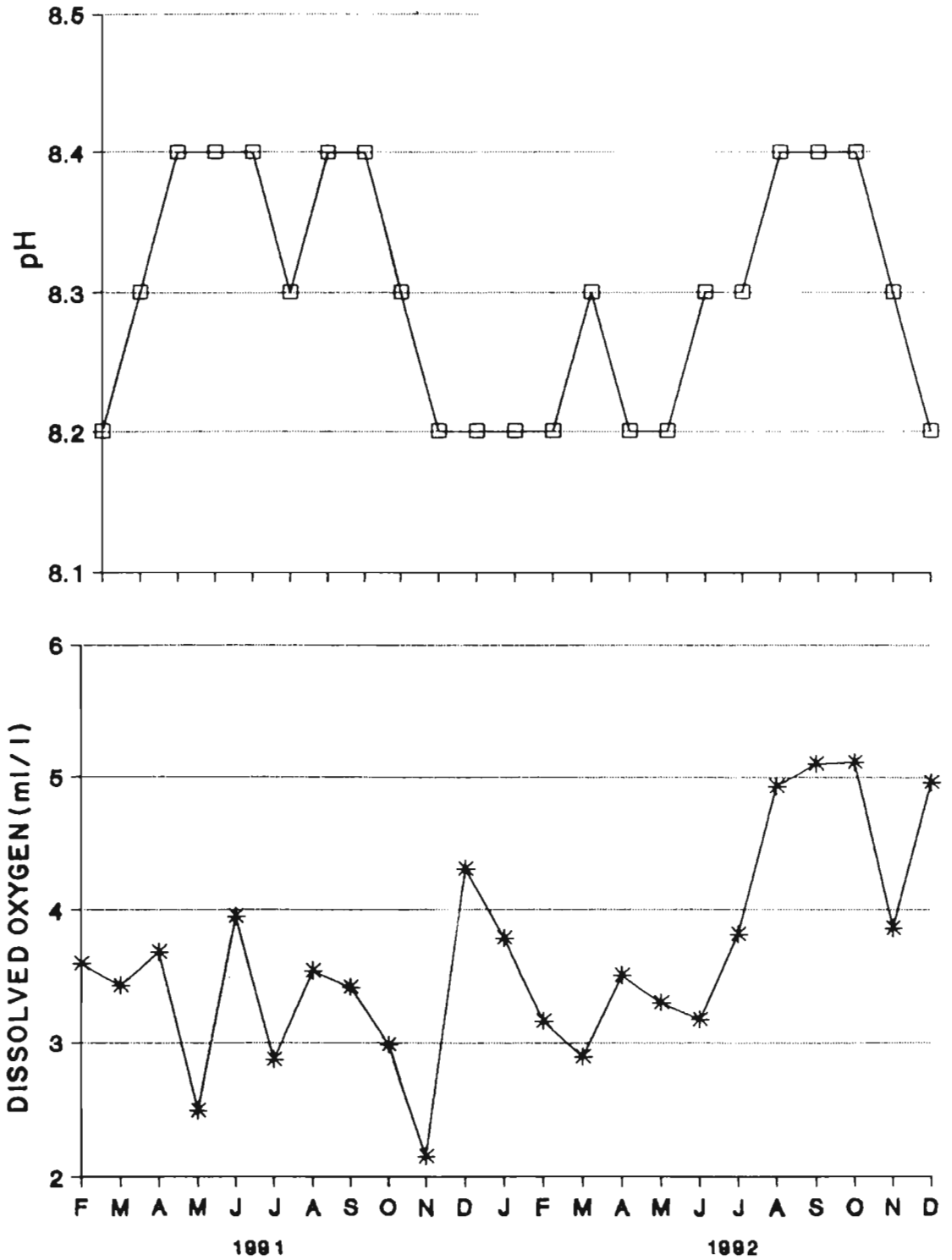


Fig. 14 : **Monthly variations in Dissolved oxygen and pH in the fishing ground of Gulf of Mannar during 1991 to 1992**

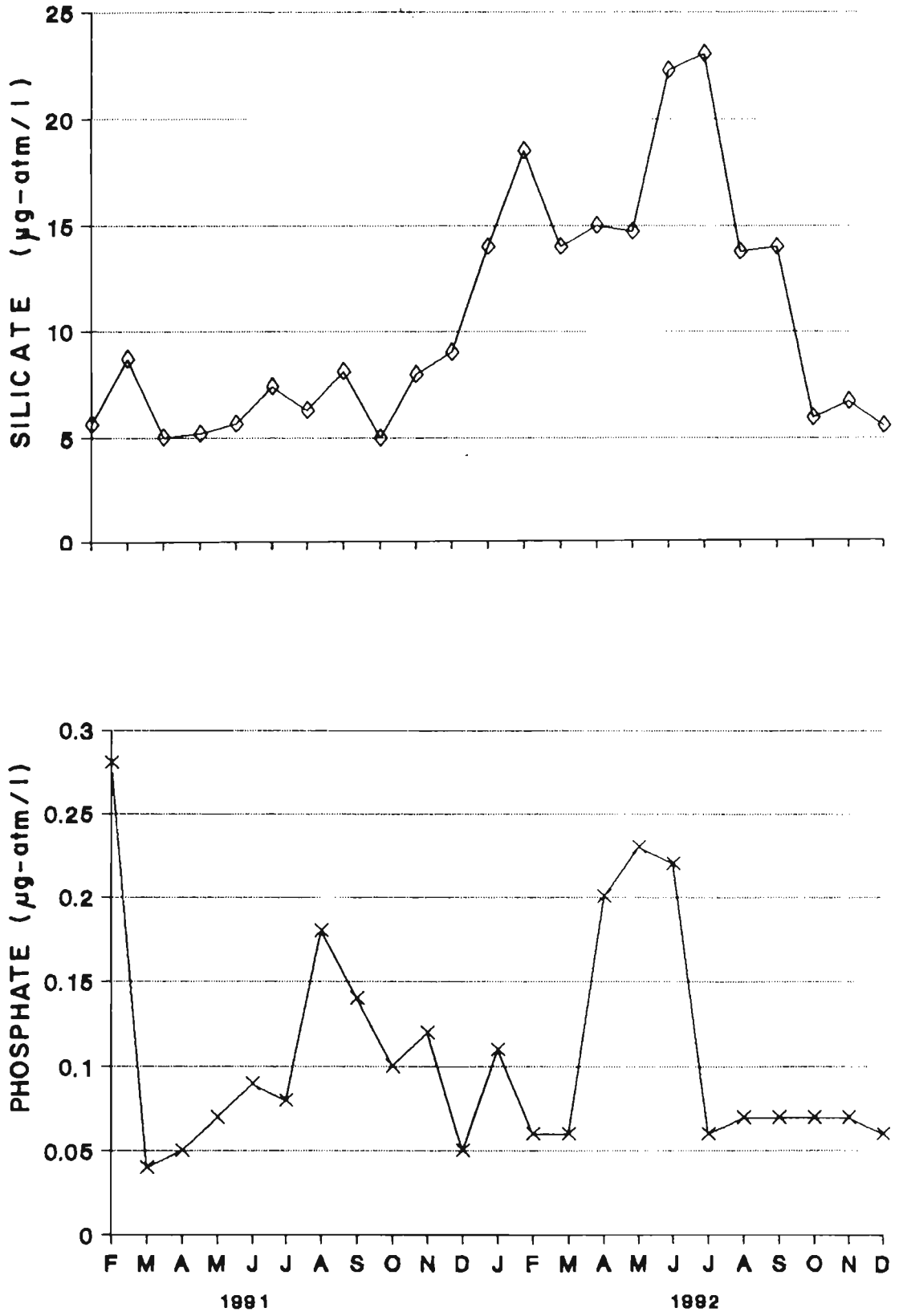


Fig.15: **Monthly variations in phosphate and silicate content of the sea water in the fishing ground of Gulf of Mannar during 1991 to 1992**

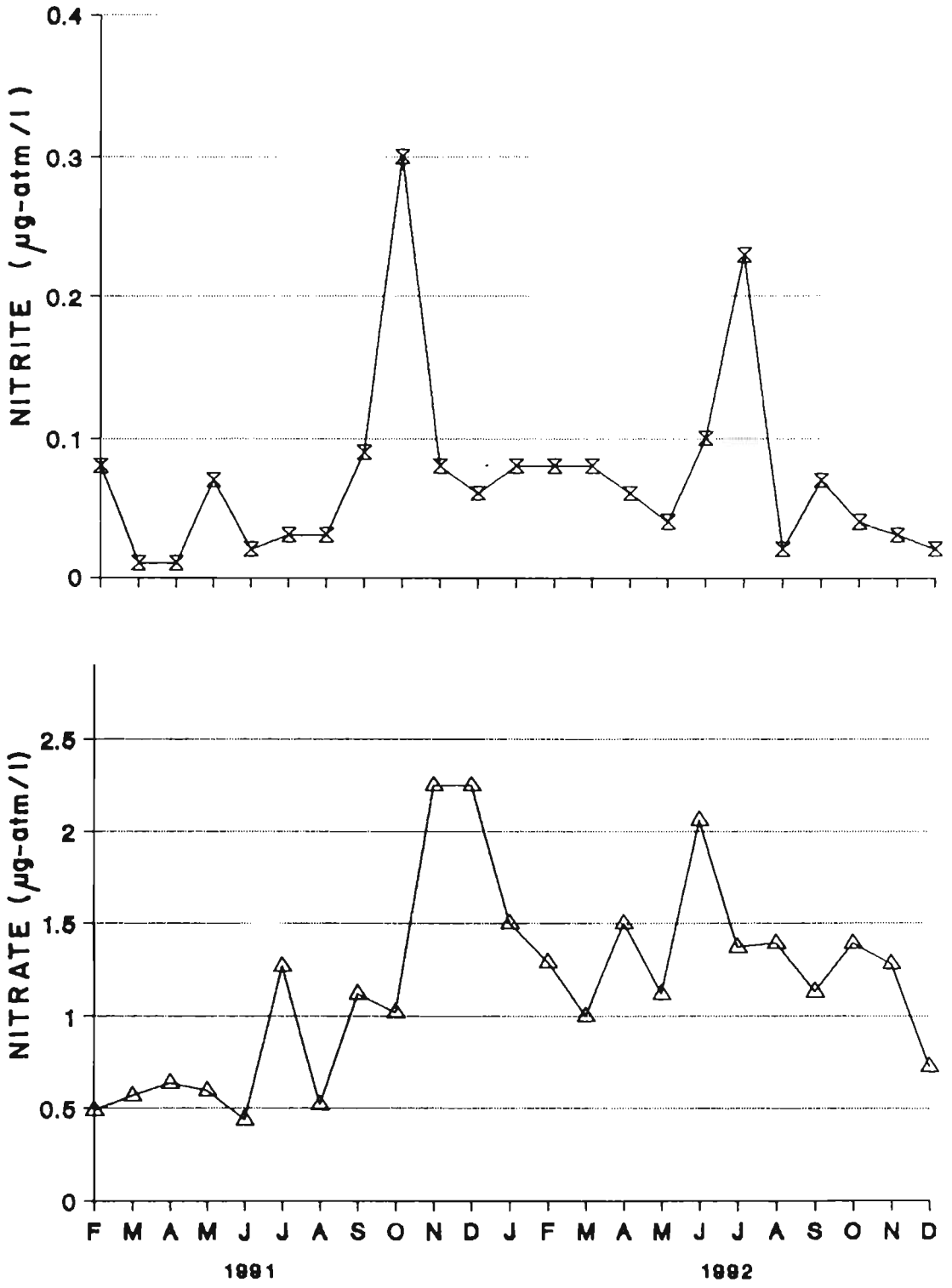


Fig. 16 : **Monthly variations in nitrate and nitrite content of the sea water in the fishing ground of Gulf of Mannar during 1991 to 1992**

that except for salinity and temperature, there is no trend in the mean values of the various parameters in the two years studied. The salinity shows a gradual increasing trend from January onwards reaching the peak in June-October. This pattern appears to be repetitive every year. A comparison of the monthly distribution of spawning population with the salinity data would reveal that the peak spawning coincides with the period of increasing of salinity from about 30‰ to 35‰ . This range of salinity prevails characteristically during March-June and May-September, thereby showing a direct relationship between this particular salinity regime and the peak spawning activities.

In the case of temperature, the highest values are recorded during April-May period which invariably corresponds with the major peak breeding season of the species. A closer scrutiny of Tables 7 and 9 and Fig. 9 and 13 would further reveal that the period of active spawning falls when relatively higher temperature (28.60-32.88°C) is noticed. pH values almost remained steady ranging narrowly from 8.2 to 8.4 (Table 7 and 9 and Fig. 10 & 14) with no perceptible relation with the change in the spawning activities in both Palk Bay and Gulf of Mannar. In the case of silicate, nitrate and nitrite, the comparatively low values recorded during March-June in the Palk Bay and May-September in the Gulf of Mannar are apparently associated with active spawning.

DISCUSSION

Information on the structure of reproductive system of penaeid prawns is available in great detail from the works of a number of authors like Heldt (1938), Hudinaga (1942), King (1948), Eldred (1958), Shaikhmahmud and Tembe (1958), Cummings (1961), Subrahmanyam (1965), Tuma (1967), Rao (1968), Tirmizi and Khan (1970), Malek and Bawab (1974 a,b) Tirmizi and Javed (1976), Motoh (1978), Motoh and Buri (1980), Champion (1987), Mohamed and Diwan (1991), Vasudevappa (1992) and Bauer and Min (1993) on different species. Considering the important role played by the secondary sexual characters such as petasma in the case of male and thelycum in the case of female, greater interest has been evinced in understanding of the structural and functional details of these characters on species of the Genus *Penaeus* by Burkenroad (1934 a,b, 1936), Heldt (1938), King (1948), Kubo (1949), Tuma (1967), George and Rao (1968), Tirmizi (1968), Perez-Farfante (1969, 1971 a,b,1975, 1982, 1985, 1988), Malek and Bawab (1974 a,b) Tirmizi and Javed (1976), Hassen (1981), Bauer (1986, 1990), Champion (1987), Ro *et al.* (1990) and Chow *et al.* (1991). In the case of *P. semisulcatus*, however, information on this aspect is very limited. The petasma (Plate 4b) of this species, especially in regard to the nature of the lateral lobes, is different from that of the closely related species *P. monodon*. In the present species, the outer surface of this structure is minutely tuberculate as compared to the unarmed outer surface of the lateral lobes in *P. monodon*. In the case of thelycum, except for some minor variations in the general shape and the major component, the structure is almost identical in both the species where the thelycum is a 'closed' one. In the early stage of formation, the petasmal halves establish fusion or union when the animal is about 115 mm in total length.

Although the structure of the reproductive system of a number of penaeid prawn species is on record, no such information is available for

P.semisulcatus from any part of the world. The present study reveals that the morphology of the reproductive system of the species conforms to the general pattern described in other species of the Genus *Penaeus* except for certain details which appear to be characteristics of the species.

In male, the internal reproductive system consists of a pair of testis, vas deferens and terminal ampoules as in all other penaeid species. The testis has a pair of long anterior lobes and seven pairs of finger-like lateral lobes (Fig. 2). In respect to the presence of a pair of anterior lobe, it is similar to the structure described for *P. setiferus* (King, 1948) and *P. monodon* (Motoh, 1978). Considerable variations are noticed in the number of lateral lobes in different species of the same Genus. While the number of lobes is four in the case of *P.indicus* (Subrahmanyam, 1965; Mohamed and Diwan, 1993), it is five in *P.monodon* (Motoh, 1978), and six in *P. setiferus*, and in the present species, it is seven (Fig. 2 & Plate 11a,b). The structure of testis of *P. semisulcatus* is distinctly different from that of *P. indicus* in which the anterior lobes are totally absent and the lateral lobes number only four pairs. A comparison of the structure of testis of *P. semisulcatus* with that of species of the other related genera would show some interesting similarities. In *Metapenaeus dobsoni*, Vasudevappa (1992) has observed a pair of anterior lobes and seven pairs of lateral lobes, while in *Trachypenaeus similis*, Bauer and Min (1993) noticed a pair of anterior lobe and only six pairs of lateral lobes. In the case of *Parapenaeopsis stylifera*, Shaikhmahmud and Tembe (1958) indicated in their illustration of the male reproductive organ, a pair of anterior lobes and three pairs of lateral lobes which may be characteristic of that genus. The accessary gland noticed by Shaikhmahmud and Tembe (1958) and Rao (1969) in the above species just below and behind the posterior lobe of testis is not noticed in *P. semisulcatus* or any other species of the Genus *Penaeus*. Vasudevappa (1992) is also of the same opinion with regard to *M. dobsoni*.

The testis is followed by a long vas deferens which has got three distinct regions namely, proximal, middle and distal regions with characteristic

size and shape for each as noticed in species like *P. monodon* (Motoh, 1978), *P.indicus* (Subrahmanyam, 1965), P. kerathurus (Malek and Bawab, 1974 a) and P. stylifera (Shaikhmahmud and Tembe, 1958; Rao, 1969). Subrahmanyam (1965) mentioned a tubular portion with two distinct regions, namely, a generative portion and a lumen inbetween testis and vas deferens in the male reproductive organ of *P. indicus*, which according to Mohamed (1989) is analogous with the proximal and mid vas deferens. Ro *et al.* (1990) studied the structure and function of the different parts of vas deferens in the shrimp *P.setiferus*. The proximal vas deferens in the present species is extremely short as against a fairly longer tube illustrated by Mohamed and Diwan (1993) for *P.indicus*.

Though the structure of ovary of *P.semisulcatus* has close resemblance to that of other penaeid prawns, clear variations are noticed in the number and arrangement of lateral lobules, when compared to the same in other species. The lateral lobules of middle lobe in this species number eight of which six are dorso-laterally placed (Plate 8a), while two on the posterior side are directed ventrally (Plates 8b & 9a). A similar arrangement of middle lobes of the ovary has also been noticed by Vasudevappa (1992) in *M.dobsoni* with a minor variation that the ventrally directed two pairs of middle lobes are located rather anteriorly. In all other species of the same genus whose ovary has been described, the number of lateral lobules of middle lobe is comparatively less ranging between five in *P. monodon* (Motoh, 1978) and seven in *P. setiferus* (King, 1948).

The oviduct in *P.semisulcatus* originates from the tip of the sixth lateral lobe (Plate 6b) as is the case in all other species of the genus (King, 1948, Subrahmanyam, 1965, Motoh 1979 and Mohamed 1989). In this respect, the present finding clearly deviates from the pattern observed by Vasudevappa (1992) in *M. dobsoni* in which the oviduct originates from the base of fifth lobule of the middle lobe.

In general, the process of maturation in prawns is judged on the basis of visual assessment of gonadal changes as well as the development of the externally visible secondary sexual characters. As a general rule, penaeid prawns initiate maturation only in the sea (Rao, 1968) or in marine condition if kept in confinement.

During the process of maturation in males of *P. semisulcatus*, four distinct maturity stages namely immature, early maturing, late maturing and mature stages have been recognised. Vasudevappa (1992) also recorded four distinct maturity stages for males in *M. dobsoni*. Based on variations in the tubular portion as well as the degree of development of testis with the age of prawn, Subrahmanyam (1965) identified five stages in *P. indicus* and categorised them by size. Rao (1978) has given a general classification of maturity stages in Penaeid prawns with five maturity stages, while Castille and Lawrence (1991) recognised only three maturity stages for males in *P.setiferus* and *P.aztecus*. The structural changes noticed during the developmental phase of male reproductive system clearly indicates the transformation from immature stage of testis with inconspicuously visible lateral lobes to fully grown testis with all the compliments of lobes completed togethter with the differentiation of the various regions of the vas deferens. In the case of *M. dobsoni* (Vasudevappa, 1992), the immature testis is rudimentary with no differentiation of lobes and the lobes are visible only from the second stage (early maturing) onwards. The fusion of petasmal endopodites and its hardening in the present species coincided with the gonadal development and hence they could be taken as a reliable indication of the initiation of the maturation process. Mere fusion of the petasmal halves without hardening of the structure showed only the beginning of testicular changes towards maturation and hardening of petasma indicated an advancement of testicular development. Studying the maturation of male in *M.dobsoni*, Vasudevappa (1992) also had a similar finding. He concluded that only after the process of hardening of petasma was complete, the testis appeared to be in fully mature state. George and Rao (1968) correlated mere

fusion of petasmal endopodites in penaeid species with attainment of gonadal maturation. However, the present study on *P. semisulcatus* as well as the study of Vasudevappa on *M. dobsoni*, would reveal that attainment of gonadal maturation takes place only after full hardening of petasma which occurs gradually after the fusion of the endopodal halves. The attainment of maturation could also be judged externally from the presence of a white mass of spermatophore at the base of the fifth pereiopod.

In female *P. semisulcatus*, five distinct maturity stages have been recognised during the present study, as in the case of most other penaeid species. Thomas (1974) also classified the maturity stages of the species into five, namely, immature, early maturing, late maturing, mature and spent stages. The minimum size at maturity of female *P. semisulcatus* at 50% level is found to be 145.07 mm total length. In the same species, Thomas (1974) reported the size at first maturity as 23 mm carapace length The corresponding length of carapace for the minimum estimated species at in the present study is 41 mm which is comparatively much larger than the size estimated by Thomas (1974), but almost equal to the size (39 mm CL) arrived at by crocos (1987 b) for *P. semisulcatus* in the Gulf of Carpentaria. Study of the gonadosomatic index (GSI) for different maturity stages (Table 1a) of *P.semisulcatus* revealed a steady increase in GSI (Fig. 7) from immature stage (0.6534 ± 0.275) to fully mature stage (8.3838 ± 1.761) followed by a sharp fall in spent stage (1.9401 ± 0.686). This is in full agreement with the observation made by Thomas (1974) in the same species. Similar observations on GSI have also been made by Kulkarni and Nagabhushanam (1979) in *Parapenaeopsis hardwickii*, Dy-Panaflorida and Millamena (1990) in *P. monodon*, Castille and Lawrence (1991) in *P. aztecus* and *P. setiferus* and Vasudevappa (1992) in *M.dobsoni*. In the present study, it is found that though changes in organ size are not as great in the digestive glands of females as in the ovaries, there are significant changes in the digestive gland organ indices or hepatosomatic indices (Fig.7) during maturation. The HSI increased from immature stage (1.9416 ± 0.545) to late maturing stage (2.6644 ± 0.774) and suddenly

decreased from late maturing stage to spent stage (2.0621 ± 1.000). Further, the hepatosomatic index was lower in spent stage than in early maturing stage. The sudden decrease of HSI in mature and spent stages would imply that the lipid content has been transferred from hepatopancreas to ovary for vitellogenin synthesis as this organ is considered as a primary organ for lipid storage (castille and Lawrence, 1991). In contrast to the present investigation, castille and Lawrence (1991) reported that the digestive glands in *P. setiferus* decreased in size during maturation, whereas, in *P. aztecus* they increased in size. In spent recovering females of both these species, the digestive gland organ indices were lower than in late maturing and mature shrimp.

The fecundity or total number of eggs present in a single prawn indicates the reproductive potential of the species and it varies from species to species. Fecundity is measured in two ways, by counting the number of eggs spawned (Hudinaga, 1942; Hassan, 1982); or by estimating the number from dissected ovaries (Rao, 1968; Thomas, 1974; Crocos and Kerr, 1983; Choy, 1987). Since the former method is less accurate as spawning in captivity is often incomplete, the latter method has been taken up for calculating fecundity in the present study. The highest fecundity of *P. semisulcatus* is estimated as 7,12,595 at 192 mm TL (Carapace length 62 mm), while Thomas (1974), estimated the same as 6,60,904, Shlagman *et al.* (1986) as 10,12,702 and Crocos (1987 b) as 7,32,000 for the same species at the carapace length 45 mm, 54 mm and 52 mm, respectively. Considerable amount of work has been carried out on the fecundity of penaeid prawn as could be seen from the contributions of Heldt (1938) on *P. trisulcatus*, Fujinaga (1963) on *P. japonicus* and Anderson (1956) on *P. setiferus* with varying estimates ranging from 10,00,000 to 13,00,000. Rao (1968) reported the fecundity in *M. dobsoni*, *M.affinis, P.indicus* and *P. stylifera* as 1,60,000 at 120 mm TL, 3,63,000 at 160 mm TL, 7,31,000 at 200 mm TL and 2,36,000 at 120 mm TL, respectively. Statistical analysis of the data on hand (Fig. 8) indicates no correlation between total length of the prawn and fecundity or between total weight of the prawn and fecundity, which is in conformity with the finding of Thomas (1974).

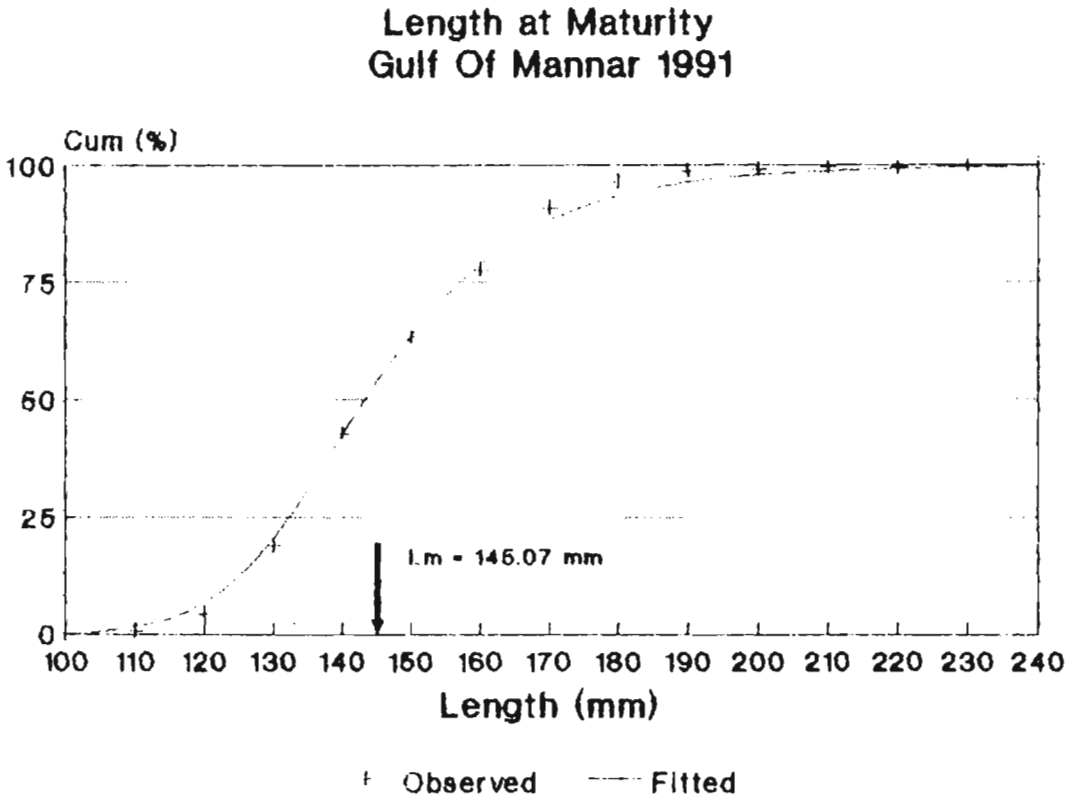
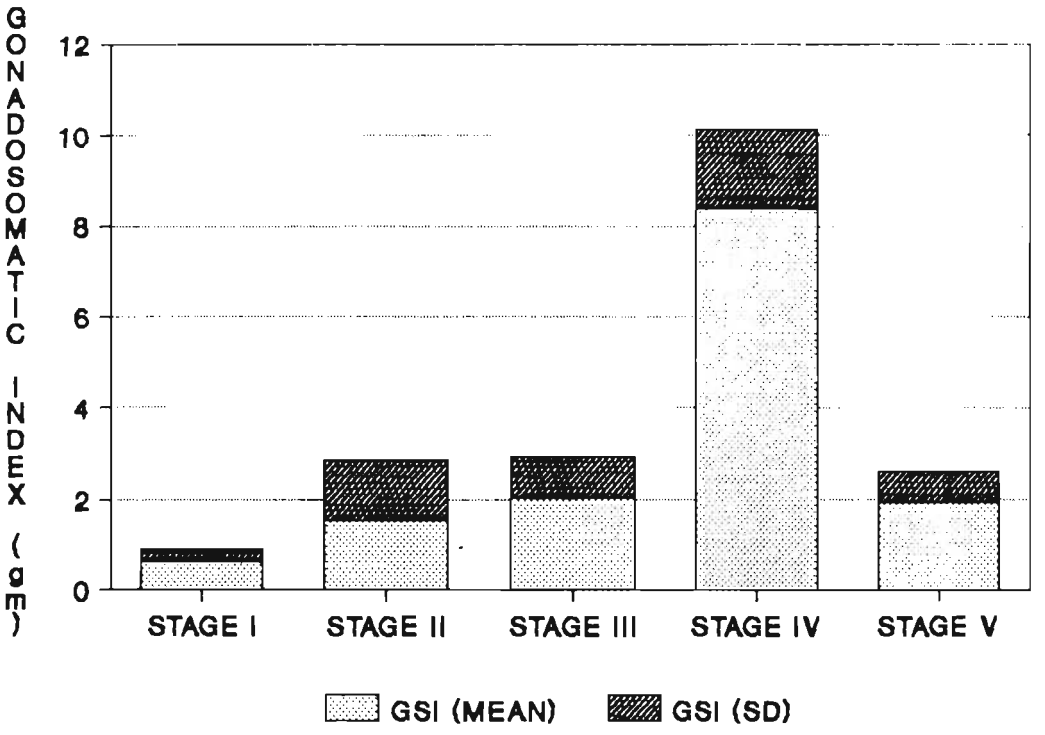


FIG. 6. Graph showing length at first maturity of female at 50% level



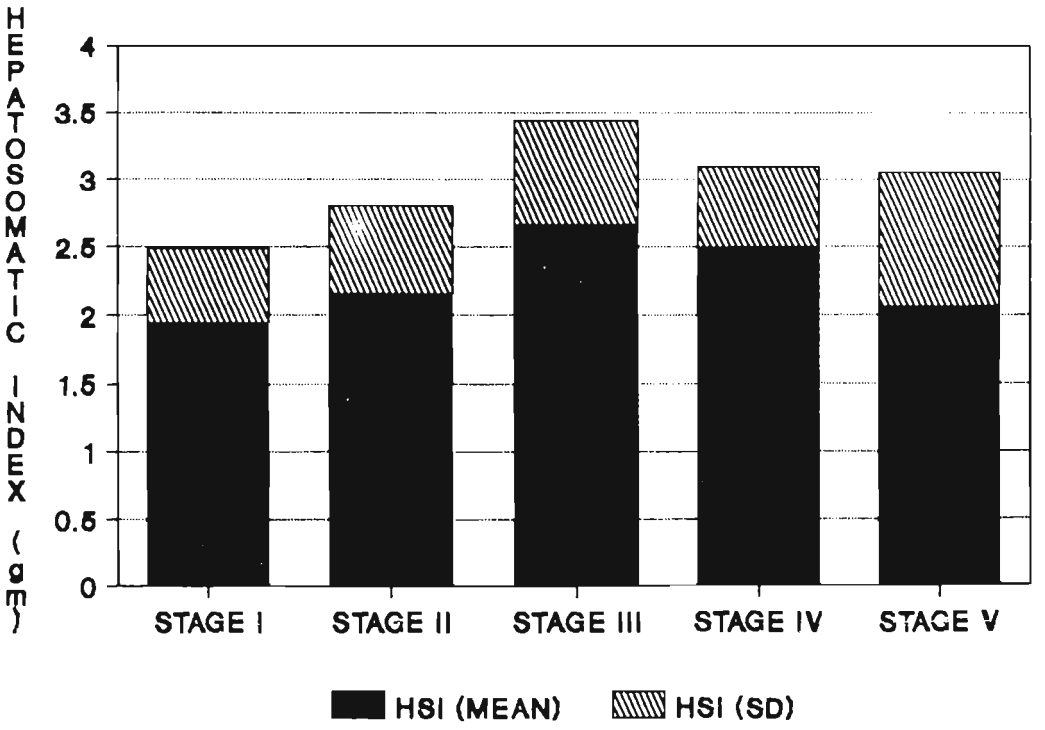
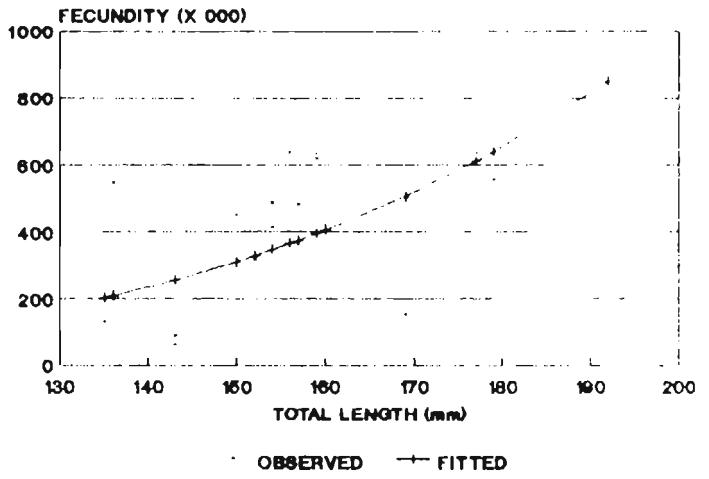
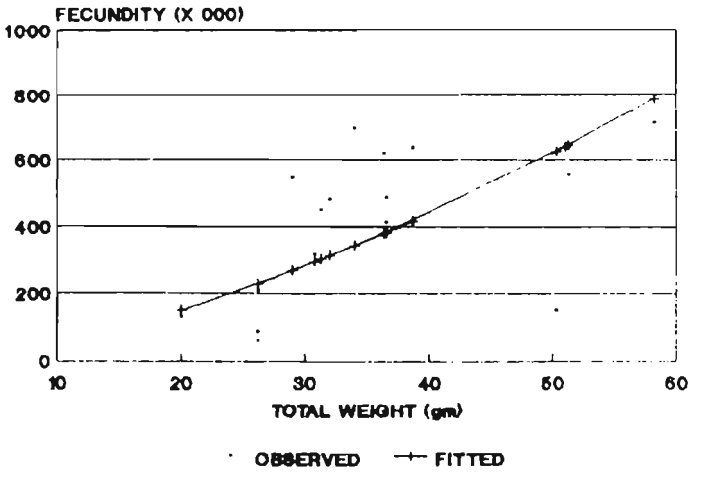


Fig. 7: Variations in gonadosomatic and hepatosomatic indices at different stages of maturity

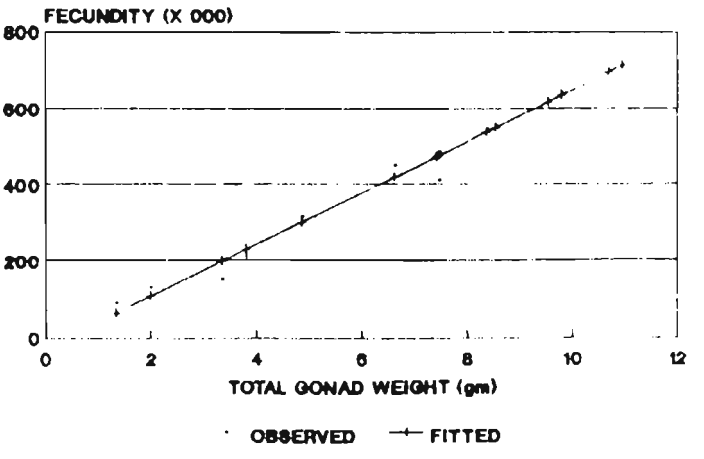
FIG. 8 FECUNDITY - TOTAL LENGTH RELATIONSHIP



FECUNDITY - TOTAL WEIGHT RELATIONSHIP



FECUNDITY - TOTAL GONAD WEIGHT RELATIONSHIP



However, in respect of the total gonad weight and fecundity, there exists a positive relationship with the r^2 value as 99%. Thomas (1974) observed no relationship between these two variables.

The data on sex ratio distribution over time and space indicates a general preponderance of females in the population of both the fishing grounds. The relative abundance of male and female prawns during different months of the year, however, shows occasional dominance of males over females and thus inconsistent for the different years of observation. Studying the fishery of juveniles of the same species along the neighbouring coast (Tirunelveli coast), Manissery (1983) observed that sexes were not equally distributed in the fishery. There was a clear preponderance of female in almost all the months contributing an average to about 56.5% of the total catch. Dominance of females in the adult population has also been reported in a number of other commercially important penaeid species by George *et al.* (1963). George and Rao (1967), analysing the sex ratio data from commercial trawl catches of Cochin, noticed that in three species - *Metapenaeus dobsoni, Penaeus indicus* and *Parapenaeopsis stylifera* - the distribution of sexes were significantly different from what could be accounted for by binomial theory. They suggested that the differential sex ratio in the fishing grounds may be brought about by the segregated sex movements for breeding. Studying the population distribution of *P. semisulcatus* in Bahrain waters, Abdulqader and Naylor (1995) observed a near 1:1 ratio for the two sexes of the species almost throughout the year with occasional preponderance of males in the fishing ground sampled, particularly in January during the presumed spawning season. They have also suggested that large, mature and spawning females move out of the sampling area into deeper waters at that time. According to Baelde (1992), the possible factors causing changes in sex ratio include: differences in growth or migration behaviour between males and females, changes in mortality or catchability, particularly after breeding, and sex-reversal pattern. Many others also opined (Kunju, 1970; Garcia and Le Reste, 1981; Penn, 1980; Garcia, 1985; Bouhlel and Hail, 1985) that spawning activity

in penaeids is often associated with marked changes in sex ratio, and with presumed behavioural differences between males and females after fertilization has occurred. Bouhlel and Hail (1985) assumed that females migrate separately from males to deeper water. As female dominance has also been noticed among juveniles in coastal waters (Manissery, 1983), the reason for the predominance of adult females in the trawling grounds of Palk Bay and Gulf of Mannar observed during the present study cannot be assigned to spawning migration of the species into deeper waters. Further, the fact that the Palk Bay is comparatively very shallow (8-14m) as against the much deeper waters of Gulf of Mannar (15-45m) and that the female dominance was noticed invariably in both these fishing grounds rule out the possibility any sex segregated breeding migration.

The present data on spawner distribution clearly reveals that *P.semisulcatus* breeds actively in both these fishing grounds. The spawning takes place throughout the year with distinct peak spawning seasons which are slightly different in the two waters. While major peak spawning season in the Palk Bay is from March-June, the same in the Gulf of Mannar is from May-September. In addition to these major peak spawning seasons, minor peaks are also noticed in some of the months of the second half of the year, the exact months of the same vary from year to year as well as from place to place. Combining the population of the Palk Bay and Gulf of Mannar, Thomas (1974) reported two peak spawning seasons, one in June-September and the other in January-February during 1967-1969 period. In Kuwait waters, the peak spawning season of *P. semisulcatus* has been reported to be from January-May (Enomoto, 1971; Al-Attar and Ikenoue, 1974; Mohamed, 1978). In Bahrain waters, however, Mohamed (1978) observed the main spawning season of this species to be from December to March. The spawning peak observed in the Palk Bay during the present investigation is more or less in agreement with the peak spawning period reported for Kuwait and Bahrain waters. In respect of the peak spawning season observed in the Gulf of Mannar, there appears to be some deviation, the peak period being May-September. In the southwest

coast of India, Rao (1968) reported peak spawning activities for *P. stylifera* more or less in the same period (June-August).

The spawning activities in penaeid prawns are correlated with Physico-chemical parameters of the environment in a number of commercially important species from different parts of the world (Anderson, 1956; Eldred *et al.*, 1961; Cummings, 1961; Idyll *et al.*, 1962 and Rao, 1968, 1978). In Indian coast, higher salinity, temperature and pressure of the depth of water have been found to influence the maturation and spawning in a number of species (Rao 1968; 1978). Among the above three parameters, the temperature has been noticed to influence closely with the spawning activities. In the southwest coast of India, Rao (1968) observed that during July-September, the bottom water temperature of the fishing ground was less than 25.0°C, when the mature females of penaeid prawns occurred only in fewer numbers. From October onwards, the temperature increased and the highest spawning in most of the species were observed during that period. During the present study also a similar relationship has been noticed, particularly between temperature and spawning. A perusal of the data on temperature (Tables 7 & 9) and the seasonal trends of the same (Figs. 9 & 13) in comparison with the monthly spawner abundance (Tables 5 & 6) would clearly indicate that the maximum spawning activity recorded during March-June in the Palk Bay synchronised with the period of highest temperature condition. However, this relationship does not appear to be so strong in the Gulf of Mannar. The only explanation that could be ascribed to this variation may be the depth difference between these two fishing grounds, the positive relationship being more applicable to the shallower ground (Palk Bay) than the deeper one (Gulf of Mannar).

CHAPTER IV

GAMETOGENESIS

RESULTS

Oogenesis

Oogenesis in *P.semisulcatus* has been studied by histological sections of ovary in different maturity stages. Examinations of the histological sections revealed that the process of oogenesis is associated with oocyte development and yolk accumulation in a graded manner. Based on the ovarian changes manifested in the cytoplasm and nucleus of the oocytes, the process of oogenesis was classified into five different stages viz. pre-vitellogenic, early vitellogenic, late vitellogenic, vitellogenic and spent oocyte stages (Plates 12-28). These oocyte phases correspond to the maturity stages I to V classified on the basis of morphological features and colour of the ovary (Plates 7-10).

The ovary is a lobular organ with thin ovarian wall having two distinct layers of epithelial cells and a layer of connective tissue in between (Plates 7-14 & 18a). In haematoxylin-eosin stain, the outer layer was found to be moderately basophilic, while the inner layer moderately eosinophilic. Blood capillaries were also noticed in the peripheral regions of the ovary (Plates 12-14). The germinal zone or the germarium was observed in all the sections as a thin band retracted into a small region of the innermost ovarian wall (Plates 12-13). This zone of proliferation was observed to persist in all maturity stages. The smaller primary oogonial cells were found at the periphery of the germinal zone. The primary oogonial cells possess a large conspicuous nucleus, stained lightly with haematoxylin. The chromatin matter was uniformly distributed in the ill defined cytoplasm. The secondary oogonial cells are formed by the mitotic division of the primary oogonial cells. The secondary oogonial cells possess a nucleus stained lightly with haematoxylin and the cytoplasm stained pale with haematoxylin (Plates 12-14).

Pre-vitellogenic oocytes (Plates 12-14 & 24-25)

This stage is characterized by the predominance of oogonia and primary oocytes with the ova diameter ranging from 32-56 µm. Previtellogenic oocytes are formed by the meiotic division of the secondary oogonial cells. The oocyte has a basophilic cytoplasm with an increased cytoplasmic volume. The nucleus is not stained with haematoxylin. The nucleoli approximately 10-15 in number are arranged in the peripheral margin as well as on the centre of the nucleus and is deeply stained with haematoxylin (Plate 13). The basophilic cytoplasm is homogeneous and agranular. The oocyte and oocyte atresia (AO) are surrounded by follicle cells.

Ultrastructurally (Plates 24-25), euchromatin and heterochromatin regions are found to be present in the nucleoplasm and the nucleoli are distributed around the periphery of the nuclear wall. The nucleoli of the oocytes are observed as electron dense bodies in the nucleus. Micropinocytotic vesicle and cytoplasmic organelles like mitochondria and smooth endoplasmic reticulum are evident in this stage.

Early vitellogenic oocytes (Plates 14-16 & 26-27)

During this phase, the oocytes increase in size rapidly with the ova diameter ranging from 96-128 um. The oocytes are almost spherical in shape with their cytoplasm and nucleus moderately basophilic and eosinophilic. The cytoplasm started increasing in volume and now appears as granular (Plates 14-16). The granular nature is mainly due to the presence of the vesicular primary yolk (Plates 14-16). A perinuclear halo of nucleolar material is observed in this phase with the basophilic nucleoli forming a circular ring around the periphery of the nucleus (Plate 15). This oocyte is surrounded by cuboidal follicle cells with its pale basophilic nucleoli (Plates 14-16).

Electron microscopic observation of the early vitellogenic oocyte reveal the abundance of mitochondria with cristae, smooth endoplasmic reticulum and free ribosomes. The nucleoplasm showed the presence of granular electron denser material (Plates 26-27) accumulated in the cytoplasm or ooplasm.

Late vitellogenic oocytes (Plates 17 & 28)

During this phase, the oocyte further increases in size with the ova diameter ranging from 144-256 µm. Characteristic of this phase is the rough or condensed granular cytoplasm which is wholly eosinophilic having lost its basophilia. The granular cytoplasm is mainly due to the formation of dense yolk platelets and accumulation of lipid or yolk globules (Plate 17). The nucleus has been slightly masked by the accumulation of yolk platelets and lipid globules. The nucleolar membrane is found to be thin and sometimes invisible. The nucleus is stained pale with haematoxylin and the nucleoli number is greatly reduced. In this stage, the maturing oocytes are compactly arranged. Because of the increase in oocyte volume, the follicle cells become elongated and appear as a narrow band of flattened cells encompassing the oocytes (Plate 17).

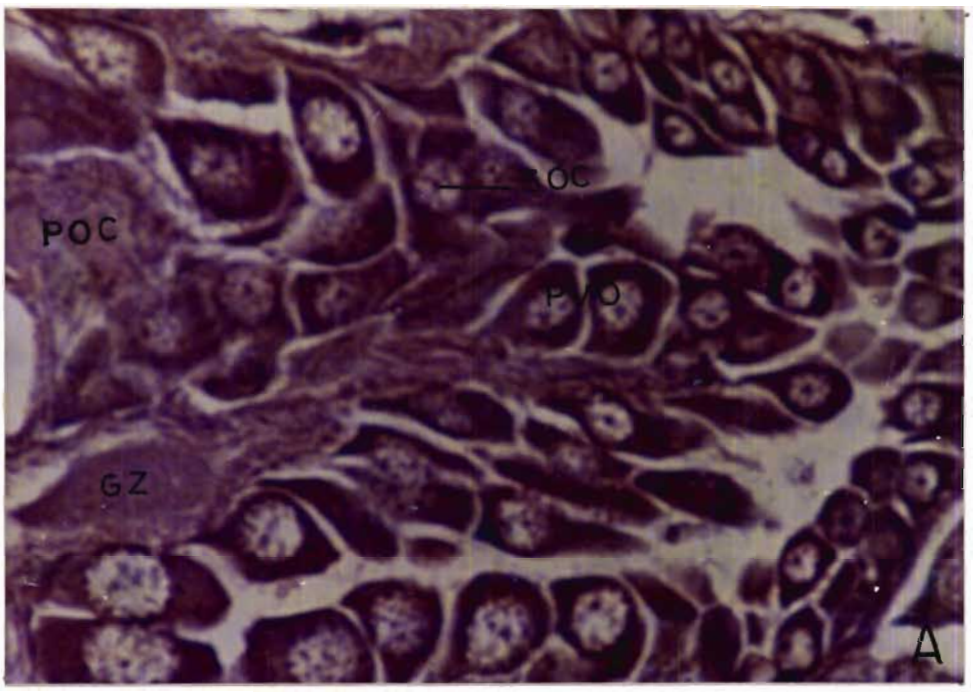
Electron microscopic studies (Plate 28) reveal the accumulation of oval shaped lipid or yolk globules and spherical yolk platelets appearing electron dense in cytoplasm. The rough endoplasmic reticulum and mitochondria are noticeable in this stage. Numerous gaps or nuclear pores are present on the nucleolar wall through which a considerable amount of exchange of materials between nucleus and the cytoplasm might take place. This phenomena is indicative of the high synthetic activity taking place within them. In this stage, the electron dense material is sparsely distributed in the nucleus compared with the cytoplasm (Plate 28).

PLATE 12

A. Section of a stage I ovary with deeply basophilic previtellogenic oocytes (PVO). The germinal zone (GZ), primary oogonial cells (POC) and secondary oogonial cells (SOC) are clearly seen. Haematoxlyin and Eosin. x 2000.

B. Section of a stage I ovary showing the oocyte atresia or Atretic oocyte (AO) along with developing oogonial cells and PVO. Haematoxylin and Eosin. x 2000.

PLATE 12



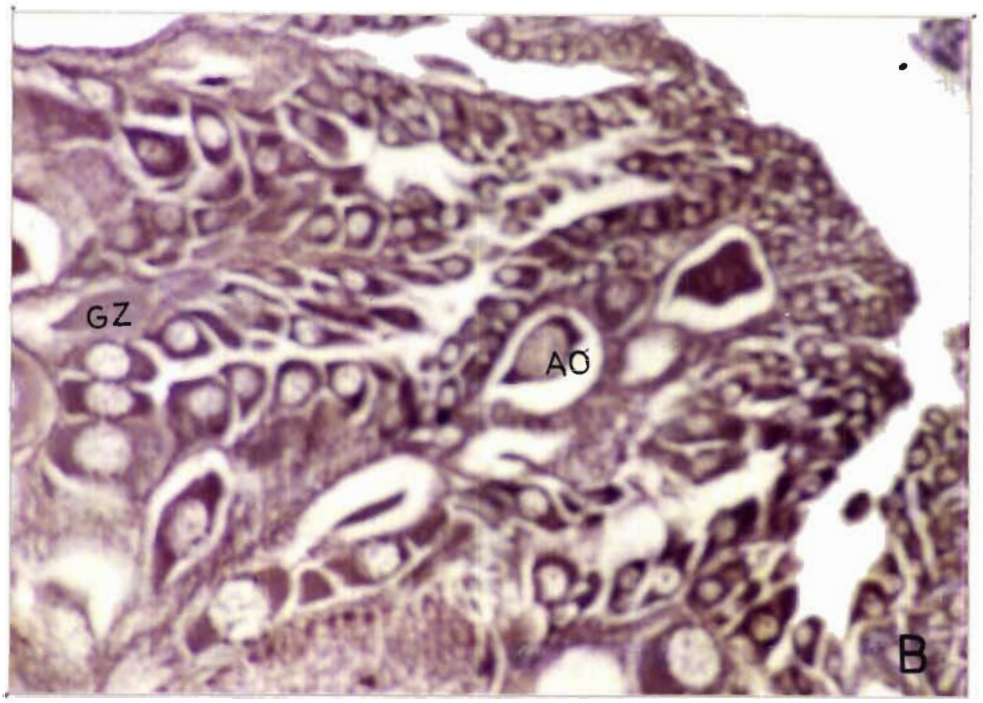
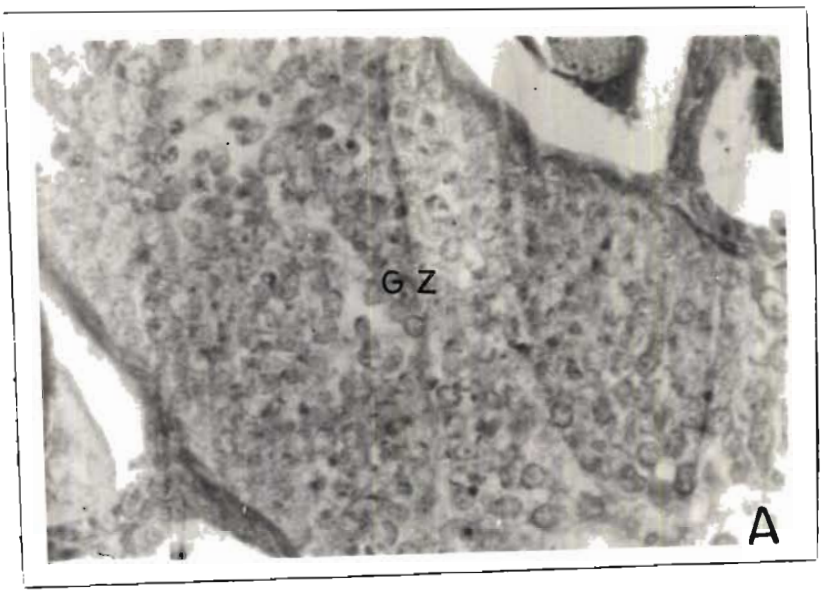


PLATE 13

A. Section of the germinal zone enlarged showing group of oogonial cells. Haematoxylin and Eosin. x 566.

B. Section of stage I ovary showing previtellogenic oocytes (PVO) surrounded by vacuolated follicle cells (FC). The secondary oogonial cells and oocyte atresia are also seen. Haematoxylin and Eosin. x 2000.

PLATE 13



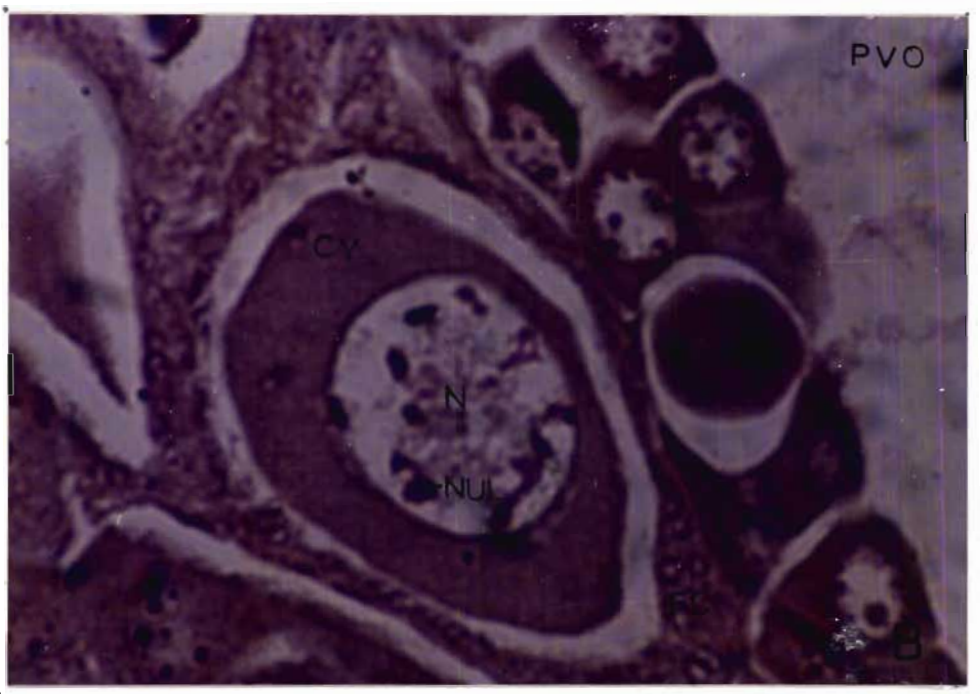
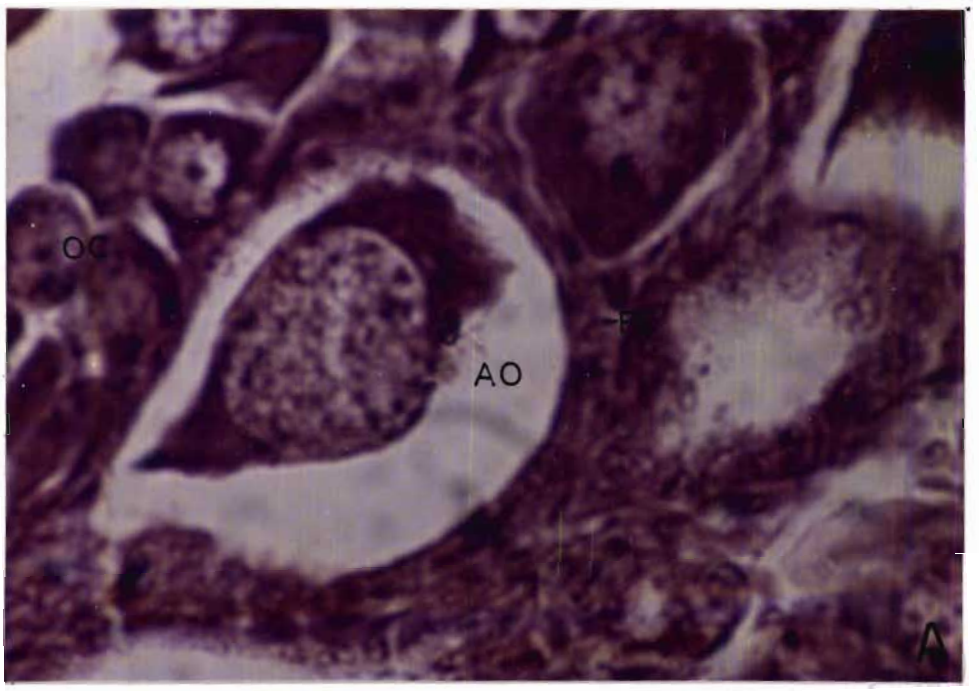


PLATE 14

A. Section of a stage I ovary showing artretic oocytes (AO) surrounded by follicle cells and oogonial cells. Haematoxylin and Eosin. x 2000.

B. Semithin section of early vitellogenic oocytes (EVO) showing part of the encircling follicle cells (FC). Toluidine blue stain. x 750.

PLATE 14



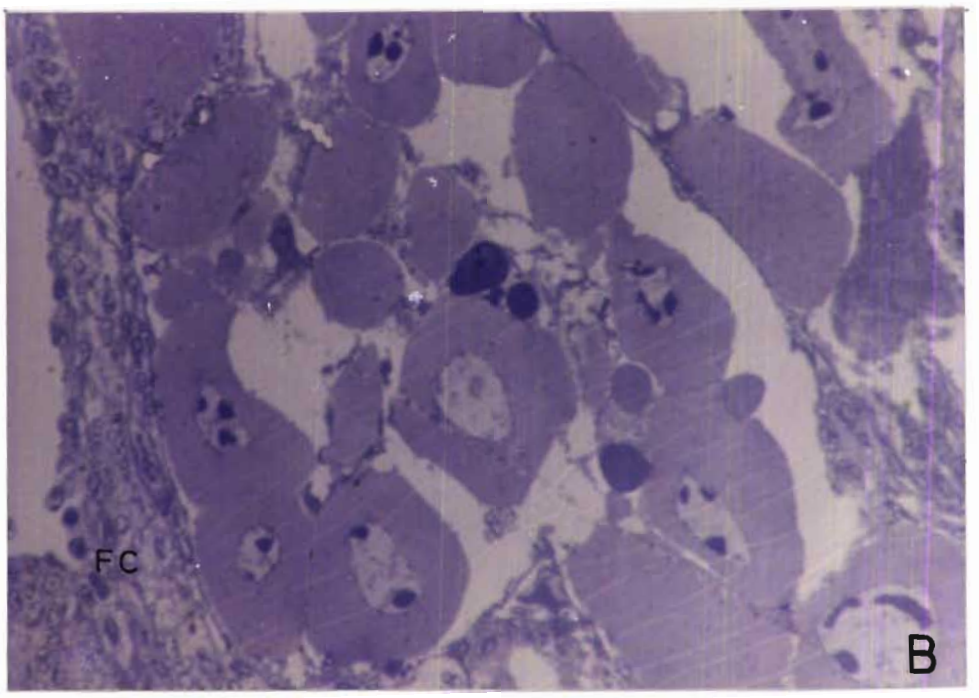
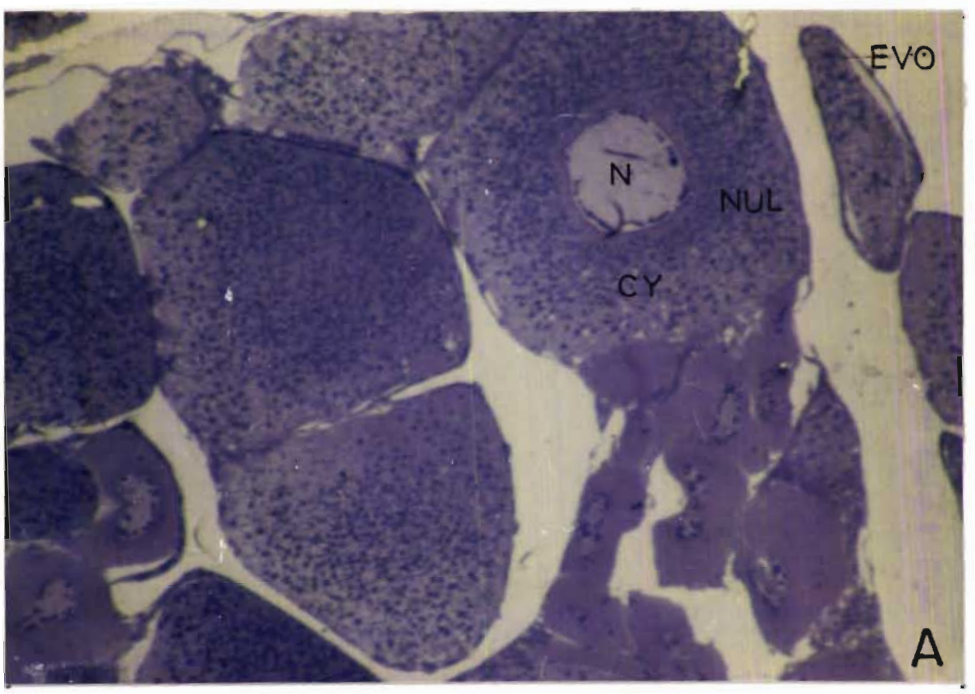


PLATE 15

A. Semithin section of EVO showing nucleolar materials (NM) distributed near the periphery of nucleus, and the granular cytoplasm. Toluidine blue stain. x 500.

B. Toluidine blue reaction at 1.09 pH in EVO showing the presence of acid mucopolysaccharides (AMP). x 1000.

PLATE 15



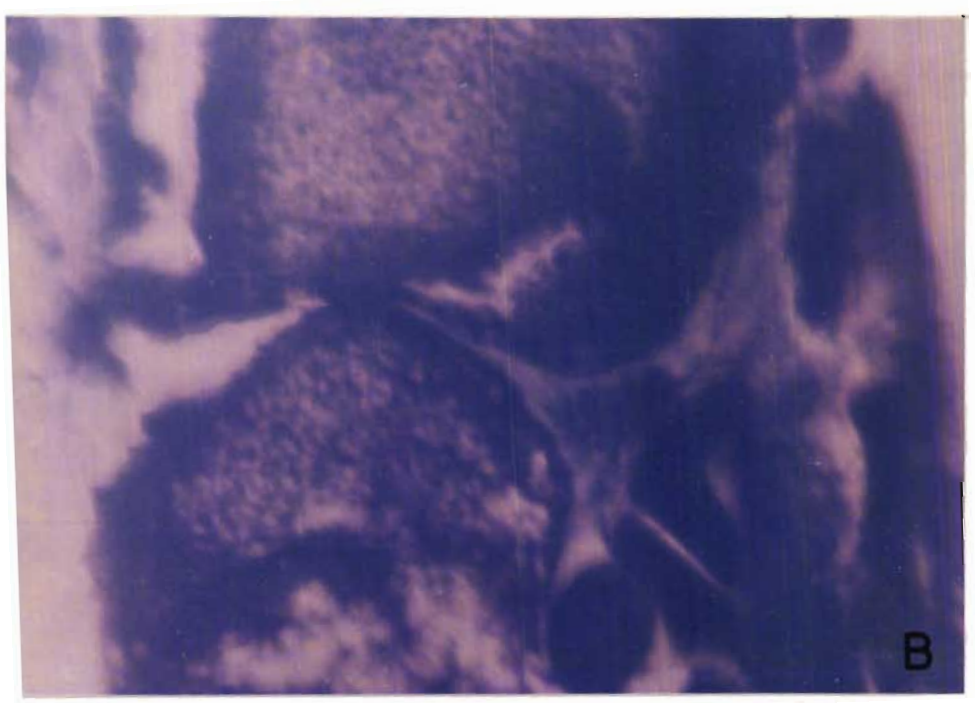
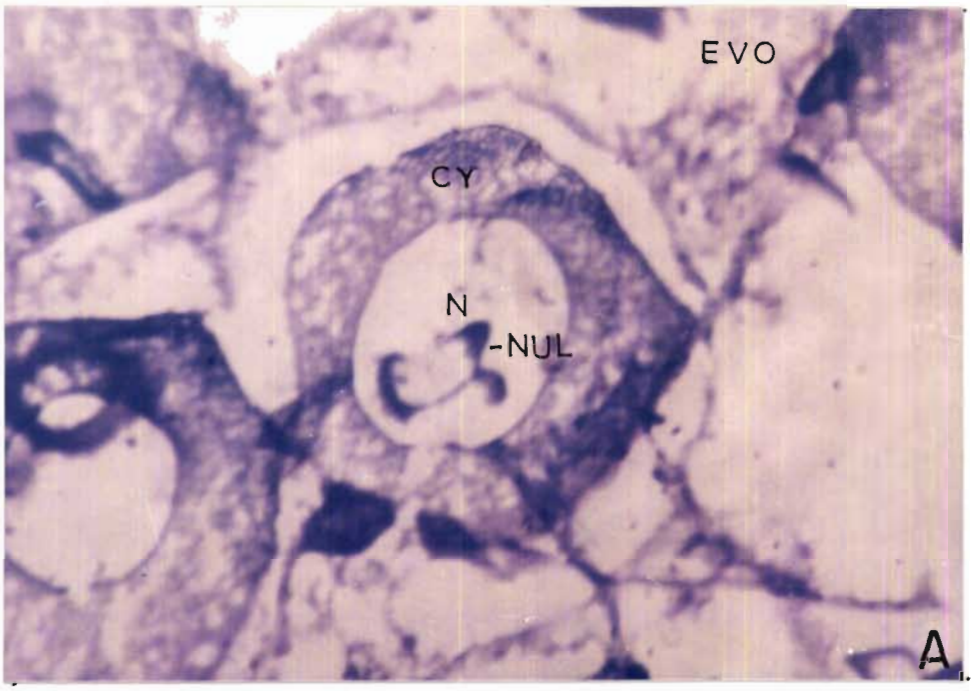


PLATE 16

A. Sudan black B reaction in EVO showing the presence of lipid in cytoplasm (CY) and nucleoli (NUL). x 1000.

B. Ninhydrin-schiff reaction in EVO showing the presence of aminogroups in cytoplasm and flattened follicle cells. x 1000.

PLATE 16



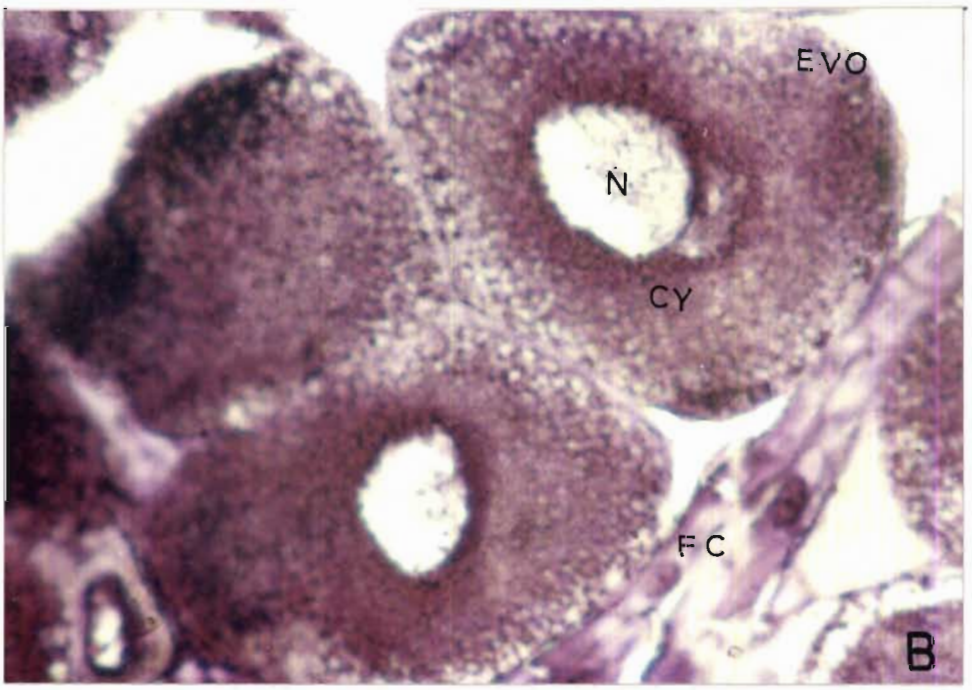
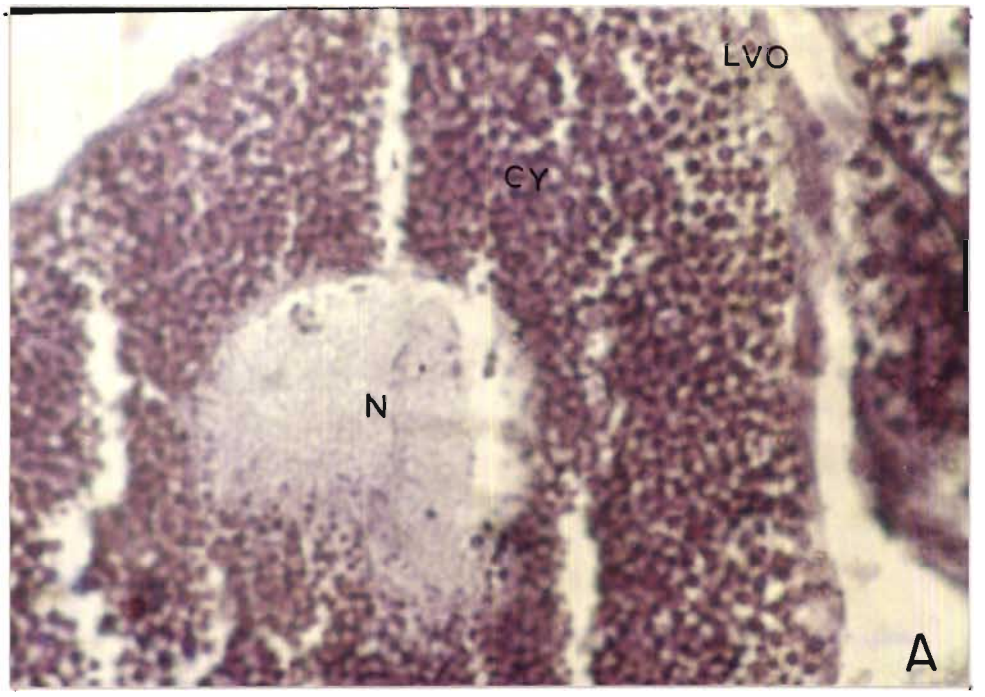


PLATE 17

A. Section of late vitellogenic oocyte (LVO) showing the granular cytoplasm filled with yolk globules and yolk platelets. The cytoplasm showing presence of aminogroups stained with Ninhydrin-schiff's reagent. Note the size decrease in nucleus and the disappearance of nuclear envelope (NE) due to the accumulation of yolk globules and yolk platelets towards nucleoplasm. x 2000.

B. Section of a LVO stained with Million's reagent showing the poor presence of tyrosyl groups in the granular cytoplasm and absence of the same in nucleus. x 1000.

PLATE 17



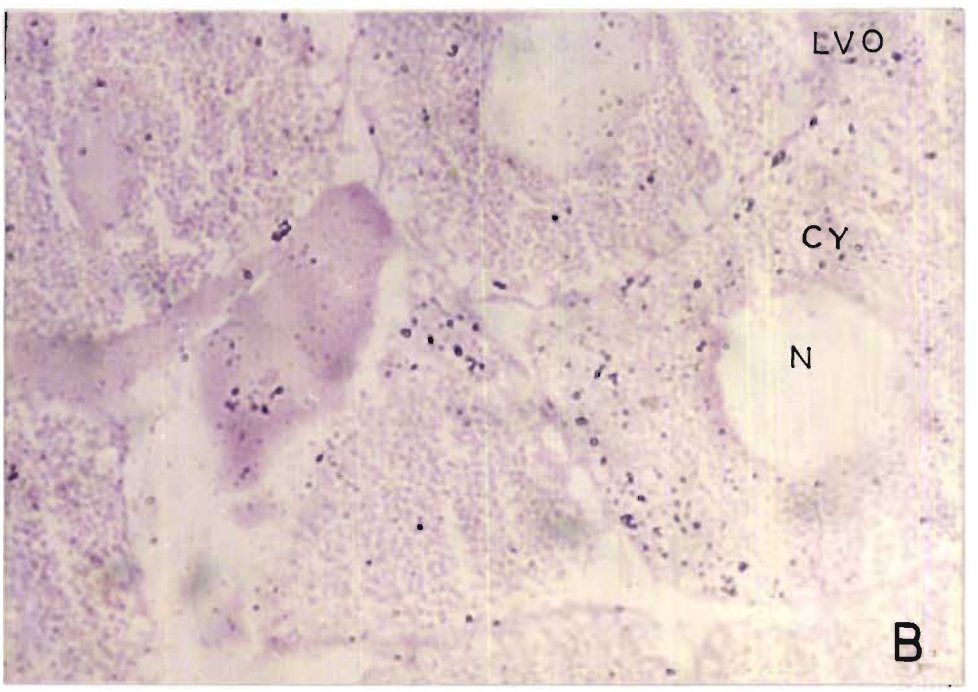
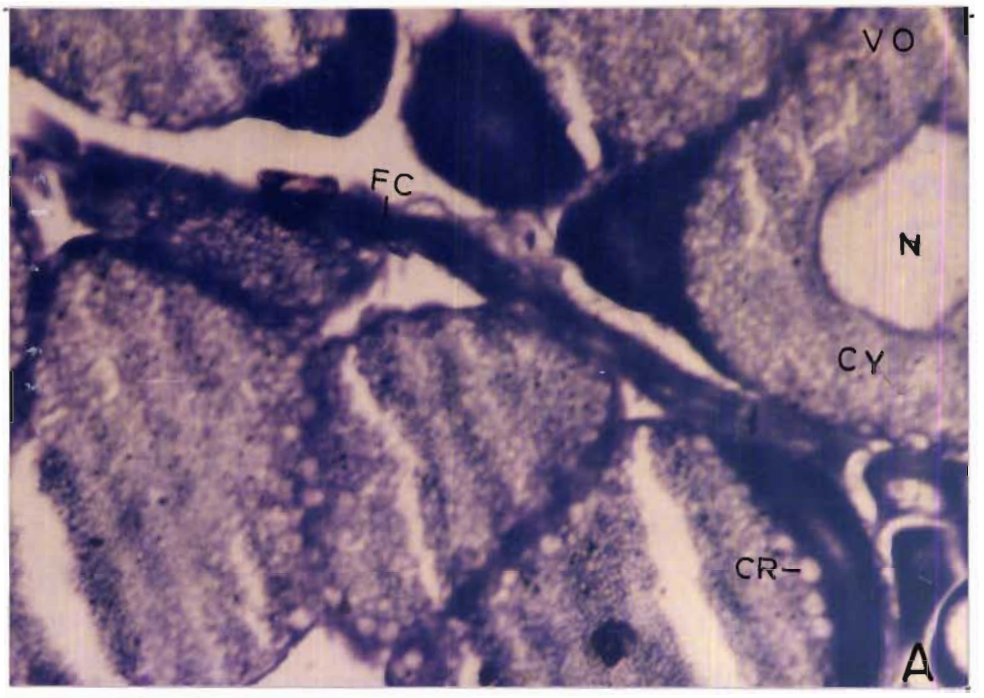


PLATE 18

A. Toluidine blue at pH 1.99 reaction in vitellogenic oocyte (VO) showing the presence of sulphated or phosphated AMP in granular cytoplasm and flattened follicle cells encircling the oocytes. Absence of the same in nucleus and at the initial stage of cortical rods present around the periphery of the oolemma is also clear. x 1000.

B. Section of the vitellogenic oocyte showing the elongated cortical rods present along the peripheral margin of ooplasm adjacent to oolemma and cell wall structure. Haematoxylin and Eosin. x 5000.

PLATE 18



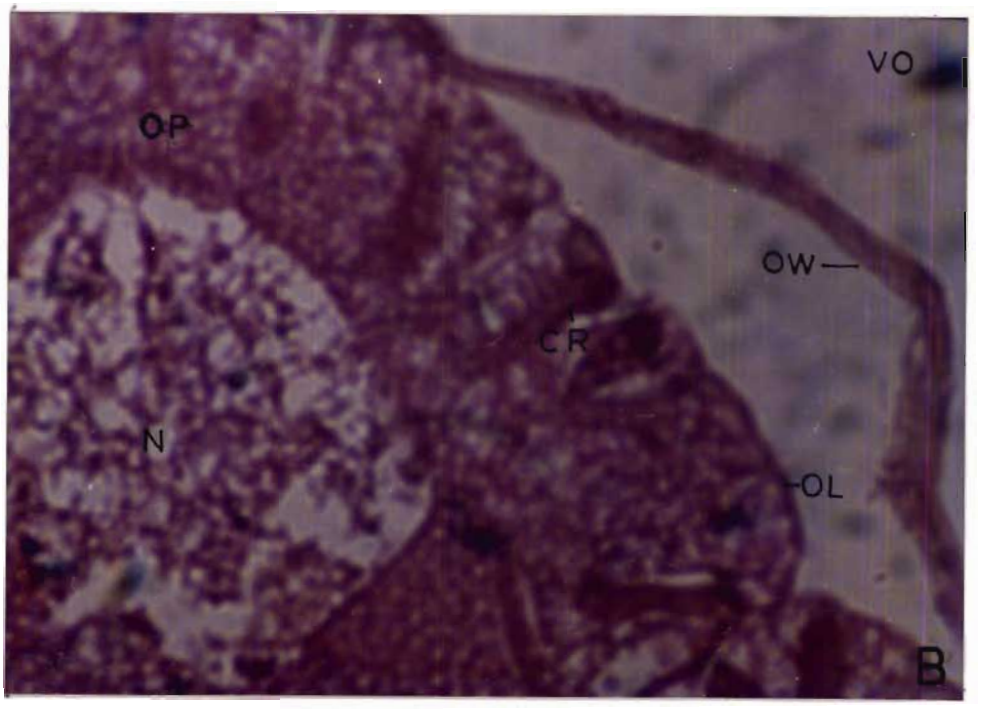
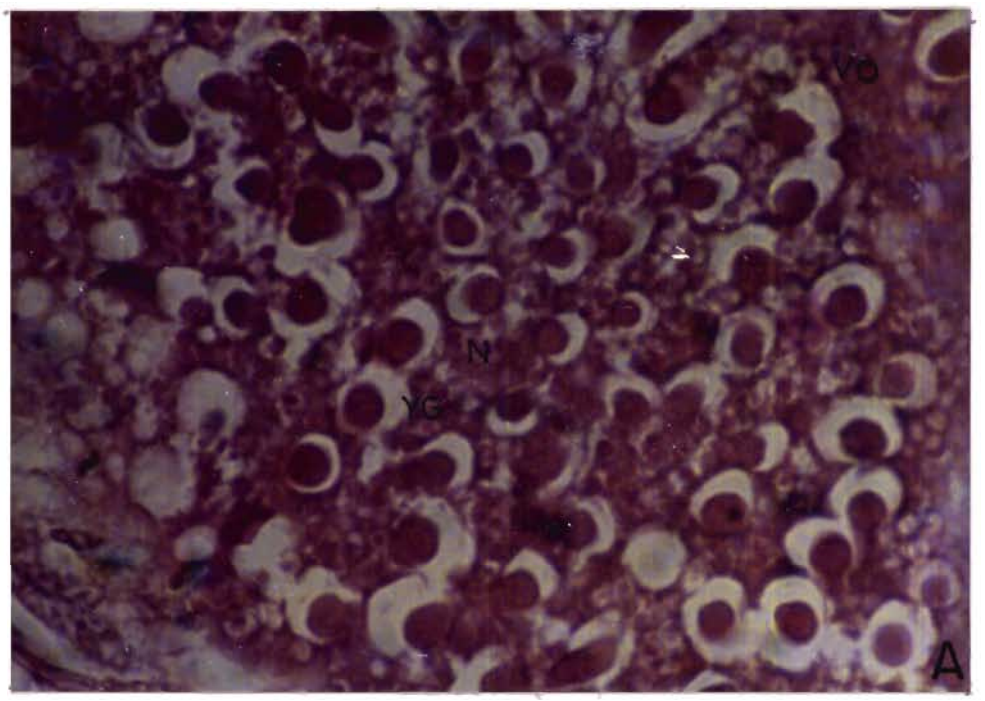


PLATE 19

A. Section of the vitellogenic oocyte showing the nucleoplasm with active yolk platelets and yolk globules (YG) and disappearance of nuclear membrane (NM →). Haematoxylin and Eosin. x 5000.

B. Semithin section of the vitellogenic oocyte showing the lightly stained nucleus (N) without inclusions and cytoplasm with yolk globules and yolk platelets and initial formation of cortical rods present in the periphery of ooplasm adjacent to oolemma (OL). The follicle cells (FC) are present only as a thin covering. Toluidine blue stain. x 1000.

PLATE 19



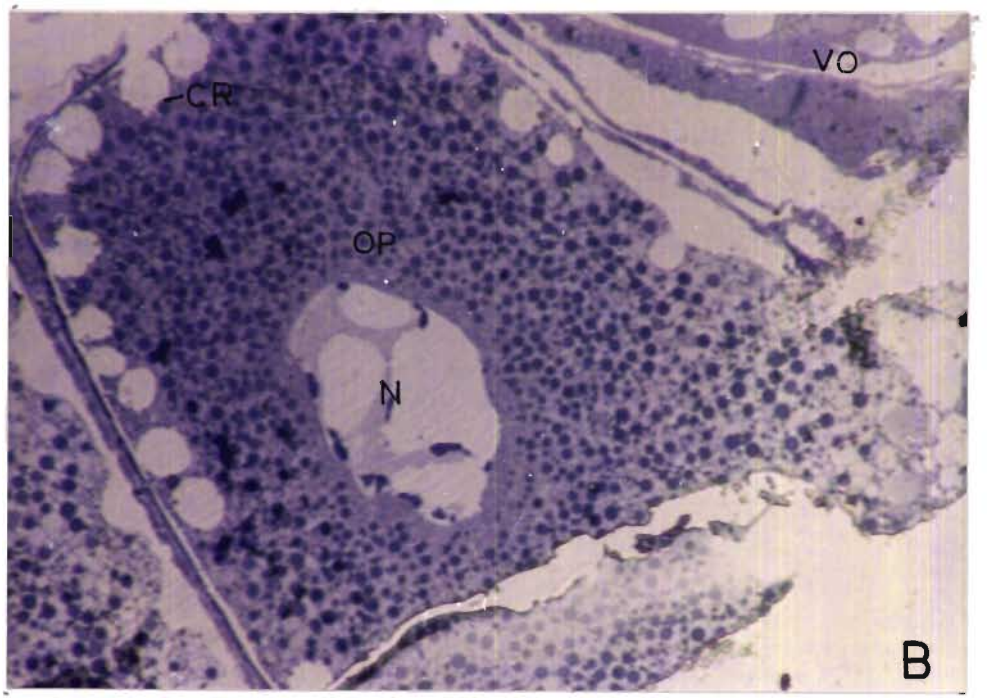
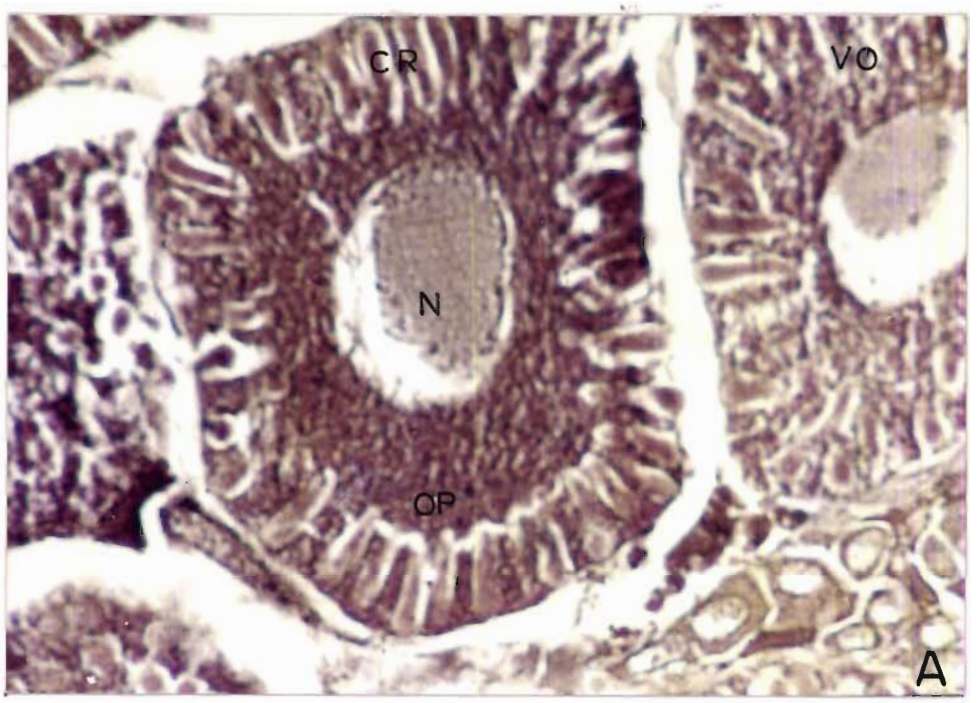


PLATE 20

A. Section of the vitellogenic oocyte stained with mercuric bromophenol blue showing the presence of protein in ooplasm, cortical rod and nucleus. x 1000.

B. PAS reaction in vitellogenic oocyte showing the aminogroups present in ooplasm and nucleoplasm except cortical rods in doubtful condition. x 1000.

PLATE 20



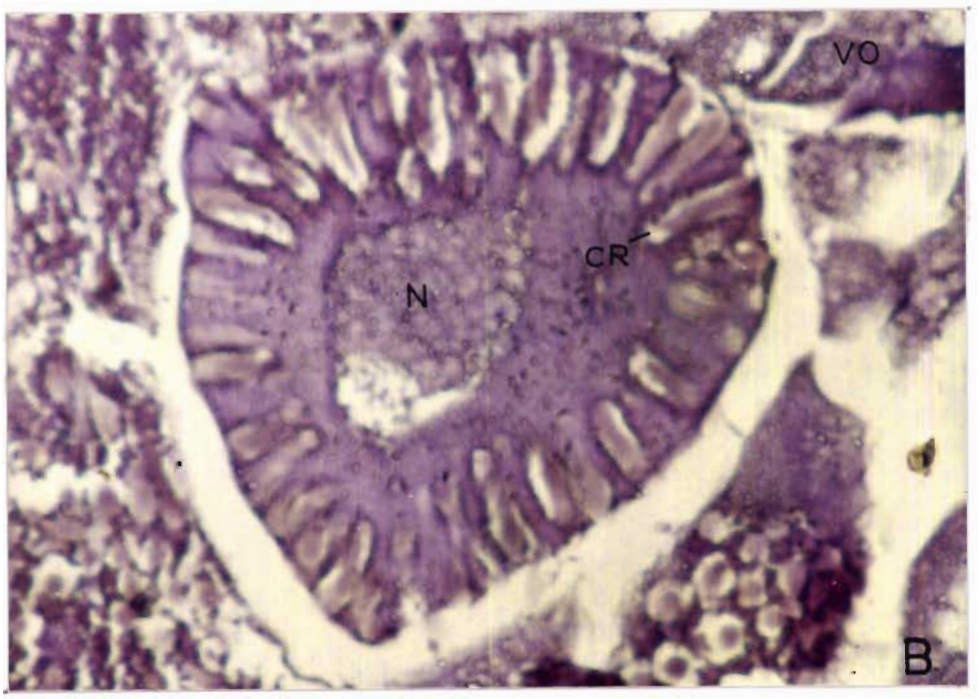
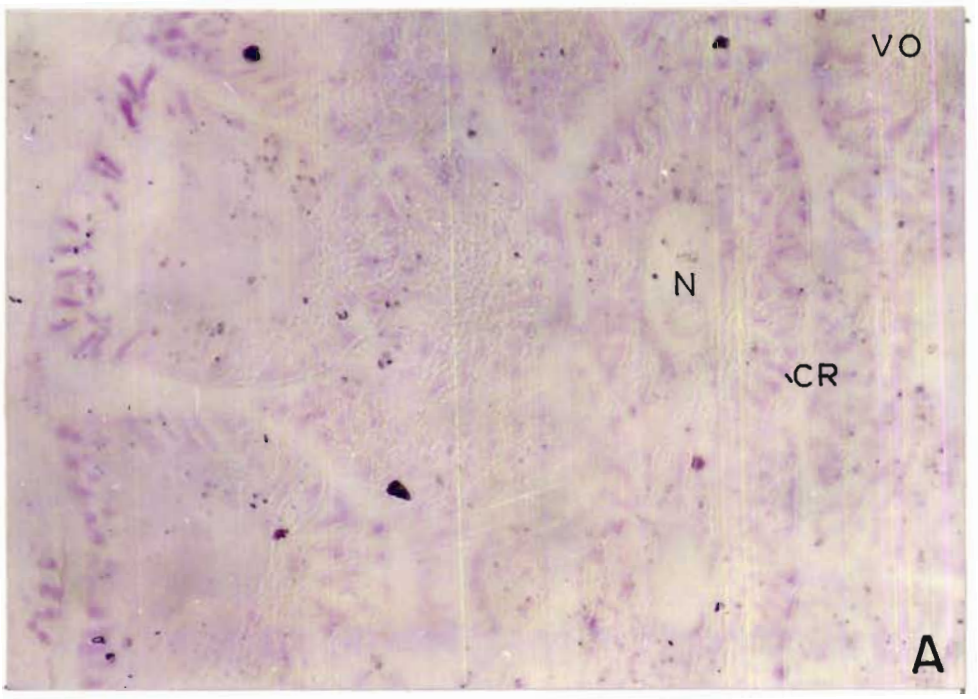


PLATE 21

A. Million's reaction in vitellogenic oocyte showing the tyrosyl groups present in cortical rods and the same absent in cytoplasm. x 500.

B. Toluidine blue reaction at pH 1.09 in vitellogenic oocyte showing the sulphated AMP present in cytoplasm. x 1000.

PLATE 21



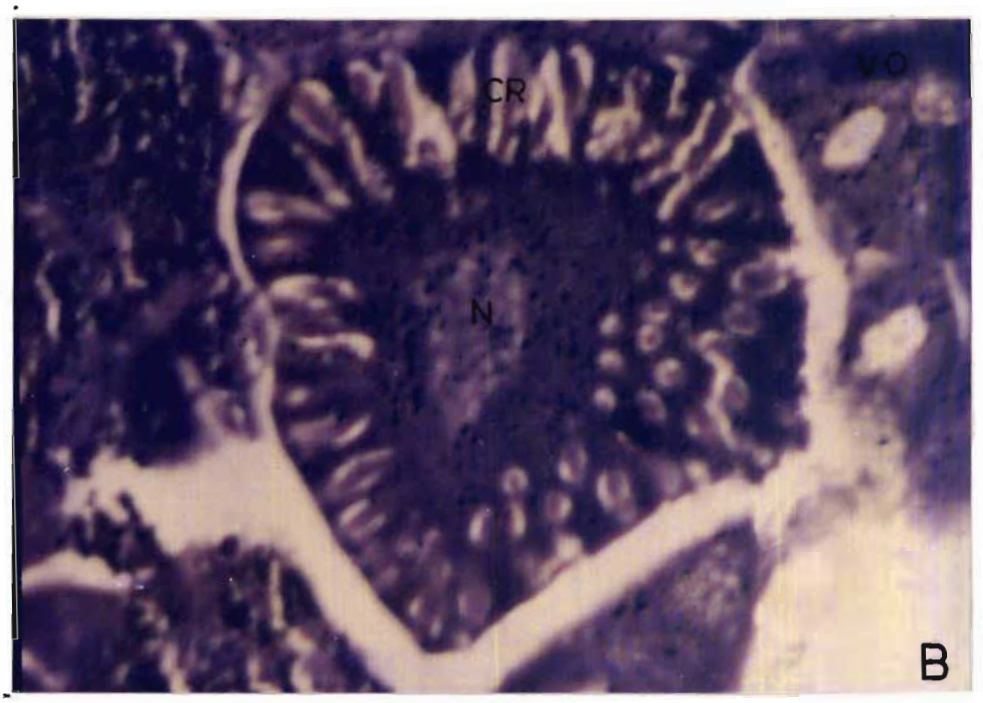
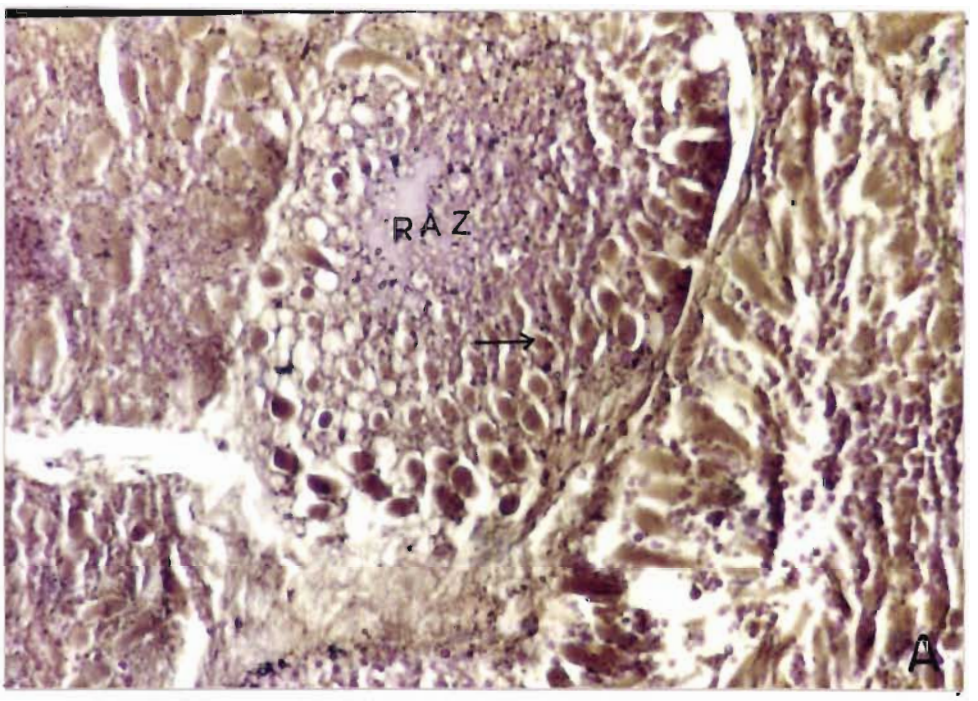


PLATE 22

A. Mercuric bromophenol blue reaction in partially spent ovary showing a disorganised zone of reabsorption (RAZ) and unsystematic orientation of cortical rods with the presence of protein in the oocyte. x 1000.

B. Section of the partially spent ovary showing atretic oocytes (AO) surrounded by follicle cells and oogonial cells (OC). Haematoxylin and Eosin. x 2000.

PLATE 22



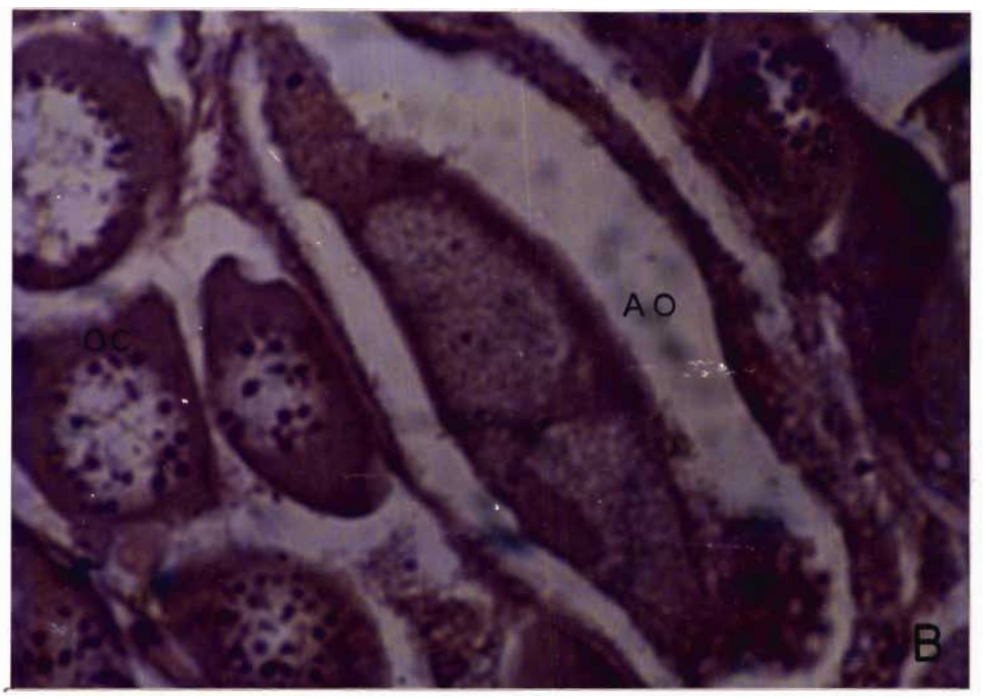


PLATE 23

A. Section of spent ovary indicating the presence of resorbing oocyte (ROC) and previtellogenic oocytes. Haematoxylin and Eosin. x 1000

B-C. Section of spent ovary. Aretic oocytes (AO) as well as pre and early vitellogenic oocytes are seen. Haematoxylin and Eosin. B: x 500, C: x 57.

PLATE 23

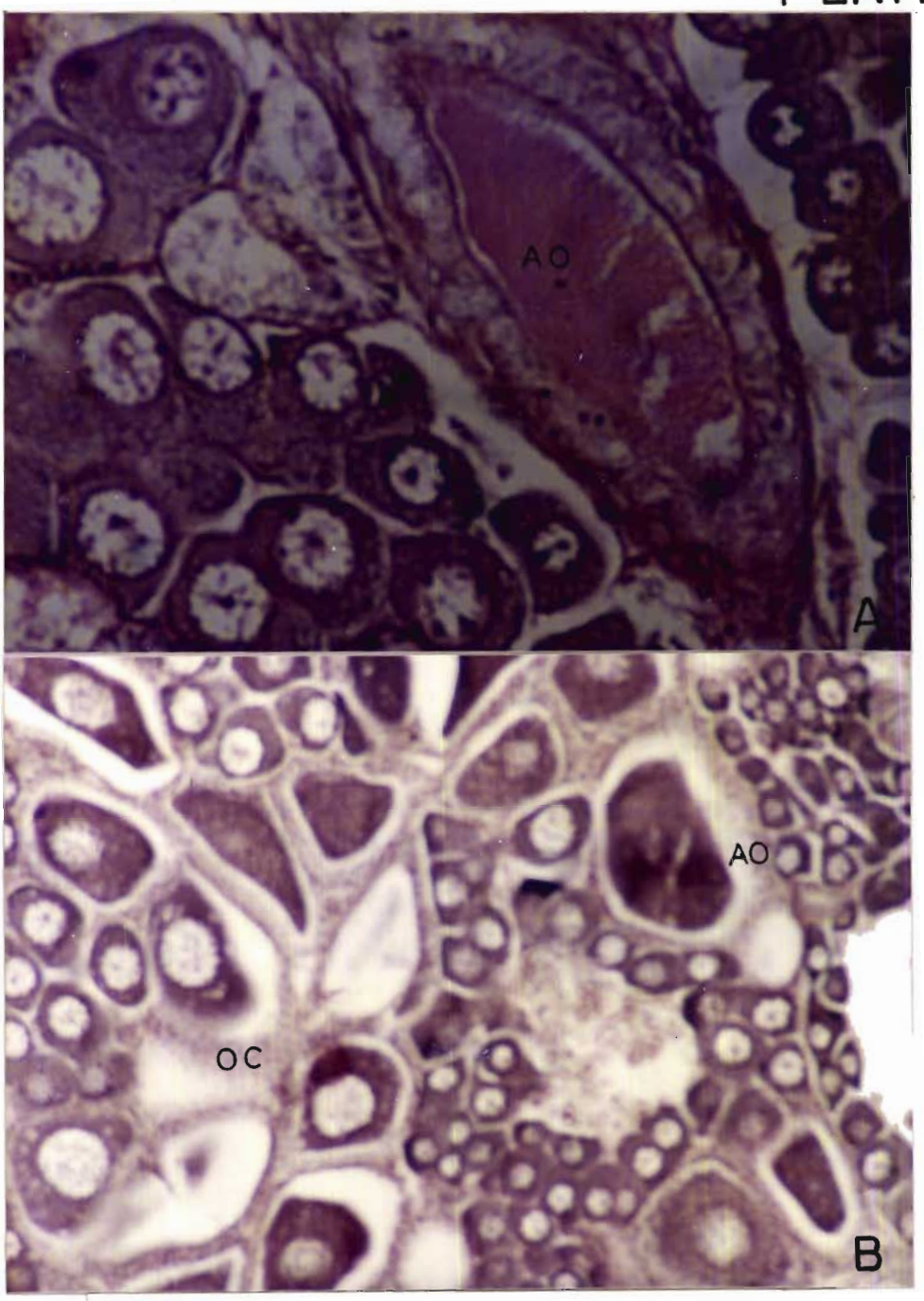


PLATE 24

A-D. Electron micrograph of an oogonial cells of immature ovary showing cellular details of cytoplasmic organelles and nucleolar material. M-Mitochondria, SER- Smooth endoplasmic reticulum A: x 2650, B: x 1550, C: x 4600, D: x 8440.

PLATE 24

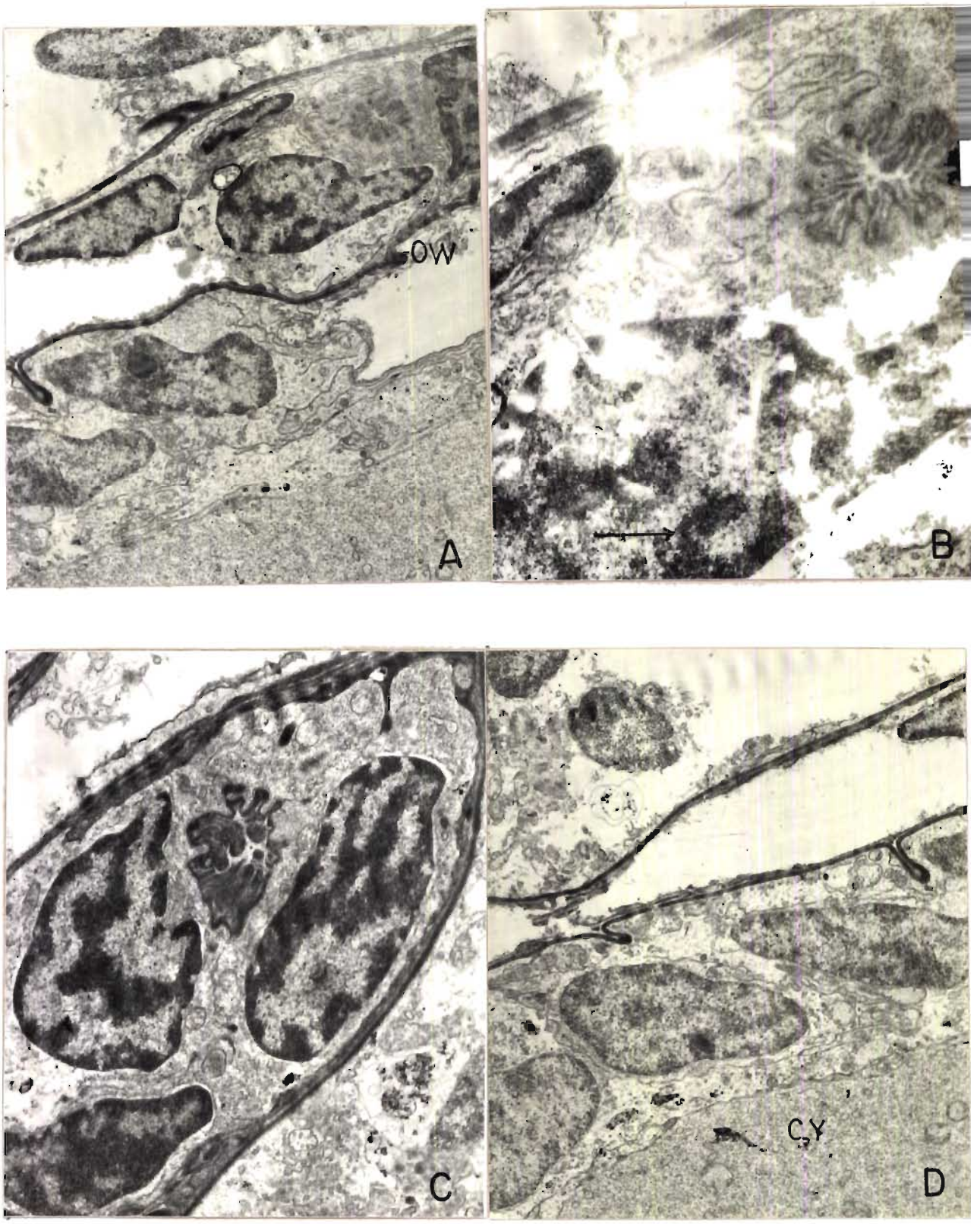
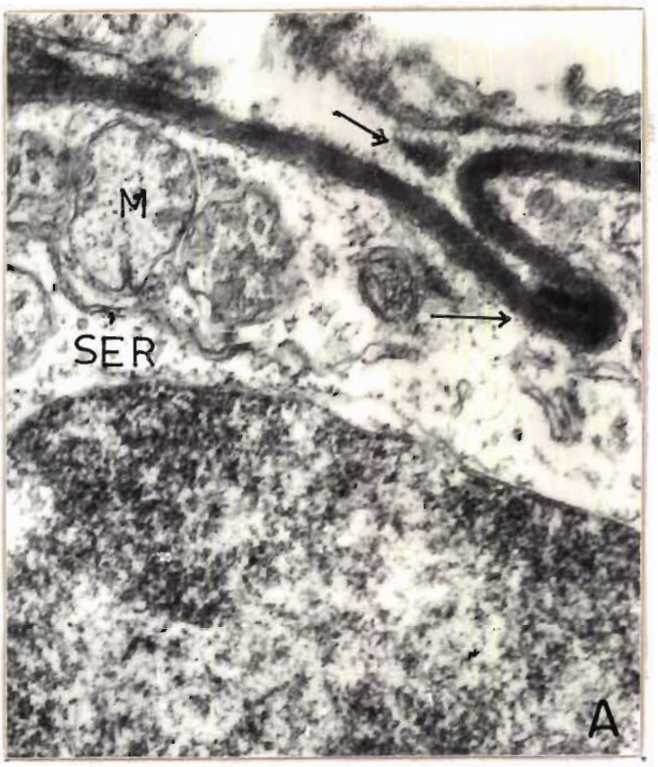


PLATE 25

A. Electron micrograph of an oogonial cell showing the details of cytoplasmic organelles like mitochondria and smooth endoplasmic reticulum. Note the formation of micropinocytotic vesicle. x 29841.

B. Electron micrograph of nucleoplasm in PVO showing the presence of nucleoli (NUL) as well as euchromatic (BCH) and heterochromatic (HCH) regions. x 6894.

PLATE 25



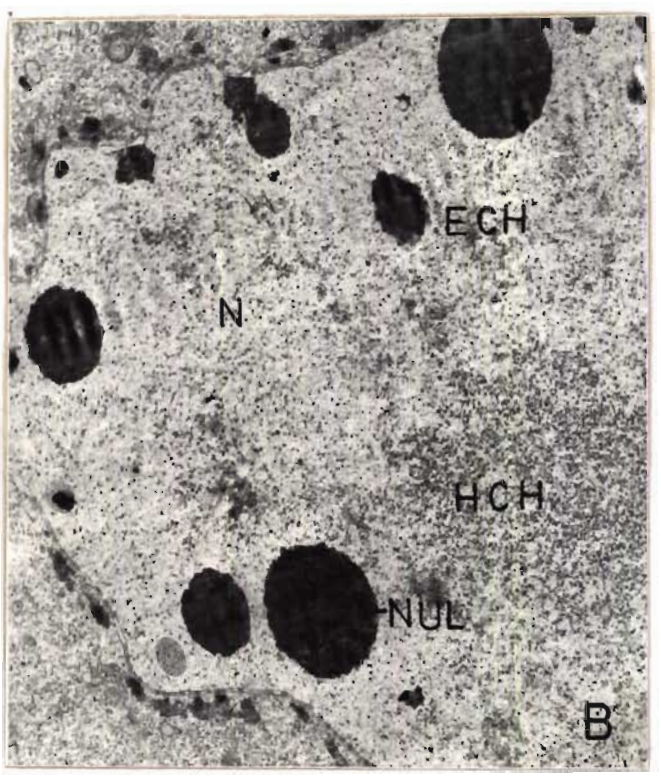


PLATE 26

A. Electron micrograph of EVO indicating the presence of yolk globules (YG), yolk vesicle (YV), yolk spheres (YS) and yolk platelets (YP) in cytoplasm and nucleoli (NUL) in nucleus. x 1950.

B. Electron micrograph of EVO showing the dense granular electron materials in cytoplasm (CY) and less dense granular electron materials in nucleus (N). CY-cytoplasm, N-nucleus, NM-nuclear membrane.

C. Electron micrograph of EVO showing the yolk globules in cytoplasm. x 16264.

D. Electron micrograph of early vitellogenic oocyte (EVO) indicating the cytoplasmic organelles like mitochondria with cristae, endoplasmic reticulum and free ribosomes in the cytoplasm. x 29700.

PLATE 26

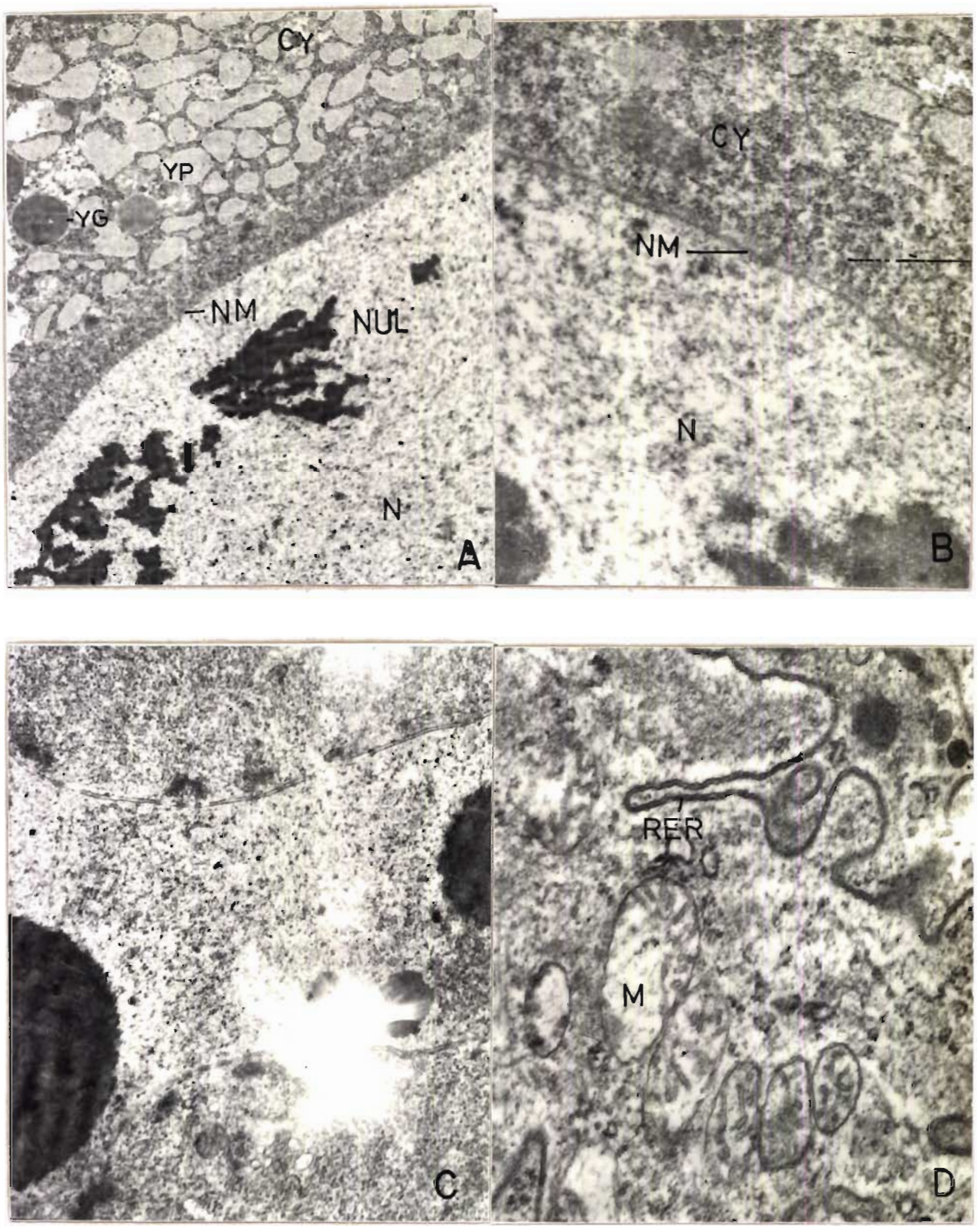
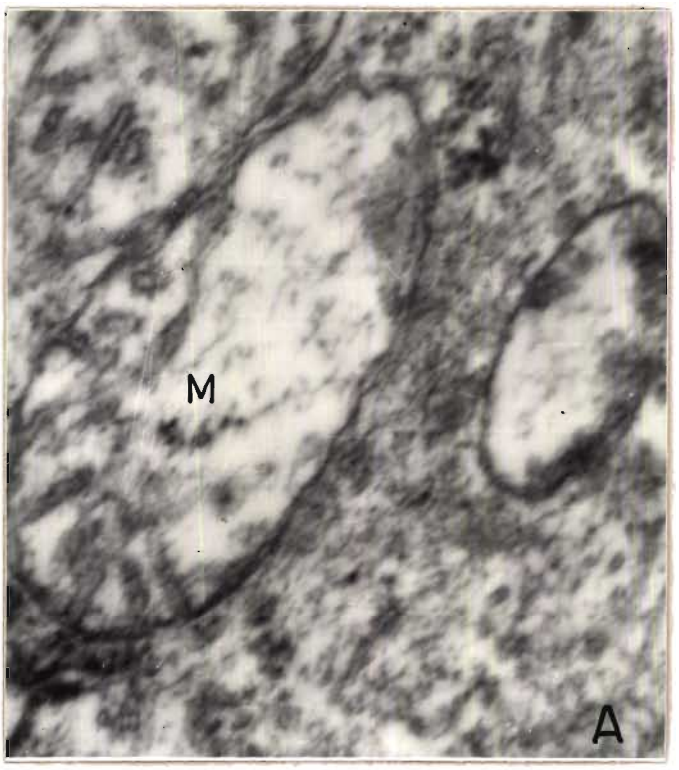


PLATE 27

A. Electron micrograph of EVO showing mitochondria in cytoplasm. x 76017.

B. Electron micrograph of nucleus of an early vitellogenic oocyte showing the presence of granular electron dense material in the nucleoplasm. x 76017.

PLATE 27



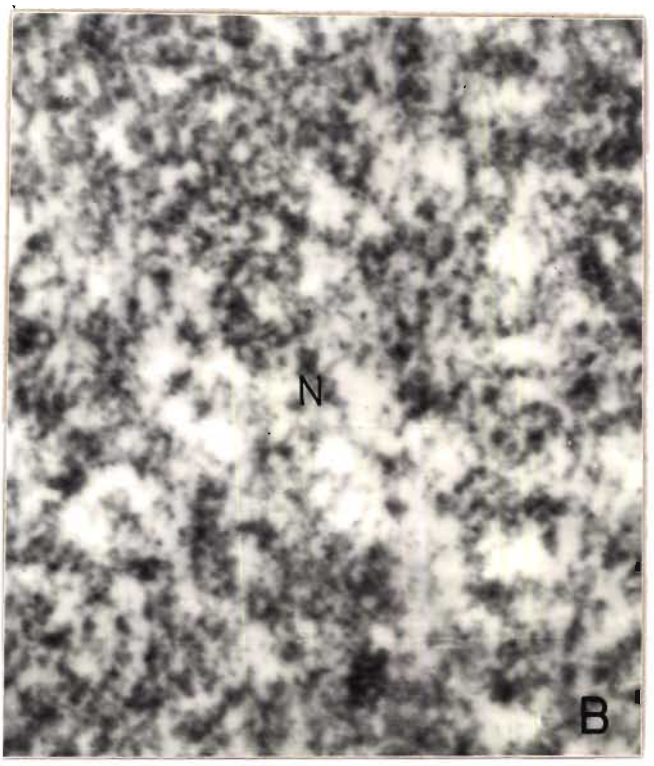


PLATE 28

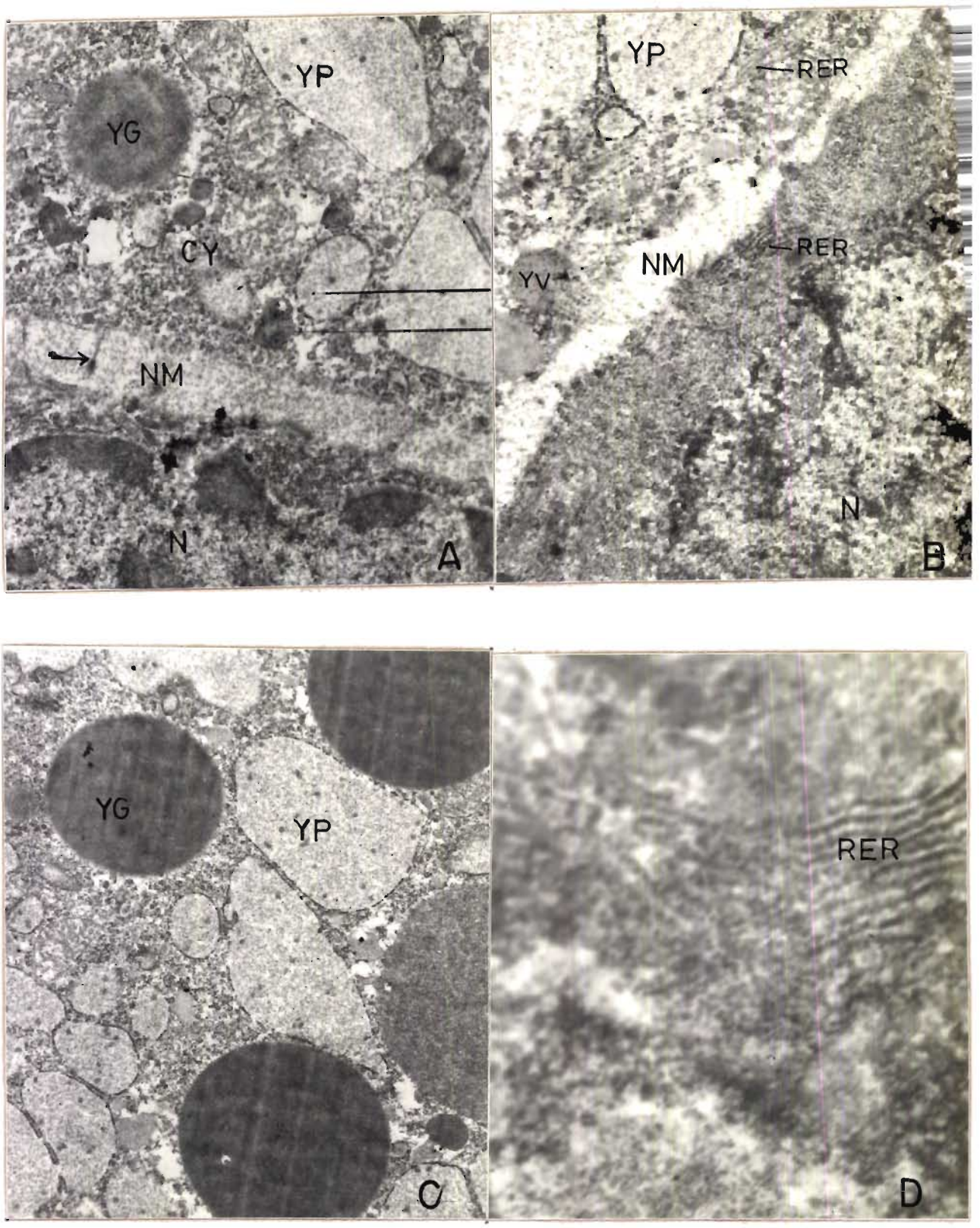
A. Electron micrograph of late vitellogenic oocyte showing the presence of yolk globules (YG) and yolk platelets (YP), and the exchange of materials between cytoplasm and nucleus (N) through pores (→) of nuclear membrane (NM). x 16264.

B. Electron micrograph of late vitellogenic oocyte (LVO) showing cytoplasm containing rough endoplasmic reticulum (RER) and yolk vesicle and nucleus containing rough endoplasmic reticulum. Note the enlargement of nuclear membrane. x 22275.

C. Electron micrograph of cytoplasm of a LVO showing the presence of yolk globules (YG), yolk vesicle (YV), yolk spheres (YS) and yolk platelets (YP). x 12021.

D. Electron micrograph of LVO indicating the presence of densely accumulated rough endoplasmic reticulum (RER) in the cytoplasm. x 54803.

PLATE 28



Vitellogenic oocytes (Plates 18-21)

Ripe or stage IV ovary is filled with vitellogenic oocytes which measure 288-416 µm in diameter. Vitellogenic oocyte is mainly characterized by the appearance of specialised elongated cortical rods or crypts, distributed along the peripheral margin of ooplasm adjacent to oolemma and abundance of yolk platelets and yolk globules (Plates 18-21). The cortical rod and ooplasm are found to be respectively pale eosinophilic and eosinophilic in nature. The nucleoplasm of oocytes is stained dull. The nucleolar membrane gets partly disappeared and the nucleoli number in the nucleoplasm gets reduced (Plate 19). The yolk globules present in the ooplasm apparently indicates the period of ovulation. The follicle cells are further flattened and appeared only as a thin covering (Plate 18-21).

Spent oocytes (Plates 22-23)

Oocytes of spawned or spent prawns showed characters identical to those of previtellogenic and early vitellogenic oocytes (Plate 23). The spent ovary displayed empty follicles, sites of oocyte resorption and areas of proliferative growth of oocyte (Plate 23). Further, a disorganized zone of reabsorption is found to be located at the core of the cyst. The oocyte atresia is commonly found to occur in the spent stage. This is a degenerative process by which the oocytes in various stages of development and differentiation are unrecognisable in the ovary (Plate 23). Partially spawned oocyte apparently indicated the mature ova undergoing breakdown and subsequently resorbed, due to its unsystematic orientation of the rod-like peripheral body (Plate 22). During this spent stage, the pre and early vitellogenic oocytes are found to have basophilic cytoplasm and nucleoli. These oocytes are surrounded by follicle cells (Plate 23).

Spermatogenesis (Plates 29-32)

Histological section of testis revealed that it is covered by a thin wall or cortex. The cortex consists of two layers: an outer epithelial layer and an inner layer of connective tissue (Plate 29). No muscular tissue is present. The secretary granules are present in the epithelial cells, which are presumed to supply the nutritive material for cellular development. The body of the testis is composed of a mass of very minute convoluted seminiferous tubules or acini (Plate 29) in which the male reproductive cells or sperm cells are produced (Plate 31). The membranous wall of acini is made of two layers (Plate 29): the outer tunica and the inner germinal epithelium. In immature prawn, the seminiferous tubules made of two layers are clearly visible (Plate 29). In the cross section of the testis, a strand-like germinal zone is seen adjacent to the acinar wall (Plate 29) and the testis is filled with primary spermatogonial cells (Plate 29) having conspicuous nuclei. The nurse cells with nuclei and clear cytoplasmic boundary are also seen. The nurse cells are found in between the spermatogonial cells and particularly on the periphery of the germinal zone. By virtue of their close association with the spermatogonial cells, it is assumed that the nurse cells have nutritive and supportive roles. The nutritive cells are also present in between the testicular acini (Plate 29) and are being supported by haemal sinuses (Plate 29). In mature prawns, the testicular acini made of outer tunica seems to be disappearing. The inner layer of germinal epithelium also partly disappears with the progression of spermatogenesis (Plate 30). Spermatogenesis is found to progress towards the central lumen of the acini and therefore subsequent development stages like spermatocytes and spermatids are formed in the central portion of the acini (Plates 30-31). Each spermatogonium passes through a period of quick growth to become a primary spermatocyte. The primary spermatocytes undergo meiotic division and result in two secondary spermatocytes (Plates 30-31). These divide again to produce four spermatids which develop without further division into spermatozoa.

Spermatogenesis involves progressive reduction of cytoplasm and chromatin material with successive divisions. The spermatogonial cells are the largest, followed by spermatocytes and spermatids.

The spermatids gradually get enlarged and move out of their cell boundaries (Plates 30-31). The cytoplasmic boundaries gradually disappear (Plate 31) and the lumen of the tubule gets filled with spermatids. These spermatids grow in size and develop into spermatozoa (Plates 31-32).

Histological section showed that the nuclei of spermatogonial cells are basophilic while the cytoplasm are eosinophilic. The spermatocytes have more condensed nuclei and the diakinetic stages are frequently seen among them as is common in the dividing cells. Spermatids undergo reduction in size and condensation of chromatin matter. The spermatozoa develops from these cells through cellular differentiation without further reduction in size. The mature sperm cell has a more or less spherical body with a long spike giving the whole structure a tack-shaped appearance. In addition to the mature spermatozoa in the above typical form, other developing stages bearing shorter spikes in different forms or no spike are also noticed in the sections. The main body of the sperm has a diffused nuclear region containing chromatin which is partially surrounded by a cytoplasmic band (Plate 36). The nucleus is separated by a nuclear band or nuclear wall from the 'Y' shaped acrosome vesicle. Thus, the sperm of *P. semisulcatus* is composed of a diffused nuclear region, a cytoplasmic band, an acrosome and a spike.

Histology of vas deferens and spermatophore formation (Plates 33-42)

A detailed histological investigation was carried out to study the structure of the vas deferens and the role played by various regions of vas deferens in the formation of spermatophore. Three successive stages can be recognished during the formation of the spermatophore. In all the three stages, the participation of certain secretions produced by the glandular epithelial cells

which line the vas deferens is involved. In the first stage, the sperm cells which are concentrated at the centre of the seminiferous tubule, are drained into the proximal vas deferens where they become a compact sperm mass. In the second stage, the sperm mass further gets compacted and the main layers of the spermatophore and the partly independent accessory wings are formed. In the third stage, the compact and convoluted spermatophore enters the terminal ampoule where it takes the final complete shape before extrusion.

The histology of the proximal, middle and distal vas deferens, terminal ampoule and the formation of the spermatophore are described below.

Proximal vas deferens (PVD) (Plate 33)

Judging from its slender appearance, the proximal vas deferens may be considered as the conducting tube. The tube consists of two layers; an outer thin layer of connective tissue and an inner thick wall of longitudinal muscle tissue (Plate 33a). The sperm mass concentrated in the testis is passed into the proximal vas deferens. Some accessary glands or glandular cells are seen in the wall of proximal vas deferens (Plate 33a). Histology section showed that the proximal vas deferens not only performs the function of conducting the sperm cells from testis to the middle vas deferens by peristaltic movement (Plate 33c), but assists through the production of some binding secretion to the sperm mass.

Middle vas deferens (MVD) (Plates 33-35)

The proximal vas deferens further dilates to form the blind pouch of middle vas deferens (Plate 33b) which in turn continues as the ascending limb. The blind pouch serves as a storage site for the sperm. The ascending limb of the MVD is divided internally into two unequal ducts by a connective tissue septum (Plate 34). The larger one is known as spermatophoric duct and small one is known as wing duct.

The wall of the sperm duct consists of glandular epithelial cells, circular muscle fibres and in between connective tissues. The sperm duct is lined with glandular cells and small typhlosoles which secrete the spermatophore matrix and spermatophore layers (Plate 34). The basophilic single round vesicular nucleus with an eccentric nucleoli is observed in the secretary cells. The sperm duct is surrounded by the secondary spermatophore layer. It consists of amorphous granular epithelial cells and is eosinophilic in nature (Plate 34). The sperm mass is surrounded by primary spermatophore layer which is amorphous agranular in nature. The cross sections of the ascending limb of the MVD showed that the wing duct secretory cells are identical to that found in the sperm duct and are involved in the secretion of the wing of the spermatophore. The glandular epithelial septum is known to be originated from the continuation of the glandular epithelial wall and further separates the small elongated wing duct and larger sperm duct (Plates 34-35). From histological section, it is apparent that the glandular epithelial cells involve in facilitating the development of wing duct and the wing to be attached to the main body of the spermatophore.

Distal vas deferens (DVD) (Plates 35-36)

The distal vas deferens is the portion of the vas deferens having long slender tubular structure that follows the descending limb of the middle vas deferens. The DVD finally opens into the terminal ampoule. The lumen of the distal vas deferens gets reduced and the spermatophore becomes a compact mass (Plate 35). The transverse section of distal vas deferens showed that the sperm mass accumulated in the lumen of the distal vas deferens is surrounded by primary and secondary spermatophore layers. The amorphous nongranular primary spermatophore layer is surrounded with glandular secondary spermatophore layer. The primary spermatophore layer is moderately stained with toluidine blue, while the secondary spermatophore layer is intensively stained with toluidine blue. In this stage, the sperm duct with glandular secretory granules becomes active in the formation of spermatophore layers.

In the sperm duct, sperms with the long spike could be clearly seen. The glandular septum located in between sperm duct and wing duct appeared to be very active.

Ultrastructure of the distal vas deferens (DVD) (Plate 41)

Transverse section of the distal vas deferens has been studied using Scanning Electron Microscope. The ultrastructure of DVD showed that it contains three chambers. Chamber I filled with sperm mass covered by primary spermatophore layer (PSL) (Plate 41). Accessary layer could be seen along with PSL. A thick secondary spermatophore layer (SSL) is found to be overlapped with PSL and forms chamber II which contains the sperm mass. Chamber III is covered with thick muscle spermatophore layer III.

Terminal ampoule (TA) (Plates 38-39)

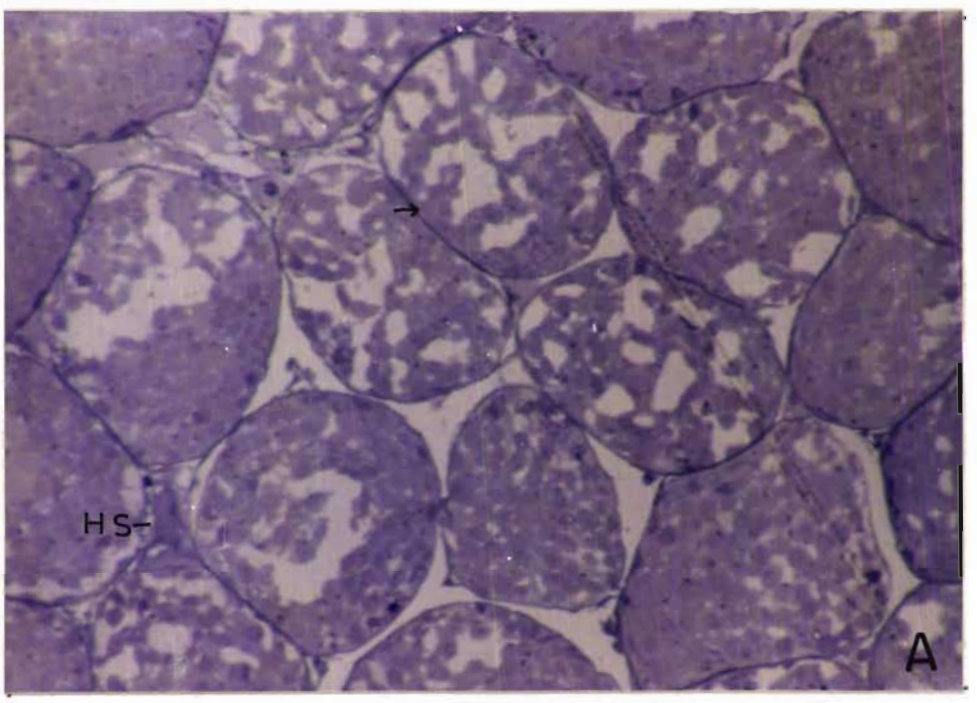
The DVD dilates to form the terminal ampoule or the ejaculatory duct which is found embedded in the coxae of the fifth pereiopod. The final and complete moulding of the spermatophore takes place in terminal ampoule. The cross section showed that the wall of the terminal ampoule is made up of an outer layer of circular muscle and an inner layer of longitudinal muscle, the inside of which is lined with secretary epithelial cells. The secretory granules found in the wall is identical to sperm duct. The secretary granules are shown to have vacuolated cytoplasm and basophilic nucleus (Plates 38-39). With the muscular contraction of the terminal ampoule, the spermatophore gets ejaculated along with the wing. The adhesive material secreted by the glandular epithelial layer helps in cementing the two spermatophores released simultaneously from the right and left terminal ampoule.

PLATE 29

A. Semithin section of immature testis showing seminiferous tubules and haemal sinuses (HS) in between. Note the presence of spermatogonial cells (SG) in the acini. Toluidine blue stain. x 185.

B. Semithin section of seminiferous tubule of the immature testis enlarged showing proliferation of spermatogonial cells (primary and secondary) from germinal zone. Seminiferous tubule made of outer tunica and inner germinal epithelium layer (→) nurse cells (NC) present outside the tunica and inside the seminiferous tubule adjacent to inner germinal epithelium layer and also in between spermatogonial cells, and haemosinuses present inbetween seminiferous tubules are clearly visible in this stage. PSG -primary spermatogonial cells, SSG - secondary spermatogonial cells, NC - nurse cells. Toluidine blue stain. x 741.

PLATE 29



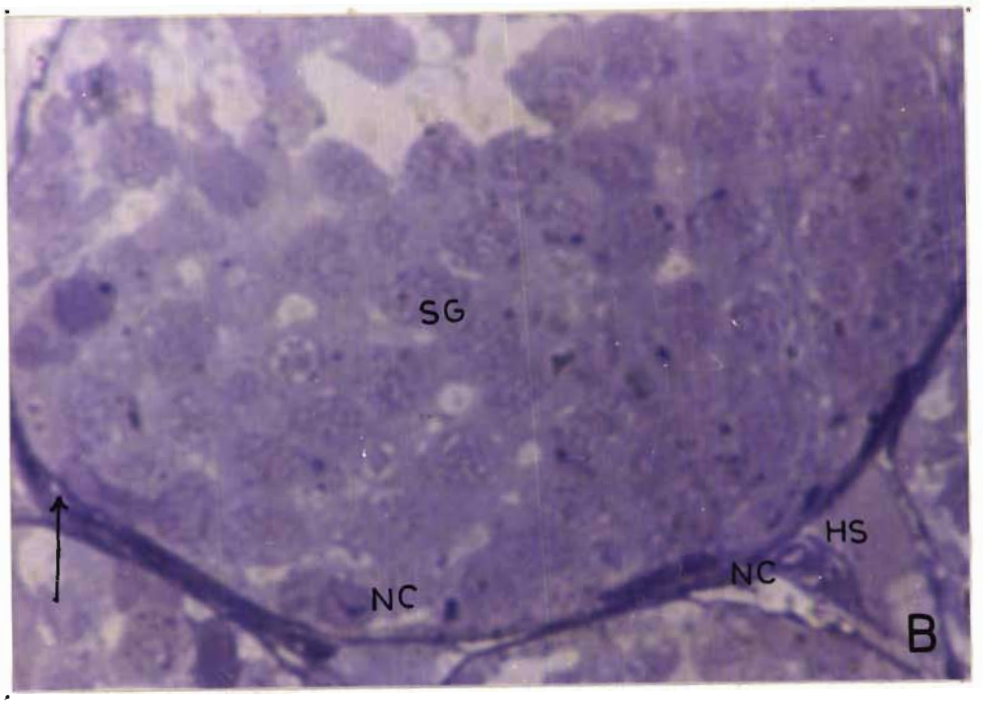
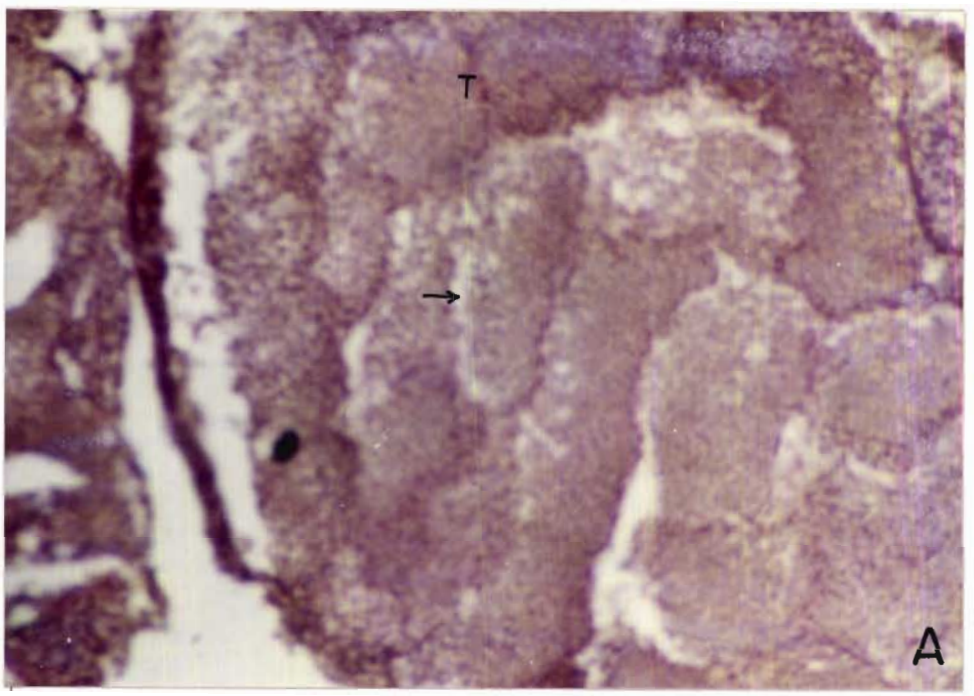


PLATE 30

A. Section of mature testis showing seminiferous tubules made of two layers in the process of disintegration (→). Haematoxylin and Eosin. x 500.

B. Section of mature testis showing seminiferous tubules filled with primary and secondary spermatocytes (PSC & SSC) and spermatids (ST). Note the metamorphosis of spermatocyte to spermatid. Haematoxylin and Eosin. x 1000.

PLATE 30



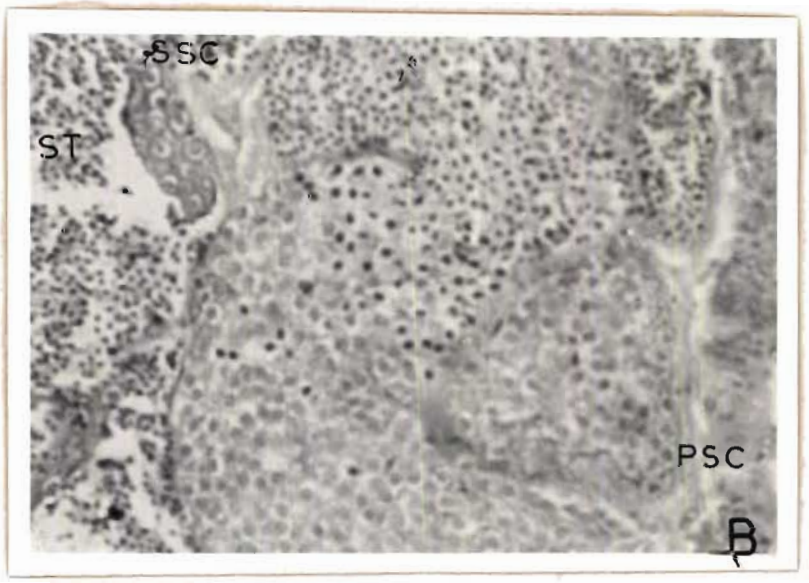
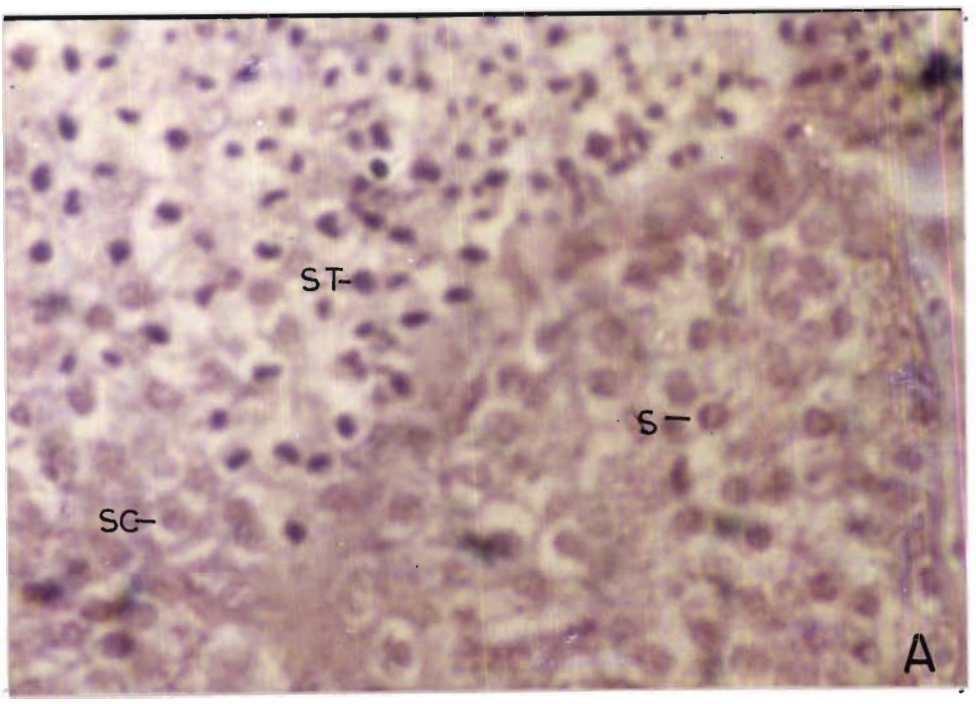


PLATE 31

A. Section of mature testis showing transformation of spermatids to spermatozoa (S). Haematoxylin and Eosin. x 2000.

B. Section of mature testis surrounded by epithelial cells (EC). Mallory's Triple stain. x 500.

PLATE 31



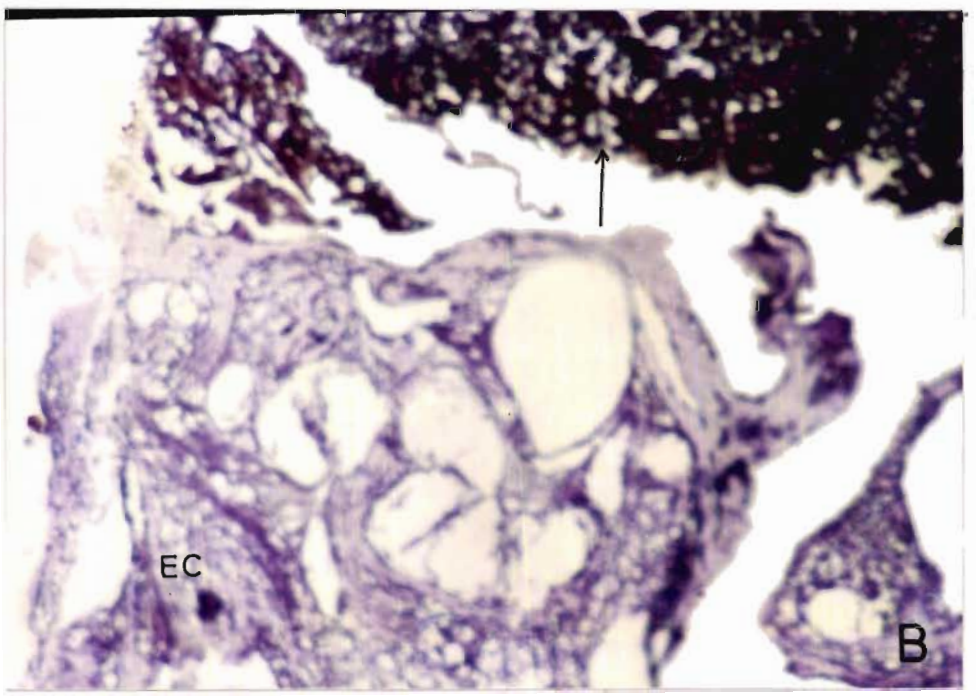
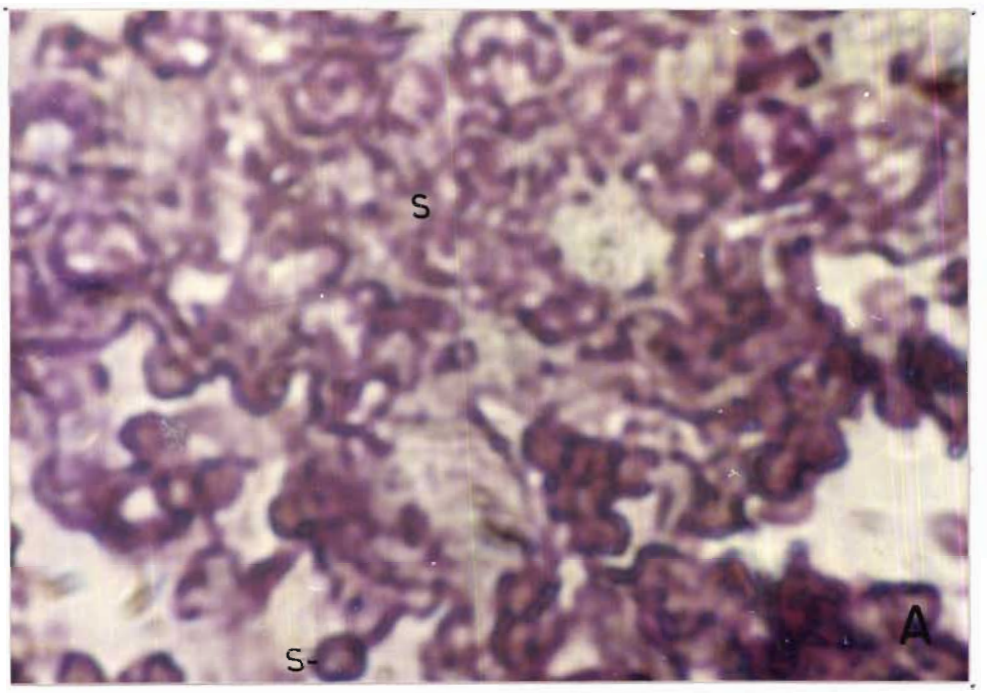


PLATE 32

A. Section of mature testis showing fully formed sperms (S) in seminiferous tubules. Note the spike (→) present in the sperm. Mallory's Triple stain. x 5000.

B. Sudan Black B reaction in mature testis showing the presence of lipid in sperms. x 1000.

PLATE 32



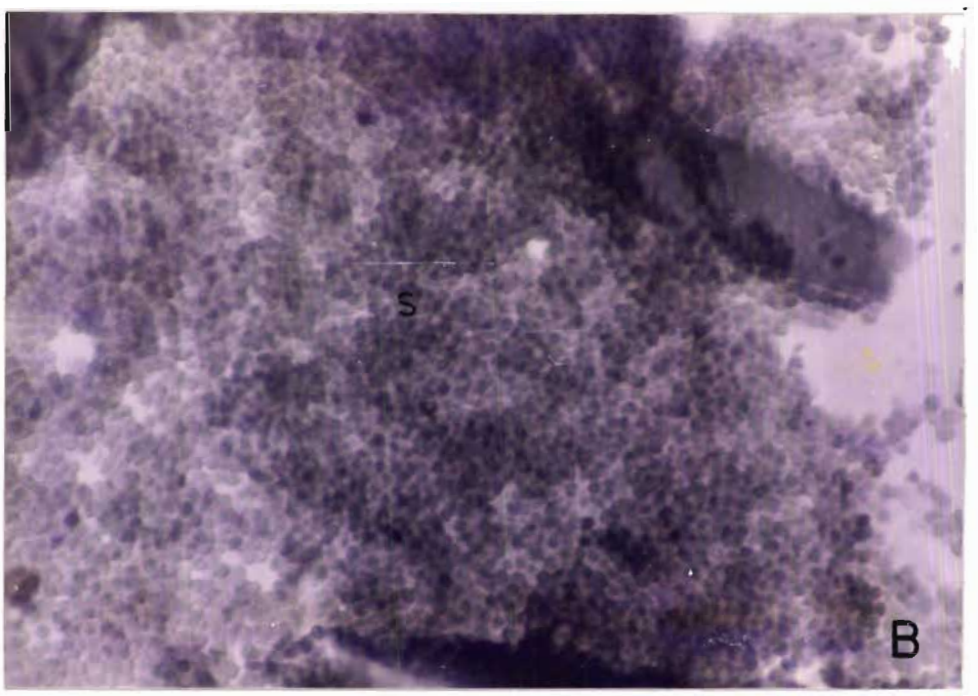
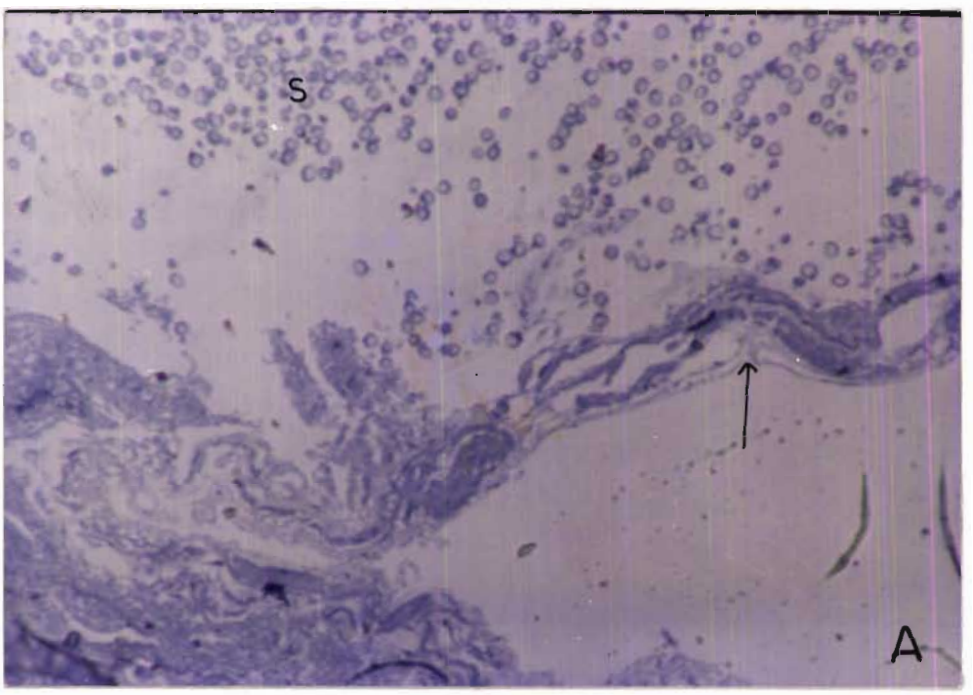


PLATE 33

A. Semithin section of proximal vas deferens (PVD) showing the draining of sperms from testis to vas deferens by peristaltic movement (→). Toluidine blue stain. x 1000.

B. Section of middle vas deferens (MVD) showing sperm duct and wing duct separated by septum (SEP), and typhlosoles (TS). Mallory's Triple stain. x 500.

PLATE 33



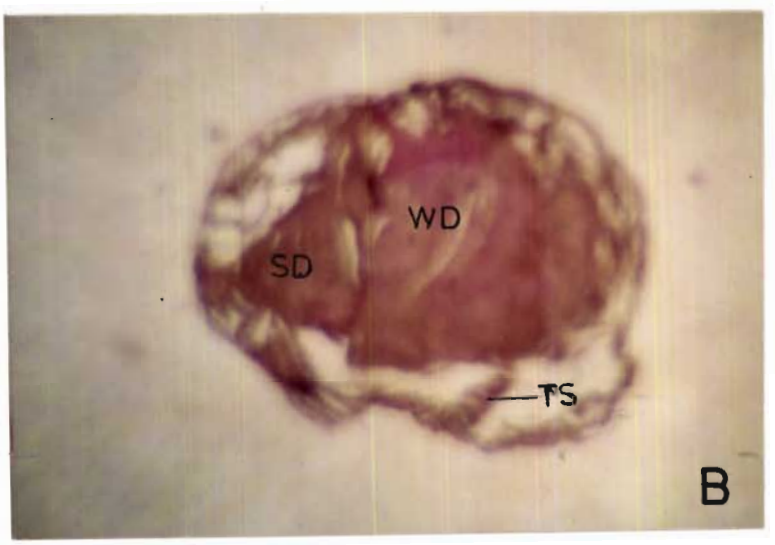
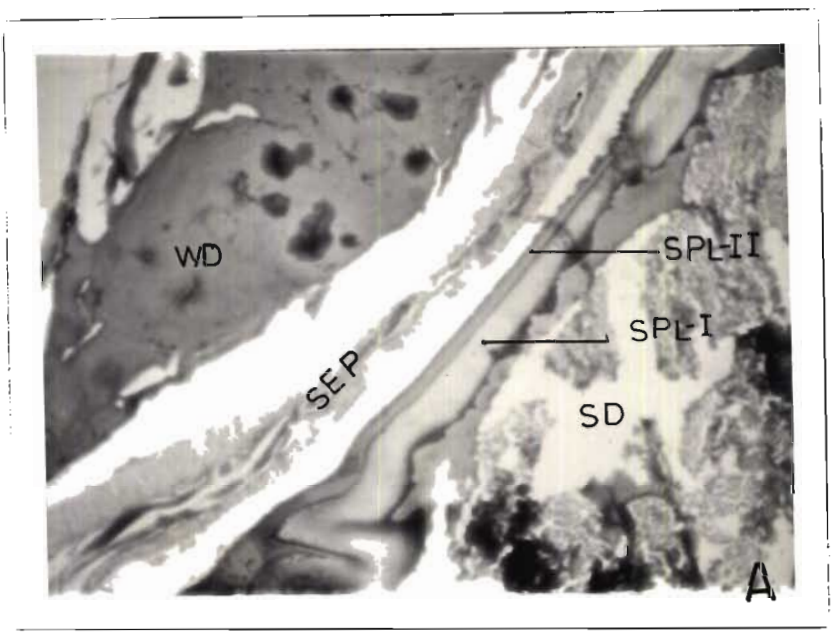


PLATE 34

A. Section of middle vas deferens showing wing duct (WD) and sperm duct (SD) seperated by SEP male up of connective tissue with gland. Primary and secondary spermatophore layers (PSL & SSL) are seen in sperm duct encompassing sperms. Haematoxylin and Eosin. x 1000.

B. Section of SD of middle vas deferens showing PSL & SSL. Note the wall of SD and WD containing connective tissue with gland and circular muscle fibre (CMF →). Haematoxylin and Eosin. x 1000.

PLATE 34



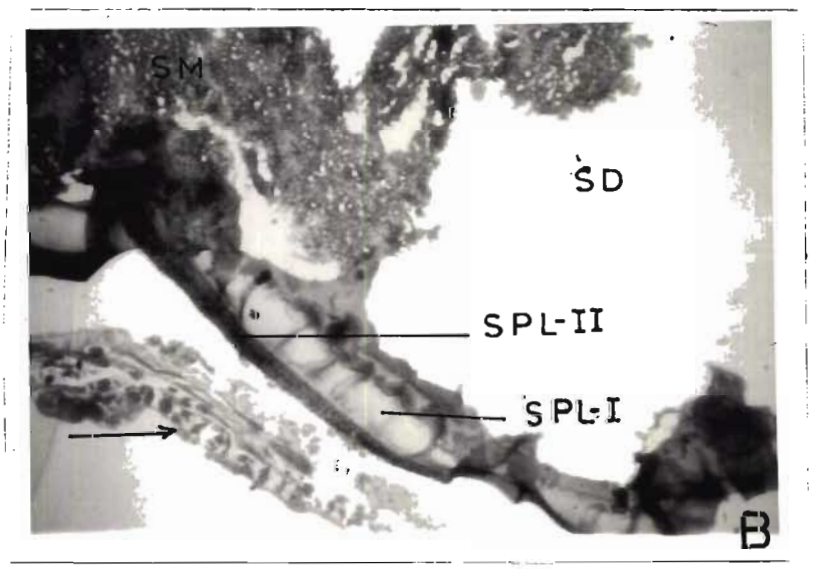
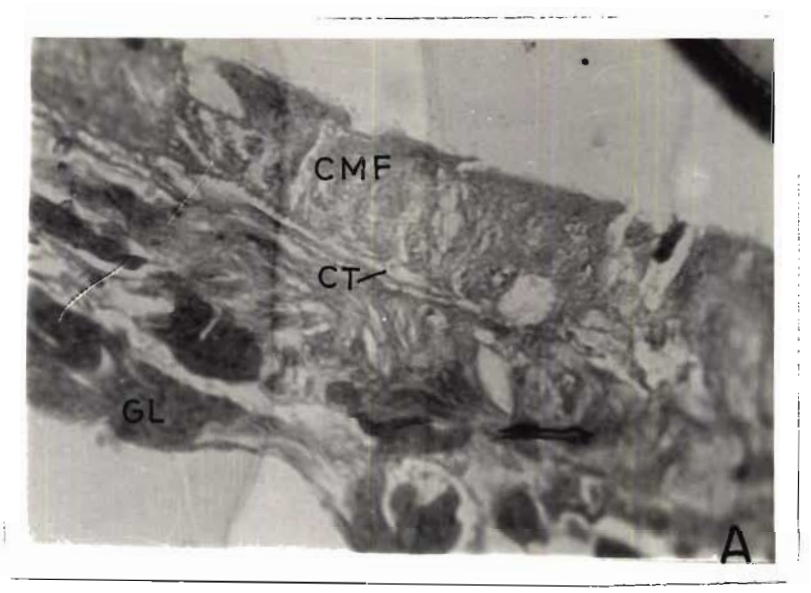


PLATE 35

A. Section of the wall of SD and WD displaying the presence of connective tissue with gland and circular muscle fibre. Haematoxylin and Eosin. x 2750.

B. Semithin section of distal vas deferens (DVD) filled with sperm mass (SM) being drained from MVD. In DVD, lightly stained PSL and deeply stained SSL are clearly seen. Toluidine blue stain. x 742.

PLATE 35



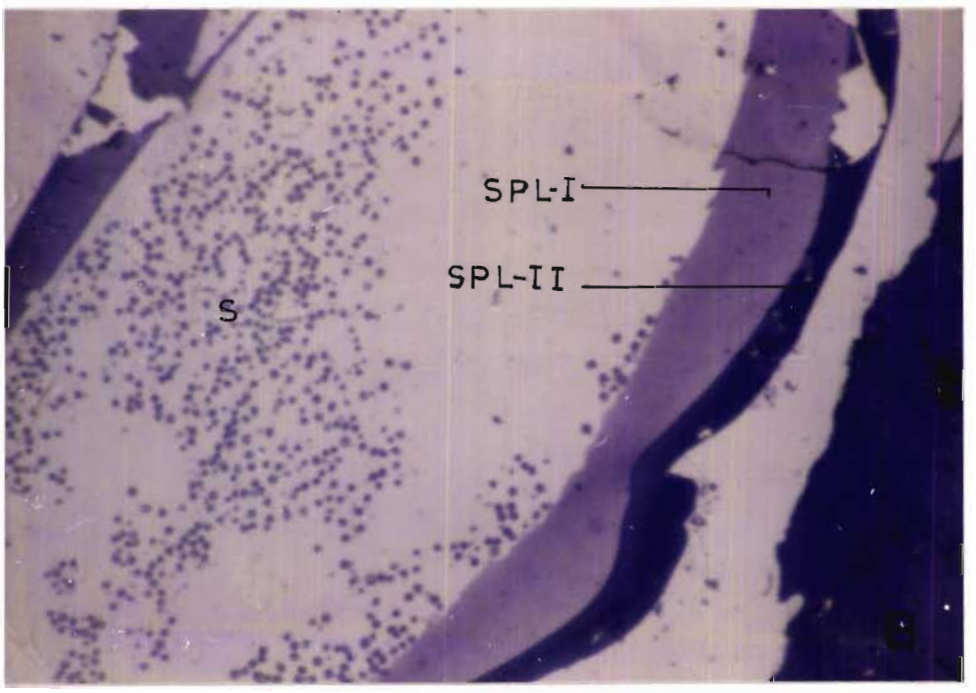
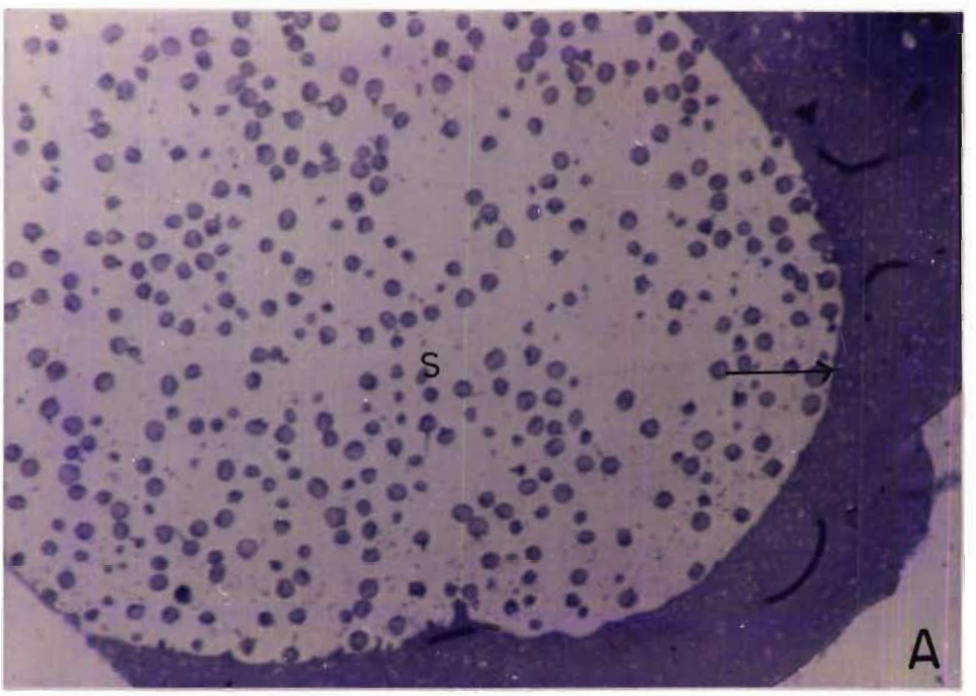


PLATE 36

A. Semithin section of DVD containing sperm mass (SM) surrounded by primary spermatophore layers (→). Toluidine blue stain. x 1854.

B. Semithin section of DVD showing sperms with short long spikes arising from 'Y' shaped acrosome vesicle (→). Toluidine blue stain. x 7500.

PLATE 36



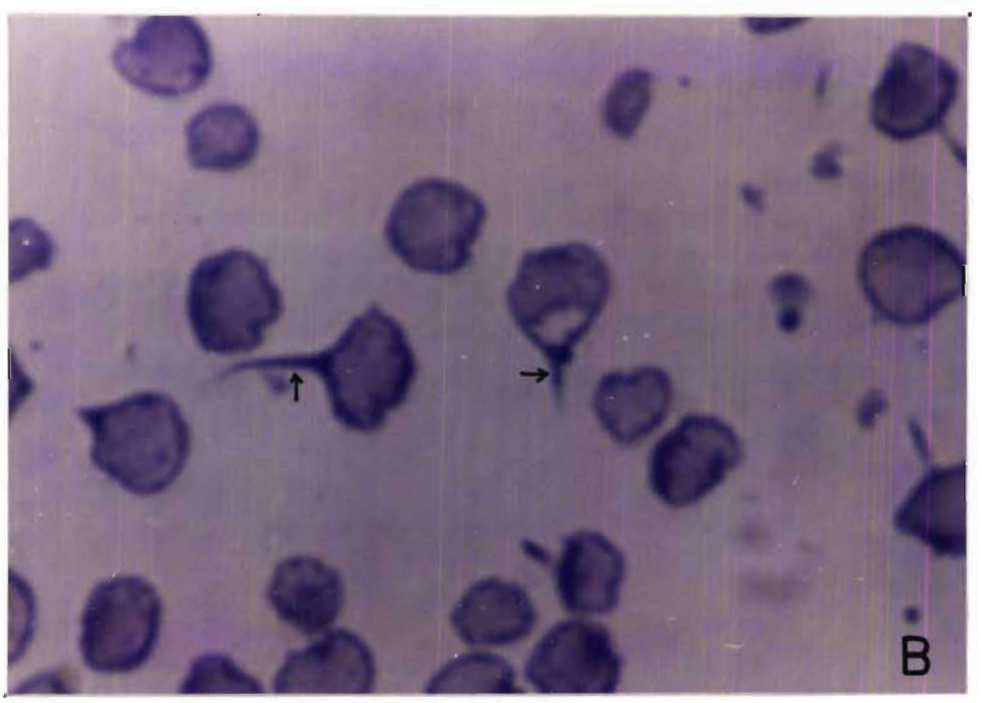
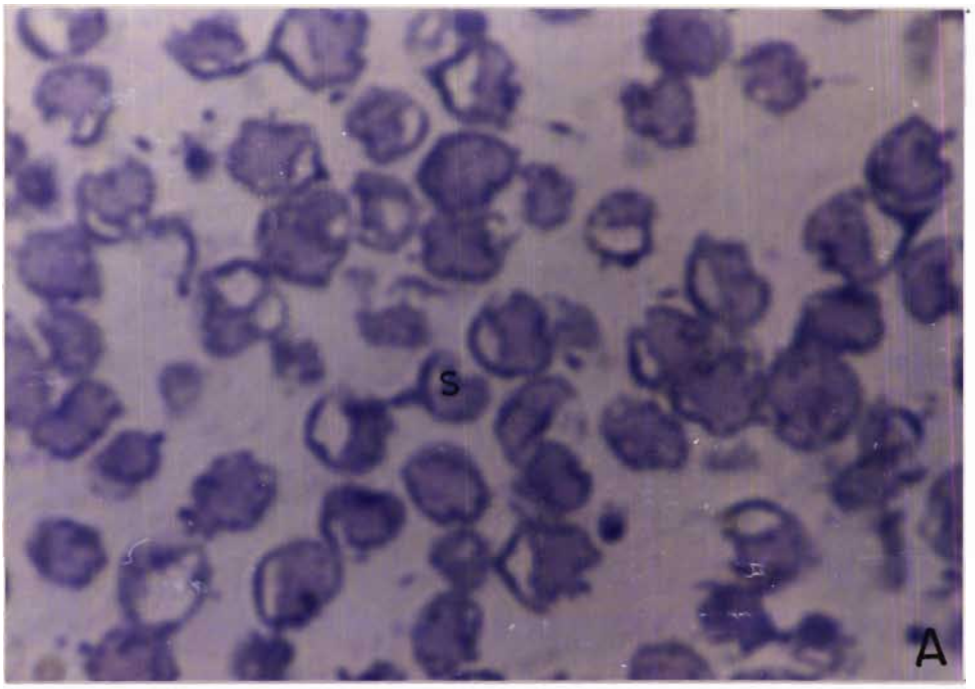


PLATE 37

A. Semithin section of extruded spermatophore showing the presence of sperms. Toluidine blue stain. x 5000.

B. Semithin section of extruded spermatophore filled with sperm mass. Toluidine blue stain. x 2250.

PLATE 37



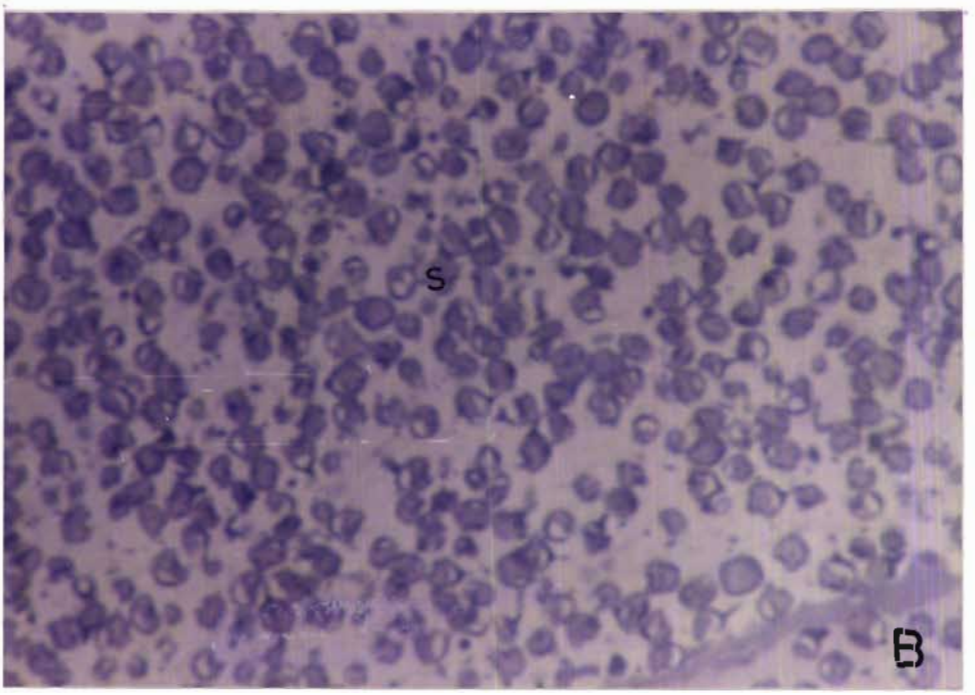
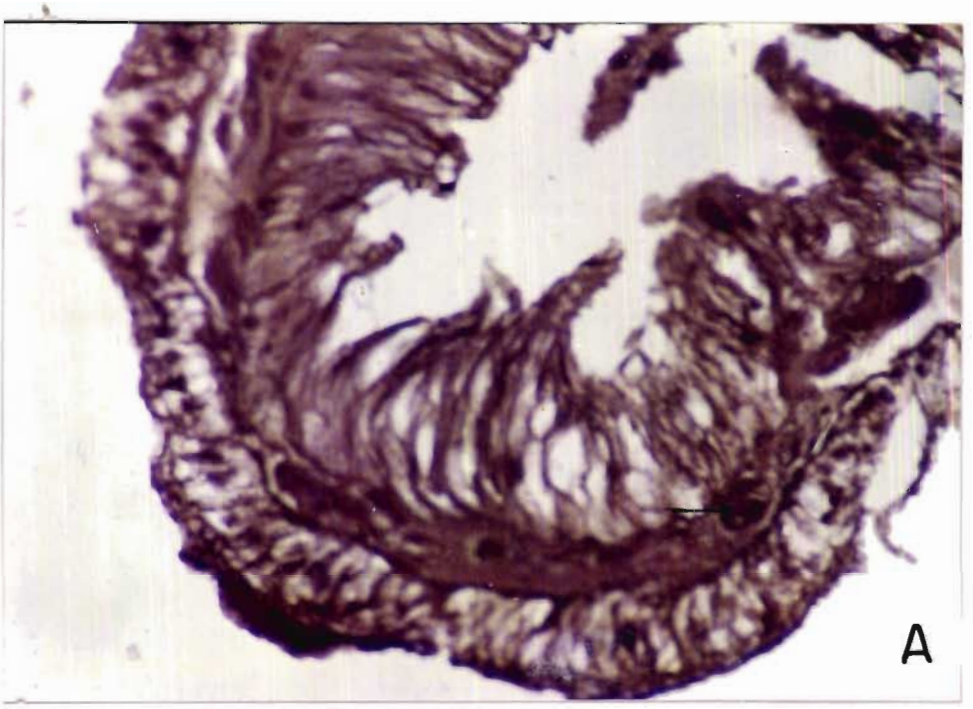


PLATE 38

A. Section of terminal ampoule (TA) showing the presence of two layers containing secretary granules (→). Haematoxylin and Eosin. x 500.

B. Section of typhlosole of terminal ampoule in active secretory phase. Note the blood vessel and glands present in the typhlosole (TS). Mallory's Triple stain. x 500.

PLATE 38



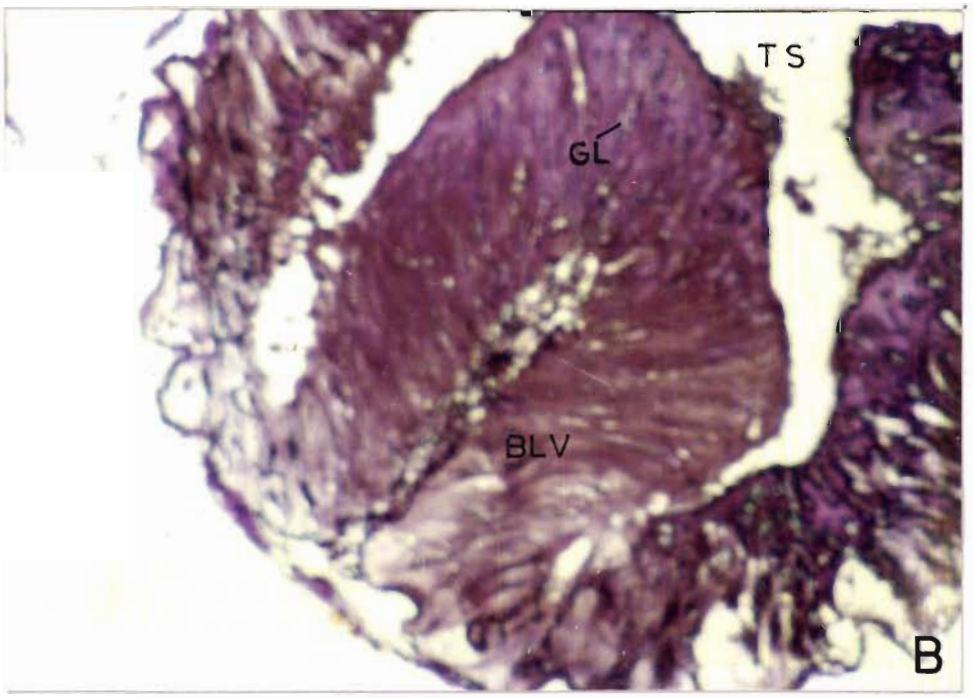
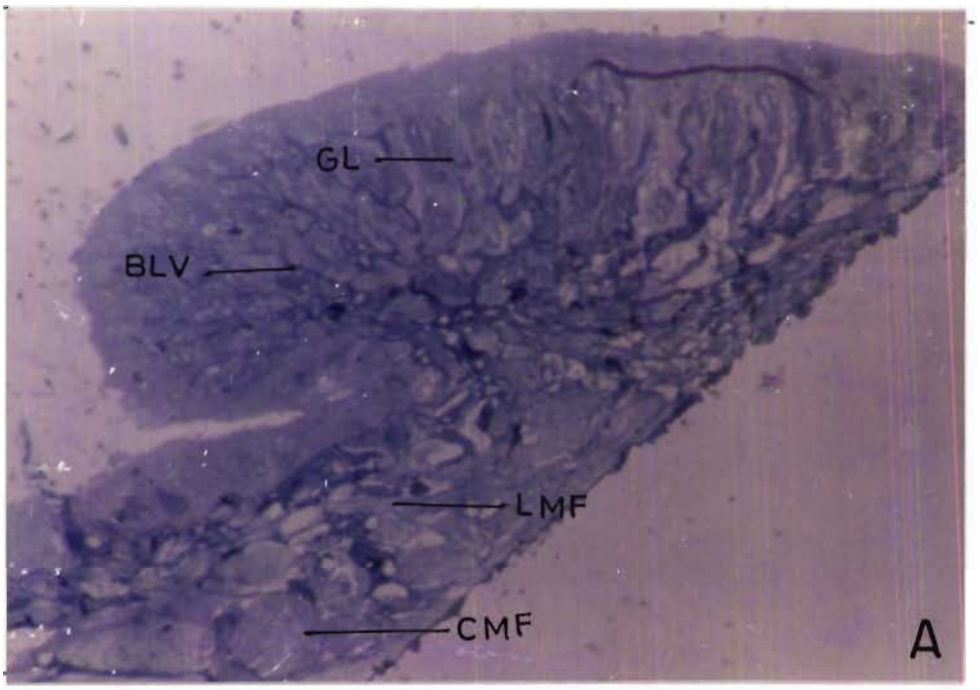


PLATE 39

A. Semithin section of terminal ampoule showing the wall made of circular muscle layer (CMF) and longitudinal muscle layer (LMF) and the typhlosole with secretory glands (GL) and blood vessel (BLV). Toluidine blue stain. x 500.

B. Section of extruded spermatophore showing three chambers containing sperm mass (→). Haematoxylin and Eosin. x 225.

PLATE 39



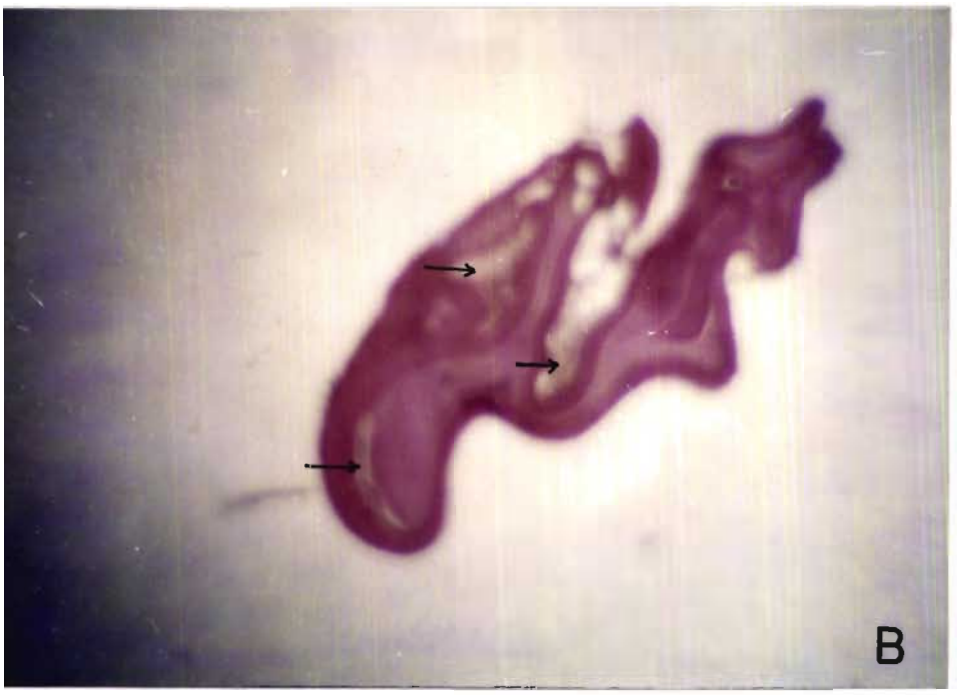
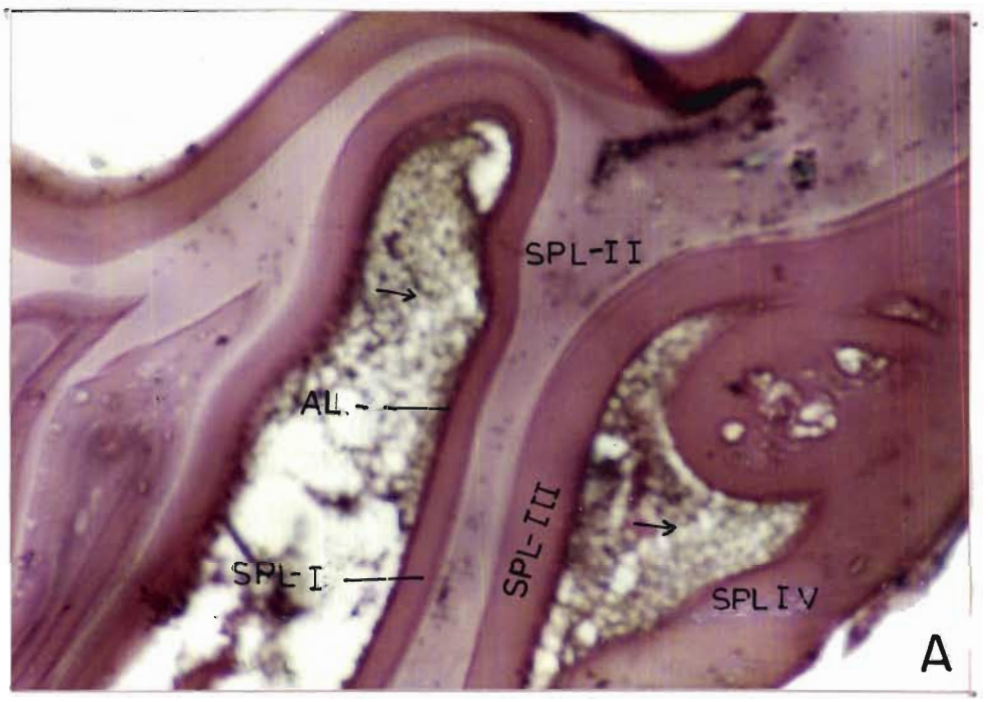


PLATE 40

A. Enlarged view of extruded spermatophore with five spermatophoric layers (SPL - I, II, III, IV) and two chambers containing sperm mass (→). Note the adhesive layer (AL) close to SPL-I. Haematoxylin and Eosin. x 500.

B. Enlarged view of SPL-II containing adhesive globules (AG). Adhesive layer (AL), SPL - III (→) and a chamber containing sperm mass are also seen. Haematoxylin and Eosin. x 1000.

PLATE 40



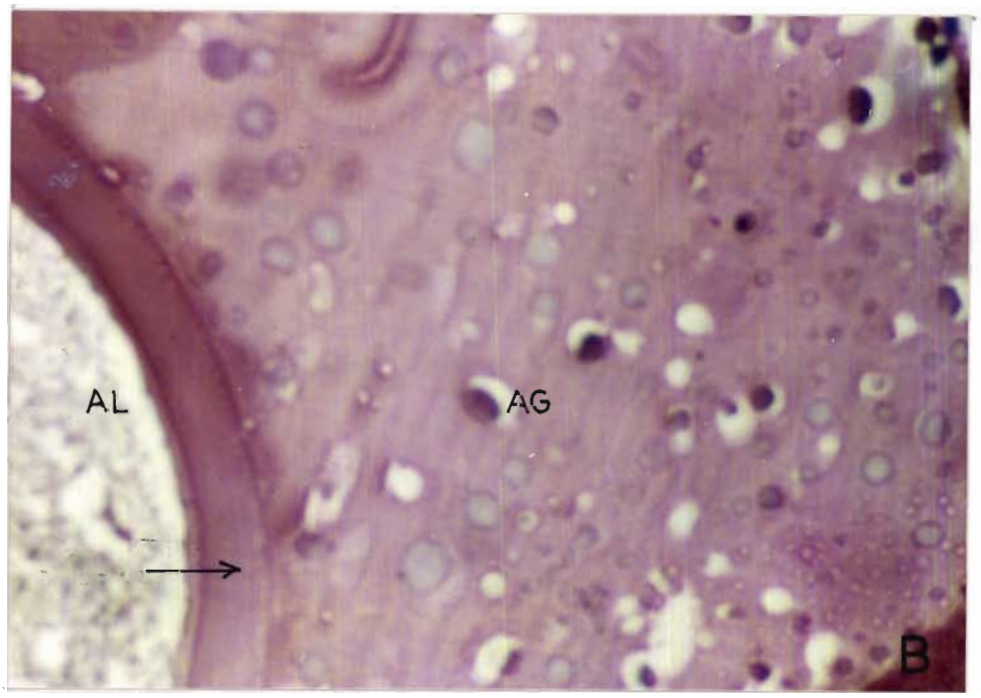
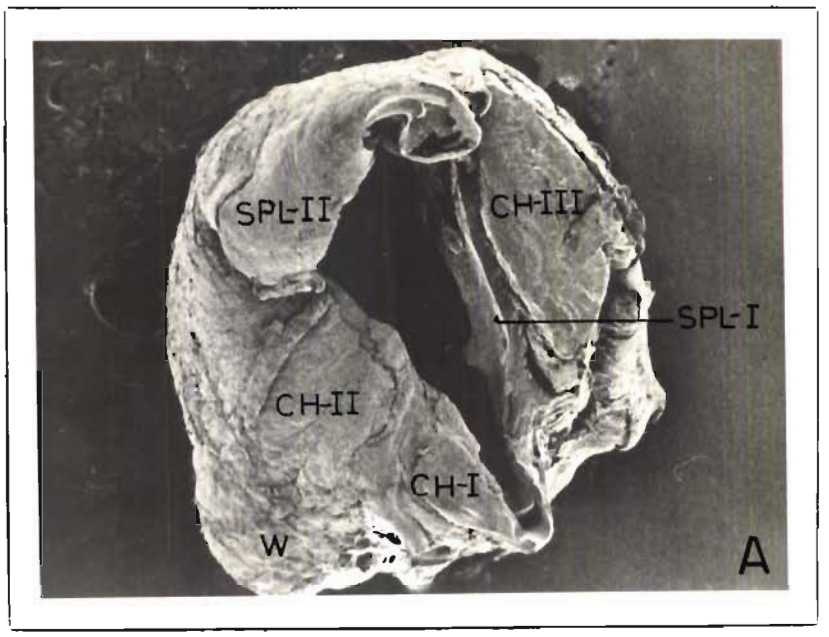


PLATE 41

A. Scanning electron micrograph of a section of the distal vas deferens (DVD) showing the presence of three chambers (CH-I,II,III) containing sperm mass (SM) and spermatophore layers. x 58.5.

B. Scanning electron micrograph of a cross section of the terminal ampoule, showing five spermatophore layers (SPL- I, II, III, IV & V) and three chambers containing sperm mass. x 92.

PLATE 41


SPL-II
CH-III
SPL-I
CH-II
CH-I
W
A

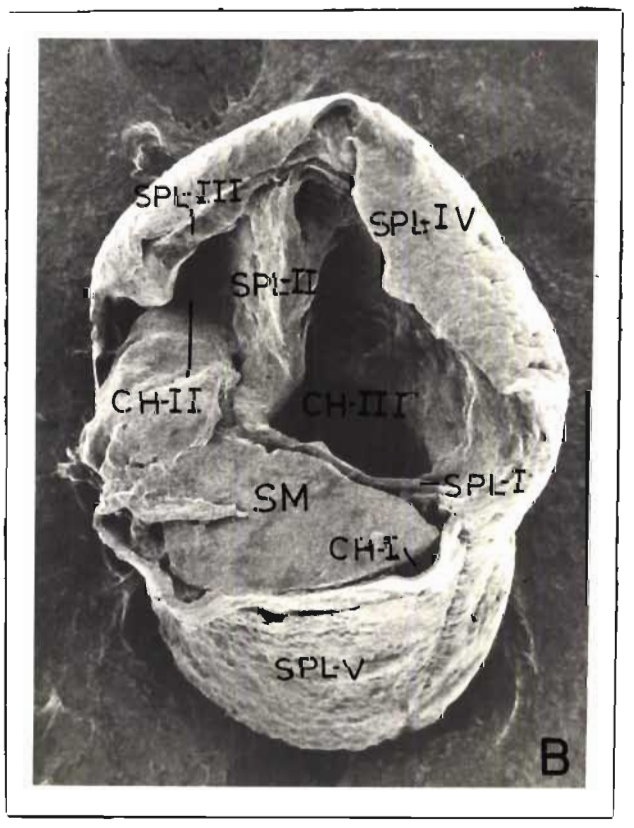
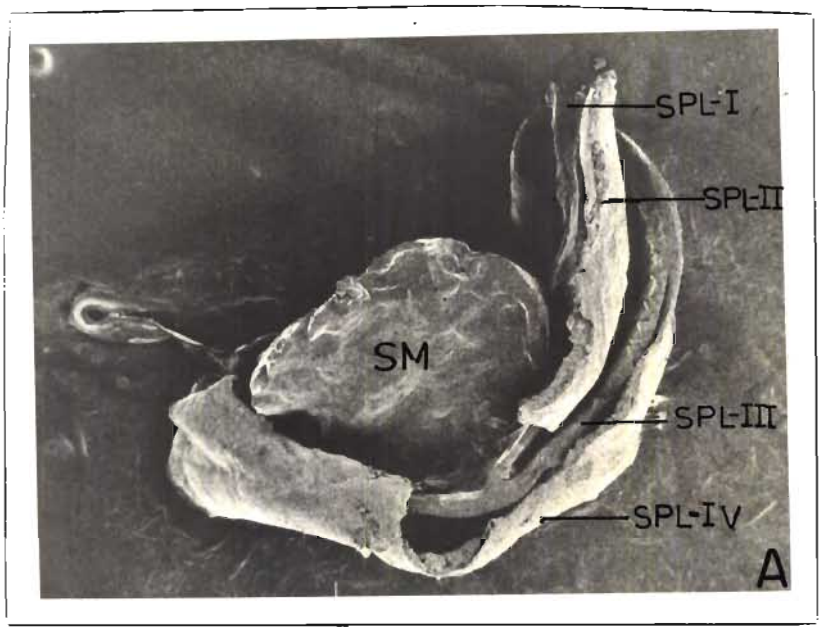

SPL-III
SPL-IV
SPL-II
CH-II
CH-III
SM
SPL-I
CH-I
SPL-V
B

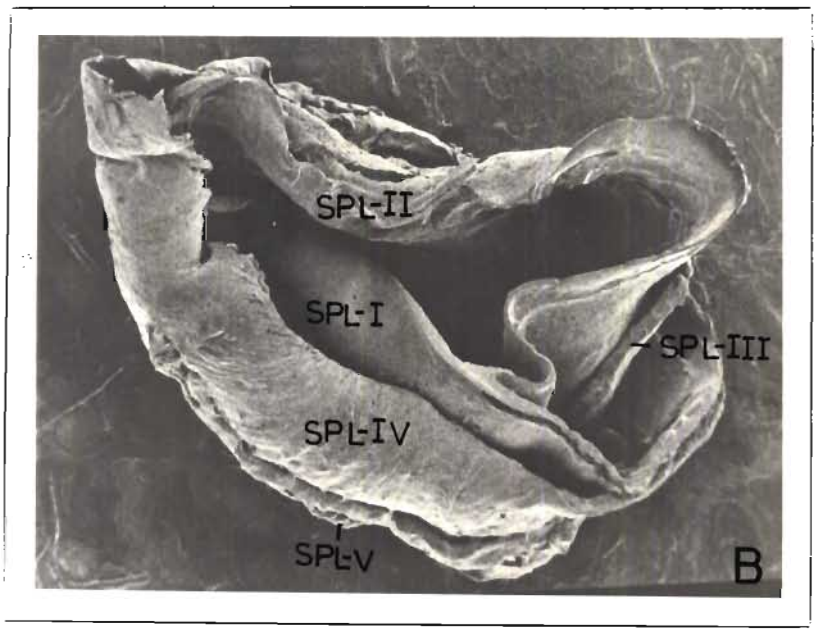
PLATE 42

A. Scanning electron micrograph of a section of terminal ampoule (TA) showing the sperm mass surrounded by spermatophore layers. x 44.

B. Scanning electron micrograph of a section of TA showing five spermatophore layers (SPL - I, II, III, IV & V). x 39.

PLATE 42





Ultrastructure of terminal ampoule (TA) (Plates 41-42)

The transverse section of the terminal ampoule has been studied in detail by Scanning Electron Microscopy. It showed that the terminal ampoule is formed of three chambers (I, II, III) and five spermatophoric layers. The sperm mass is accumulated in chambers I and II. chamber I contains sperm mass which is surrounded with PSL on which the adhesive layer is deposited (Plate 41). With the continuing invagination of primary spermatophore layer, chamber II is found with full of sperm mass. The spermatophore layer III is deposited on the SSL and is made up of glutinous material. Chamber III is an empty concave pouch without sperm mass, but surrounded by thick plate-like spermatophore layer IV. Chamber IV is located in the distal region of the terminal ampullae. This chamber contains the wing portion of the spermatophore and is made of spermatophore layer V which is reticulate or corky in appearance. The respective accessary layer I or II located on the chambers I and II are known to function as a supportive sheath for the sperm mass and the spermatophore layers I and II. The function of the dorsal plate made of spermatophore layer IV is to attach the spermatophore onto the thelycum, while the anterior portion of the spermatophore is anchored by the wing or the spermatophore layer V.

Structure and histology of spermatophore (Fig. 3 & Plates 5,32, 39-40)

The fully extruded spermatophore of *Penaeus semisulcatus* (Fig. 3 & Plates 39-40) has more or less an oval-shaped body measuring approximately 7-7.5 mm in length and 4-5 mm in breadth, and a parachute or umbrella-like wing attached to middle of the body by a short stalk. The entire spermatophore in fresh condition is whitish in colour. The sperm mass accumulated in the spermatophore is clearly noticeable externally by the two-third opaque area of the body which is bordered by a relatively transparent region for about half of its periphery. The wing when stretched fully is much larger than the size of the body proper and appears to have two major folds, each one partly folded

many times along the border. When the spermatophore is placed in water. the wing spreads quickly like a white translucent parachute. The size of the wing ranged 10-12 mm in length and 20-21 mm in breadth in spread condition. The stalk is very short not exceeding 0.5 mm in length.

The transverse section of an ejaculated spermatophore shows five spermatophoric layers and 3 chambers containing sperm mass. The PSL is composed of a homogenous flocculent material. Beneath the PSL the adhesive layer could be clearly seen covering chamber I (Plate 40). The spermatophore layer II occupies a larger area as a thin plate composed of adhesive globules or secretary granules. Spermatophore layer III is a thick layer made of homogenous flocculent material similar to the primary spermatophore layer. This is larger than PSL and it covers the chambers II and III containing full of sperm mass. Chamber II is covered with spermatophore layers II and III, while chamber III is covered with spermatophore layer III and IV. Each spermatophore layer is known to have a specific function and serves to transfer, storage and protect the sperm as well as to anchor the spermatophore onto the thelycum.

DISCUSSION

Oogenesis

The overall goal of maturation in crustacea is the investment of the oocyte with egg yolk protein. Maturation of ovary has been identified and classified based on light and electron microscopy and it was found to vary from one species to another. Duronslet *et al.* (1975) identified 5 stages of oogenesis in *P. aztecus* and *P. setiferus*. Oka and Shirahata (1965) classified ovarian maturity in *P. orientalis* into 8 stages. Yano (1988) further demonstrated 10 stages of oocyte development in *P. japonicus*. Oocyte development was classified into 4 stages in *P. monodon* (Tan-Fermin and Pudadera, 1989) and *P. chinensis* (Matsuyama and Matsura, 1983). Quinitio and Millamena (1992) identified 4 maturity stages in *P. indicus*. In the present study the process of oocyte development was classified on the basis of ovarian change manifested in the cytoplasm and nucleus of the oocyte, into five maturity stages viz. previtellogenic, early vitellogenic, late vitellogenic, vitellogenic and spent oocyte stages, and resembles to that of the other penaeid prawns reported by Mohamed (1989) in *P. indicus*, Browdy *et al.* (1990) in *P. semisulcatus* and Vasudevappa (1992) in *M. dobsoni* with some variations.

The egg maturation has traditionally been divided into two phases: primary and secondary vitellogenesis. In general, primary vitellogenesis is characterized by little growth in egg diameter. Secondary vitellogenesis is characterized by a massive increase in oocyte size and weight, and the development of cortical granules (Quackenbush, 1991). The ovary of *P.semisulcatus* is observed to be encompassed by two distinct layers of epithelial cells with a layer of connective tissue in between. This is identical to the condition observed in *P. setiferus* by King (1948), in *P. indicus* by Subrahmanyam (1965) and in *M. dobsoni* by Vasudevappa (1992).

The presence of small blood vessels seen on the inner wall of the ovary of *P. semisulcatus* might help in the transportation of the nutrients to ovary as described in penaeid prawns (King, 1948; Mohamed, 1989; Vasudevappa, 1992). A wide variation has been reported in the placement of germinal zone in the ovary of crustaceans (Adiyodi and Subramonian, 1983). In the present study, it is found that the germinal epithelium which produces a continuous crop of oogonia is confined to a certain well defined areas on the inner ovarian wall referred to as the zone of proliferation (Gutsell, 1936). This agrees with the observations made by King (1948) in *P. setiferus*, Subrahmanyam (1965) and Mohamed (1989) in *P. indicus* and Vasudevappa (1992) in *M. dobsoni*. The germinal zone is observed in ovaries at all maturity stages indicating that the ovary is active throughout the reproductive period of the female. Identical observations have been made in other crustanceans (Adiyodi and Subramoniam, 1983) including the penaeids *P. japonicus* (Yano, 1988), *P. indicus* (Mohamed, 1989) and *M. dobsoni* (Vasudevappa, 1992). As in other crustaceans, the process of oogenesis in *P. semisulcatus* is completed in two phases; first, the proliferative phase wherein the primary oogonial cells are multiplied by mitotic division to form secondary oogonial cells and the second phase is the differentiative phase wherein the immature ova accumulates yolk and develops into mature oocytes. Similar observations have been reported in many other decapod crustaceans (Adiyodi and Subramoniam, 1983; Yano, 1988).

The cytological changes observed during different maturity stages of the ovary in *P. semisulcatus* is almost similar to that occurring in the oocytes of *P. setiferus* (King, 1948), *P. stylifera* (Shaikhmahmud and Tembe, 1958) and *P. indicus* (Subrahmanyam, 1965; Mohamed, 1989). In *P. semisulcatus*, the presence of numerous ribosomes, rough endoplasmic reticulum and mitochondria in the cytoplasm indicated an autosynthetic capabilities during immature to late maturing stages of ovary. In immature ovary, micropinocytotic absorption is presumed to occur for the transportation of nutrients to the ooplasm. In *P. semisulcatus*, there is no clear evidence in late

vitellogenic oocyte for heterosynthesis as to compare with the observations made by Mohamed (1989) in *P. indicus*. The formation of yolk platelets in *P.semisulcatus* is almost similar to that observed in *P. indicus* (Mohamed, 1989).

A striking feature of the mature oocyte in *P. semisulcatus* is the appearance of rod-like cortical body in the periphery of the oolemma. This term rod-like cortical body has been used in different forms in the penaeid prawns as marginal bodies by Subrahmanyam (1965), cortical rods by Duronslet *et al.* (1975) and Mohamed (1989), peripheral bodies by King (1948) and Rao (1968), Jelly substance by Hudinaga (1942) and cortical crypts by Yano (1988) and Browdy *et al.* (1990). These are unique to the genus *Penaeus*. They do not occur in the ovaries of *Parapenaeopsis stylifera* (Shaikhmahmud and Tembe, 1958), *M. ensis* (Yano, 1985) and *M. dobsoni* (Vasudevappa, 1992). In *P. aztecus*, Clark *et al.* (1980) demonstrated that the cortical bodies are responsible for the jelly layer which surrounds the egg during early development. These cortical rods are characteristics of mature ova of *Penaeus* and are indicators of imminent spawning (Anderson *et al.*, 1984). In the oocytes of penaeid shrimp, distinct cortical granule precursors begin to develop at the end of secondary vitellogenesis. These cortical granule precursors would eventually form the characteristic penaeid cortical crypts (Clark *et al.*, 1984). In penaeid prawn, Anderson *et al.* (1984) and Clark *et al.* (1984) reported that the cortical granules eventually fuse with the egg oolemma and contribute to egg jelly which surrounds a fertilized egg. Anderson *et al.* (1984) reported that in penaeid prawn, the stage called the platelet stage, bridges the transition to secondary vitellogenesis. During the platelet stage the oocytes increase in size and yolk spheres appear in the cytoplasm for the first time, micropinocytosis activity also increases during this transition. Further, he reported that the germinal vesicle breaks down antecedent to ovulation (24 hrs in penaeid). Joshi *et al.* (1982) reported that in *P. stylifera* the germinal vesicle breaks down when oocytes are released into the water during spawning. The present study agrees with the above observations made by many authors in penaeid prawn.

The basophilic reaction of cytoplasm of ova of pre and early vitellogenic stages and gradual shift to acidophilic nature in late vitellogenic and vitellogenic oocytes noticed in this species is similar to that noticed in *P. setiferus* (King, 1948), *P. monodon* (Tan-Fermin and Pudadera, 1989), *P. indicus* (Mohamed, 1989) and *M. dobsoni* (Vasudevappa, 1992). During the process of oogenesis in *P. semisulcatus*, the number of nucleoli get reduced and finally in the vitellogenic stage, the yolk platelets or globules are densely accumulated in the cytoplasm. This almost agrees with the observations of Mohamed (1989) in *P.indicus* and Browdy *et al.* (1990) in *P. semisulcatus*.

In *P. semisulcatus*, folliculogenesis or the investment of follicular cells around the oocytes is initiated in previtellogenic oocytes and continued upto vitellogenic phase. The flattening of the follicle cells with the growth in volume of the oocyte observed in the present study appears to be characteristic of this process. According to Charniaux-cotton (1975), follicle cells facilitate vitellogenic activity by aiding in the uptake of yolk protein from external sources. In *P. japonicus*, the follicle cells are implicated as the possible cell type responsible for ovarian vitellogenic synthesis (Yano and Chinzei, 1987). Similar observation is also made by Mohamed (1989) in *P. indicus*.

During previtellogenic and spent oocyte stages in *P. semisulcatus*, the atretic cells are commonly seen as reported in other penaeid prawns like *P.indicus* (Mohamed, 1989), *P. monodon* (Tan-Fermin and Pudadera, 1989) and *M. dobsoni* (Vasudevappa, 1992). Follicular atresia is a degenerative process by which oocytes in various stages of their development and differentiation are lost from the ovary (Guraya, 1973).

Spermatogenesis

The process of spermatogenesis observed in *P. semisulcatus* appears to be similar to that reported in other crustaceans (Pochon-Masson, 1983). A general feature of the process is the reduction in cytoplasmic volume and

condensation of the chromatin matter as spermatogenesis progresses through cellular stages like spermatogonia, spermatocytes and spermatids to form the spermatozoa. The sperm of *P. semisulcatus* (Plates 36-37) has a spherical body composed of a diffused nuclear region, a cytoplasmic band and an acrosome complex and a long spike giving it a tack-shaped appearance. The nature of the spike indicates that the sperm is non-motile. Histological section of mature testis has also indicated almost fully formed sperms with slight variations in shape and structure along with fully formed typical spermatozoa. Some of such sperms showed more or less spherical body with relatively shorter spike in different forms and others with no spike at all. It is probable that these stages are in the transitional phase from spermatid to spermatozoa. Studying spermatogenesis and sperm structure in the shrimp *Parapenaeus longirostris*, Medina (1994) came across more or less the same forms of spermatozoa through scanning electron microscopic studies. He considered the forms identical to those observed during the present investigation as transitional stages and named them as early spermatids, mid spermatids and late spermatids, the morphology of fully formed spermatozoa being tack-shaped. Pochon-Masson (1983) distinguished flagellate and non-flagellate sperms in crustaceans. The structure of sperm in penaeid prawns has been studied in detail by many workers. According to King (1948), the spermatozoa of *P.setiferus* is composed of a head, middle piece and a tail. Shaikhmahmud and Tembe (1958) and Joshi *et al.* (1982) described the sperm of *P. stylifera* to be short and cylindrical with a small head and very short tail.

Subrahmanyam (1965) observed the shape of the spermatozoa of *P.indicus* to be oval. Mohamed (1989) in *P. indicus* reported an oval or spherical main body of sperm encompassed by a morphologically diverse cap region containing the acrosomal complex from which a single short spike arises. More or less similar structure has been reported for the sperm of *Sicyonia ingentis* by Kleve *et al.* (1980) and *P. setiferus* by Lu *et al.* (1973). Nath (1942) described and illustrated the spermatozoa of *P. indicus* as lacking rays or pseudopodia although every other speices of *Penaeus* has a distinct

spike. Felgenhauer and Abele (1990) reviewing the morphological diversity in decapod crustaceans reported that most of the crustacean spermatozoa are aflagellate and non-motile. Demestre and Fortuno (1992) did not show the characteristic structure of the spiked sperm in deep water shrimp *Aristeus antennatus* examined by SEM. King (1948), based on the structure of the sperm, has logically assumed that the spermatozoan in *P. setiferus* is capable of movement as presumed by Subrahmanyam (1965) in the case of *P. indicus*. Agreeing to this finding, Vasudevappa (1992) reported the sperm of *M. dobsoni* as tadpole like and capable of motility as judged from the shape and size of the tail. The observations made in the present study differs from those of King (1948), Subrahamanyam (1968) and Vasudevappa (1992) and are in agreement with the finding of Malek and Bawab (1974 a) who expressed doubt about the motility of the sperms of crustaceans aided by any nourishing fluid in the seminiferous tubules. Malek and Bawab (1974 a) opined that in crustaceans the sperm cells lack discrete vibratile organelles and their conduction along channels free from secretion is still possible even though these channels may further be devoid of muscle.

In mature *P. semisulcatus*, the testicular acini made of outer tunica apparently disappears and the inner layer of germinal epithelium also disappears as the sperm mass moves to the proximal vas deferens. But, in other penaeid prawns the disappearance of outer tunica and the inner layer of germinal epithelium has not been detailed.

The vas deferens of decapod crustaceans conveys sperms from the testis to the exterior in the form of a spermatophore (Dudenhausen and Talbot, 1983). The present study showed that the sperm mass drained from testis to proximal vas deferens is transferred to middle vas deferens by peristaltic movement. In the middle vas deferens, the spermatophore is partly formed enclosing the sperm mass. The sperm and wing duct are also observed to be active in this process with the help of the epithelial glandular cells and the typhlosole. Thereafter, the spermatophore is transported into the distal vas

deferens where the spermatophore becomes a compact mass, and in the terminal ampoule the complete shape of spermatophore containing all the five spermatophore layers is attained. The present study fully agrees with the observation made by Mohamed (1989) in *P.indicus*.

Considerable amount of work has also been done on the formation of spermatophore and wing in proximal vas deferens, middle vas deferens and distal vas deferens as reported by Perez-Farfante (1975) in the Subgenus *Litopenaeus*, Chow (1982) in *Macrobrachium rosenbergii*, Radha and Subramoniam (1985) in *P. homarus*. Martin *et al.* (1987) in *Panulirus interruptus* and Berry and Heydorn (1970) in *P. homarus* opined that the middle region of vas deferens is for storage of fully developed spermatophores. Chow *et al.* (1991) studied the middle and distal vas deferentia and terminal ampullae of *P. setiferus* and *P. vannamei* by light and electron microscopy to assess their roles in spermatophore formation and reported that the passage of spermatophoric materials from the middle vas deferens to the terminal ampullae is apparently discontinuous. Leung-Trujillo and Lawrence (1991) studied the spermatophore developments in *P. setiferus*, *P.vannamei* and *P.stylirostris* and reported that the PSL and SSL are secreted in the vas deferens and the third and fourth spermatophore layers are completed in the terminal ampoule. In the present study, the PSL and SSL are formed in the middle vas deferens and remaining spermatophore layers (III to V) are completed in the terminal ampoule as shown by scanning electron microscopy in terminal ampoule. This is almost in agreement with the observation of Mohamed (1989) in *P. indicus*. Malek and Bawab (1974 b) have described the five successive stages involved in the formation of the complete spermatophoric layer in *P. kerathurus*. Agreeing with the histological observations of Malek and Bawab (1974 a,b) in *P. kerathurus* and Bizot-Epiard (1980) in *P. japonicus*, Heitzmann *et al.* (1993) suggested that the sperm mass in *P. vannamei* joined the spermatophore wings at the end of the middle vas deferens before reaching the terminal ampullae. In *M. dobsoni*, Vasudevappa (1992) observed the formation of four spermatophore layers and the presence of three conspicuous

typhlosoles in the sperm duct and one small typhlosole in the wing duct in the middle vas deferens. Malek and Bawab (1974 b) in *P. kerathurus*, Mohamed (1989) in *P. indicus* reported the presence of a typhlosole each in the sperm duct and wing duct as encountered in the present study. The presence of blood supply to the typhlosole and the active secretion of the latter (Plates 38-39) indicate the high metabolic rate.

Penaeid spermatophores exhibit considerable variation. At one extreme they are structurally complex external spermatophores, characterized by various wings, flanges, plates and adhesive materials (families Aristeidae, Solenoceridae and the subgenus *Litopenaeus*, genus *Penaeus* in the penaeidae). At the other extreme they are highly internalised simple spermatophoric mass as found in Sicyoniidae (review-Bauer, 1990). Most workers have investigated spermatophores of crustaceans by using material teased out from the distal vas deferens (Malek and Bawab, 1974; Chow 1982; Subramoniam, 1984 and Radha and Subramoniam, 1985). The fully formed spermatophore of *P. semisulcatus*, extruded using the electro-ejaculation technique (Kooda-Cisco and Talbot, 1982), during the present study is parachute or umbrella-like and the pattern of the attachment of the wing-like structures on the main body of spermatophore by means of short stalk is characteristically different from that of other penaeid prawns like *P. indicus* (Mohamed, 1989) and *M. dobsoni* (Vasudevappa, 1992). The wing-like structure in *P. semisulcatus* is attached in the middle region of the main body of spermatophore, whereas in *P. indicus* (Mohamed, 1989; Mohamed and Diwan, 1993) and *M. dobsoni* (Vasudevappa, 1992) the same is attached to one end of the body. Subramoniam (1993) reviewed spermatophore and sperm transfer in marine crustaceans and described and discussed spermatophore morphology and origin of spermatophore in penaeid shrimp. In the present study, the transverse section of ejaculated spermatophore showed that it consists of five spermatophoric layers and 3 chambers containing sperm mass.

CHAPTER V

BIOCHEMICAL AND MINERAL CHANGES IN RELATION TO OVARIAN MATURATION

RESULTS

BIOCHEMICAL CHANGES

Concentrations of the biochemical components such as protein, lipid, carbohydrate, carotenoid, free amino acid and moisture showed highly significant variations in the different tissues of *P. semisulcatus* during the process of maturation. The levels of biochemical concentration in hepatopancreas, haemolymph, ovary and muscle in different maturity stages are shown in Tables 11-16 and the trends are graphically represented in (Figs. 17-24).

Protein

The protein content in the hepatopancreas decreased from 18.91 mg/100 mg in stage I to 12.53 mg/100 mg in stage III. Then from stage III to V, the values sharply increased to 21.24 mg/100 mg.

The protein content in the haemolymph showed fluctuations during different stages of maturity. From stage I to stage III, the values gradually decreased from 63.54 to 34.18 mg/ml and suddenly increased in stage IV to 96.46 mg/ml. In spent stage, the value again decreased to a very low level of 34.38 mg/ml which was equal to that of late maturing stage.

The protein content in the ovary increased gradually from stage I and attained the maximum value of 54.22 mg/100 mg in stage IV. In the spent stage (Stage V), protein content declined sharply to 21.87 mg/100 mg (Fig. 21).

The protein content in the muscle recorded in stage I was 60.15 mg/100 mg and it slowly decreased to 57.96 mg/100 mg in stage II. In stage III, there was an increase to 61.06 mg/100 mg and the value again came down in stages IV and V. The minimum protein value of 47.31 mg/100 mg was observed

in stage V. Muscle protein content was comparatively high in all maturity stages and the values showed no definite pattern.

The variations in the concentration of proteins observed in the hepatopancreas, ovary, haemolymph and muscle (Table 11) in relation to different maturity stages were highly significant ($p < 0.01$).

Free amino acid

Hepatopancreatic free amino acid content was maximum in stage IV (3.49 mg/100 mg) and minimum (1.68 mg/100 mg) in stage V. From stage I to IV, there was a sharp increase (1.81 - 3.49 mg/100 mg).

High level of 1.56 mg/ml total free amino acid content in the haemolymph was observed in stage IV, whereas the lowest value of 0.75 mg/ml was seen in stage V (spent). From stage I to IV (0.93 - 1.56 mg/ml), the value sharply increased and then abruptly decreased in stage V (0.75 mg/ml).

Total free amino acid contents in ovary behaved as in hepatopancreas and haemolymph. Maximum value of 2.94 mg/100 mg was observed in stage IV and the lowest value of 0.81 mg/100 mg in stage V.

Total free amino acid content of muscle was high in stage I (1.24 mg/100 mg) and stage II (2.87 mg/100 mg) and it suddenly fell in stage III. Again slight increase was noticed in stage IV (1.09 mg/100 mg). During spent stage, the value suddenly decreased to 0.49 mg/100 mg.

The changes in total free amino acid content in hepatopancreas, haemolymph, ovary and muscle (Table 12) were statistically proved to be highly significant at 1% level ($p < 0.01$).

Lipid

Maximum lipid level of 42.08 mg/100 mg in the hepatopancreas was observed in stage IV, while the lowest level of 6.29 mg/100 mg was seen in stage I. In stage V, a sudden decrease in lipid content (32.78 mg/100 mg) was noticed.

Maximum lipid content in the haemolymph was recorded in stage III (15.15 mg/ml), while the lowest value of 7.47 mg/ml was seen in stage I and in stage IV. In spent stage, however, there was a sudden increase to 13.29 mg/ml.

In the ovary, minimum lipid content of 6.66 mg/100 mg was recorded in stage I and maximum value of 22.93 mg/100 mg in stage IV. From stage I to stage IV there was a sharp increase in lipid content. In stage V, the value declined to 8.77 mg/100 mg which was close to the value of stage I.

Lipid content in muscle tissues was found to be uniformly lower, the recorded levels being 4.00,6.80, 4.75, 7.40 and 5.09 mg/100 mg in stages I, II, III, IV and V, respectively. During the process of maturation, the highest value of lipid (7.40 mg/100 mg) was noticed in mature stage and lowest (4.00 mg/100 mg) in stage I.

The variations of lipid content in hepatopancreas, haemolymph, ovary and muscle (Table 13) were highly significant at 1% level ($p<0.01$).

Carbohydrate

The carbohydrate content in the hepatopancreas was found to increase sharply from stage I to IV. The minimum value observed was 0.30 mg/100 mg in stage I, while the maximum value of 2.37 mg/100 mg was noticed in stage IV. In spent stage, a sudden decrease to 1.79 mg/100 mg could be noticed.

The maximum carbohydrate content (2.75 mg/ml) in the haemolymph was observed in stage IV, whereas, the lowest value of 0.22 mg/ml was noticed in stage I, showing a steady increase from stage I to IV. In stage V, a slight decline was observed (2.01 mg/ml).

In the ovary, the lowest value of 0.01 mg/100 mg was recorded in stage I, while the maximum value of 0.63 mg/100 mg was seen in stage IV. In the ovary, carbohydrate constituents were generally poor. The carbohydrate content showed a gradual increase from stage I to IV (0.01 to 0.63 mg/100 mg) and a subsequent decline in stage V (0.44 mg/100 mg).

Muscle carbohydrate content showed an erratic behaviour without a definite pattern. The minimum carbohydrate content of 0.86 mg/100 mg was observed in stage II and the value increased slowly up to stage V through stage II, where the maximum value of 1.16 mg/100 mg was noticed. During immature stage, the carbohydrate content of muscle increased to the value of 1.39 mg/100 mg which was higher than the value of late maturing stage. The carbohydrate values recorded in various tissues as well as in different maturity stages were generally lower.

The variations of total carbohydrate content in the hepatopancreas, haemolymph, ovary and muscle (Table 14) were highly significant at 1% level ($p<0.01$).

Carotenoids

The changes in the total carotenoid content of hepatopancreas, haemolymph, ovary and muscle during the different maturity stages are given in (Table 15).

In the hepatopancreas, a rapid increase in the carotenoid content was observed from stage I to II (72.61-103.17 µg/g) and the value slowly shot down

to 21.15 µg/g in spent stage through the stages III & IV. The maximum value of 103.17 µg/g was observed in stage II.

The total carotenoid contents in haemolymph showed a gradual increase from stage I to III where the maximum value of 30.67 µg/ml was observed. From stage III to V, a slow decline in values from 30.67 to 18.39 µg/ml was observed.

In the ovary, the carotenoid contents showed a drastic increase from stage I to IV. Minimum value of 24.40 µg/g was observed in stage I, where as a seven-fold higher value of 141.00 µg/g was recorded in stage IV. In spent stage, there was a sudden fall to 46.60 µg/g.

The total carotenoid content of muscle showed an erratic pattern. The maximum and minimum carotenoid values of 10.52 and 3.42 µg/g were observed in stages I and V, respectively.

ANOVA showed that the differences in the carotenoid levels in the ovary, haemolymph, hepatopancreas and muscle (Table 15) for the various maturity stages were highly significant at 1% level ($p<0.01$).

Moisture

The variations in the moisture or water content of the hepatopancreas, ovary and muscle during the different maturity stages are given in (Table 16).

In hepatopancreas, the variations in moisture content in the different maturity stages showed an irregular pattern. Maximum moisture content of 78.17% was noticed in stage I and the minimum (69.08%) in stage II. Subsequently the moisture level stabilized at 72.26 and 72.24% in stages IV

and V respectively. The moisture content of hepatopancreas, ovary and muscle showed remarkable fluctuations in relation to maturity stages.

In ovary, the maximum moisture content of 81.59% in stage I, declined to the minimum water content of 75.49% in stage IV. Again there was a sudden increase to 83.94% in stage V.

The variations in moisture content of muscle in all the stages showed an irregular pattern. In stage I and IV, the maximum moisture content of 77% was observed. The moisture contents in the maturity stages II, III and V were 74.94, 75.85 and 74.45%, respectively.

ANOVA showed that the variations (Table 16) in moisture levels in hepatopancreas and ovary during different maturity stages were statistically significant ($p < 0.05$). However, the changes in the muscle water content were not significant ($p < 0.05$).

MINERAL CHANGES

Trace element components viz. calcium, magnesium, copper, manganese, sodium, potassium, iron, zinc, nickel and lead were analysed from the tissues like ovary, hepatopancreas and muscle in different maturity stages of *P. semisulcatus*, and the results are given in (Tables 17-19).

Hepatopancreas

Among the macrominerals (Ca, Mg, Na, K) analysed in the hepatopancreas, potassium content recorded the maximum in all maturity stages and particularly in stage I it showed the highest value of 18750 ppm. The value decreased to 10004 ppm in stage III and again increased through 11874 ppm in stage IV to 17505 ppm in stage V. Following potassium, sodium content also recorded the maximum value of 16,873 ppm in stage I. From stage

I to IV (16.873 to 10,006 ppm) a steady decline was observed. In stage V, there was a slight increase to 12505.4 ppm. Among the microminerals, the values of Mn, Fe and Ni were interestingly high in stage III, the values being 163, 13605 and 21799 ppm, respectively. Cu and Zn showed the maximum values in stages V (418.1 ppm) and Stage I (697.1 ppm) respectively. The values of lead content ranged from 30.5 ppm in stage III to 51.1 ppm in stages IV and V. In general, the fully mature stage showed relatively low values for all minerals except lead. There was absolutely no cadmium content in the hepatopancreas.

The changes in the contents of all the macro and microminerals except Ca in hepatopancreas in the different maturity stages (I to V) were found to be statistically significant at 1% level ($p<0.01$) as evident from (Table 17a).

Ovary

In the ovary, the values of all minerals showed a drastic decline during the process of maturation. Potassium registered a maximum value of 75001 ppm in stage I, while in the subsequent stages the values declined. Sodium content was maximum (62500 ppm) again in stage I and it drastically declined through the maturity stages II to IV (15200 to 5479 ppm). During spent stage,it suddenly increased to 6750 ppm. The maximum value for calcium was 46230.4 ppm recorded in stage I followed by a drastic decline through stages II to IV (4772 - 582 ppm). Magnesium content showed a maximum value of 32619 ppm in stage I and declined from stages II to V (3262 to 351.2 ppm) as noticed in the other minerals. For iron, the maximum value of 13636.2 ppm was recorded in stage I and thereafter declined from stage II to III (1676.3 - 392.5 ppm) and again from stages IV to V (761 - 449 ppm). Zinc recorded a maximum value of 5120.1 ppm in stage I and in the remaining stages the values were low, the lowest (124 ppm) being recorded in stage III. Nickel content was maximum (12004.4 ppm) in stage I and minimum (131 ppm) in stage III. Other minerals like Cu, Mn and Pb recorded low values in

Table 11. Variations in the concentration of total protein during the different maturity stages of *P. semisulcatus*

Tissues		Maturity stages				
		I	II	III	IV	V
Hepatopancreas (mg/100 mg) dry weight	N	5	5	5	5	5
	$\bar{X}$	18.91	16.09	12.53	18.22	21.24
	±SD	0.70	0.66	0.80	1.25	0.66
Haemolymph (mg/ml)	N	5	5	5	5	5
	$\bar{X}$	63.54	56.03	34.18	96.46	34.38
	±SD					
Ovary (mg/100 mg) dry weight	N	5	5	5	5	5
	$\bar{X}$	16.35	28.27	42.49	54.22	21.87
	±SD	0.37	1.82	2.12	2.27	3.59
Muscle (mg/100 mg) dry weight	N	6	5	5	5	5
	$\bar{X}$	60.15	57.96	61.06	53.21	47.31
	±SD	0.51	0.63	0.41	0.38	0.33

Analysis of variance

Tissues	Source	D.F.	Mean SQRS	F-value
Hepatopancreas	Treatment	4	529.3645	1.3144^{NS}
	error	24	402.7542	
Haemolymph	Treatment	4	3291.7813	1453.8912^{**}
	error	24	2.2641	
Ovary	Treatment	4	1203.9546	231.6260^{**}
	error	24	5.1978	
Muscle	Treatment	4	162.4652	756.6022^{**}
	error	24	0.2147	

** Highly significant ($p < 0.01$)
NS Non-significant

Table 12. Variations in the concentration of total free amino acids during the different maturity stages of *P. semisulcatus*

Tissues		Maturity stages				
		I	II	III	IV	V
Hepatopancreas (mg/100 mg) dry weight	N	5	5	5	5	5
	$\bar{X}$	1.81	2.94	3.15	3.49	1.68
	±SD	0.07	0.05	0.03	0.15	0.16
Haemolymph (mg/ml)	N	5	5	5	5	5
	$\bar{X}$	0.93	1.15	1.37	1.56	0.75
	±SD	0.05	0.19	0.01	0.08	0.01
Ovary (mg/100 mg) dry weight	N	5	5	5	5	5
	$\bar{X}$	1.47	1.70	2.56	2.94	0.81
	±SD	0.05	0.02	0.02	0.09	0.02
Muscle (mg/100 mg) dry weight	N	5	5	5	5	5
	$\bar{X}$	1.24	2.87	0.69	1.09	0.49
	±SD	0.02	0.04	0.02	0.01	0.05

Analysis of variance

Tissues	Source	D.F.	Mean SQRS	F-value
Hepatopancreas	Treatment	4	3.3350	287.5966**
	error	24	0.0116	
Haemolymph	Treatment	4	0.5361	59.7503**
	error	24	0.0090	
Ovary	Treatment	4	3.6367	1415.5612**
	error	24	0.0026	
Muscle	Treatment	4	4.4060	4620.8705**
	error	24	0.0010	

** Highly significant ($p < 0.01$)

Table 13. Variations in the concentration of total lipid during the different maturity stages of *P. semisulcatus*

Tissues		Maturity stages				
		I	II	III	IV	V
Hepatopancreas (mg/100 mg) dry weight	N	5	5	5	5	5
	$\bar{X}$	6.29	21.70	38.30	42.08	32.78
	±SD	0.67	0.44	0.70	0.40	0.52
Haemolymph (mg/ml)	N	5	5	5	5	5
	$\bar{X}$	7.47	12.02	15.15	7.47	13.29
	±SD	0.20	0.91	0.40	0.20	0.25
Ovary (mg/100 mg) dry weight	N	5	5	5	5	5
	$\bar{X}$	6.66	12.00	21.54	22.93	8.77
	±SD	0.19	0.19	0.30	0.19	0.26
Muscle (mg/100 mg) dry weight	N	5	5	5	5	5
	$\bar{X}$	4.00	6.80	4.75	7.40	5.09
	±SD	0.16	0.17	0.07	0.26	0.17

Analysis of variance

Tissues	Source	D.F.	Mean SQRS	F-value
Hepatopancreas	Treatment	4	1047.0485	3333.1906**
	error	24	0.3141	
Haemolymph	Treatment	4	60.6247	267.7699**
	error	24	0.2264	
Ovary	Treatment	4	276.3829	5204.1671**
	error	24	0.0531	
Muscle	Treatment	4	10.1991	321.0082**
	error	24	0.0318	

** Highly significant ($p < 0.01$)

Table 14. Variations in the concentration of total carbohydrate during the different maturity stages of *P. semisulcatus*

Tissues		Maturity stages				
		I	II	III	IV	V
Hepatopancreas (mg/100 mg) dry weight	$\underline{N}$	5	5	5	5	5
	$\overline{X}$	0.30	0.80	1.17	2.37	1.79
	±SD	0.005	0.04	0.07	0.37	0.10
Haemolymph (mg/ml)	$\underline{N}$	5	5	5	5	5
	$\overline{X}$	0.22	0.65	1.43	2.75	2.01
	±SD	0.33	0.07	0.19	0.16	0.07
Ovary (mg/100 mg) dry weight	$\underline{N}$	5	5	5	5	5
	$\overline{X}$	0.01	0.11	0.51	0.63	0.44
	±SD	0.001	0.02	0.05	0.02	0.20
Muscle (mg/100 mg) dry weight	$\underline{N}$	5	5	5	5	5
	$\overline{X}$	1.39	0.86	0.94	1.51	1.16
	±SD	0.11	0.038	0.004	0.30	0.05

Analysis of variance

Tissues	Source	D.F.	Mean SQRS	F-value
Hepatopancreas	Treatment	4	3.3333	107.9030**
	error	24	0.0309	
Haemolymph	Treatment	4	5.1869	367.1896**
	error	24	0.141	
Ovary	Treatment	4	0.3627	39.0814**
	error	24	0.0090	
Muscle	Treatment	4	0.3916	18.3805**
	error	24	0.0213	

** Highly significant at 1% level ($p < 0.01$)

Table 15. Variations in the concentration of total carotenoids during the different maturity stages of *P. semisulcatus*

Tissues		Maturity stages				
		I	II	III	IV	V
Hepatopancreas (µg/g) wet weight	N	5	5	5	5	5
	$\bar{X}$	72.61	103.17	80.95	42.00	21.15
	±SD	4.90	11.38	1.68	2.70	2.90
Haemolymph (µg/ml)	N	5	5	5	5	5
	$\bar{X}$	12.93	16.94	30.67	28.73	18.39
	±SD	1.16	0.43	1.25	0.87	0.44
Ovary (µg/g) wet weight	N	5	5	5	5	5
	$\bar{X}$	24.40	61.20	72.00	141.00	46.60
	±SD	2.07	1.79	2.74	6.52	4.10
Muscle (µg/g) wet weight	N	5	5	5	5	5
	$\bar{X}$	10.52	5.03	6.98	8.20	3.42
	±SD	0.60	0.49	0.33	0.23	0.53

Analysis of variance

Tissues	Source	D.F.	Mean SQRS	F-value
Hepatopancreas	Treatment	4	5269.5606	153.1892**
	error	24	34.3990	
Haemolymph	Treatment	4	300.1708	369.5992**
	error	24	0.8122	
Ovary	Treatment	4	9680.9400	651.4764**
	error	24	14.8600	
Muscle	Treatment	4	38.0672	182.8534**
	error	24	0.2082	

** Highly significant ($p < 0.01$)

Table 16. Variations in the concentration of moisture during the different maturity stages of *P. semisulcatus*

Tissues		Maturity stages				
		I	II	III	IV	V
Hepatopancreas	N	5	5	5	5	5
	$\bar{X}$	78.17	69.08	74.84	72.26	72.24
	±SD	7.32	0.52	0.34	2.41	4.04
Ovary	N	5	5	5	5	5
	$\bar{X}$	81.59	80.02	76.96	75.49	83.94
	±SD	3.53	4.19	2.84	5.20	0.18
Muscle	N	5	5	5	5	5
	$\bar{X}$	77.05	74.94	75.85	77.00	74.45
	±SD	2.59	2.63	1.04	1.28	1.07

Analysis of variance

Tissues	Source	D.F.	Mean SQRS	F-value
Hepatopancreas	Treatment	4	57.6984	3.7883*
	error	24	15.2306	
Ovary	Treatment	4	58.6108	4.4974*
	error	24	13.0321	
Muscle	Treatment	4	6.9339	1.9830NS
	error	24	3.4966	

* Significant ($p < 0.05$)
NS Non-significant

all the stages, and more particularly between stages II and V. Cadmium was absent in the ovary.

Macro and microminerals changes in the ovary during all the stages from I to V were statistically highly significant at 1% level ($p<0.01$) and the values are given in Table 18a.

Muscle

In the muscle, potassium and sodium registered the maximum values of 60002 and 2004 ppm in stage II respectively. The minimum value of potassium (281234 ppm) recorded in stage I shot up to 60002 ppm in stage II and then gradually declined to 43753 ppm in stage IV. In stage V, slight increase was observed. The trend in sodium content of muscle was more or less the same as potassium. Mn and Pb showed the least values of 8.0 and 3.1 ppm in stages I and III respectively. Cu, Ni and Pb recorded the maximum values of 25, 87 and 4.1 ppm respectively in fully mature stages (IV) whereas Mg, Na, K, Fe and Pb showed the maximum values of 186.4, 2004, 60002, 468 and 4.1 ppm respectively in stages II. Ca and Zn (393 and 116.4 ppm) recorded the maximum values in stage I, while the minimum values were seen in stage V (296 and 87 ppm). Mn was at the maximum (9.2 ppm) in stage V. Cd was totally absent in muscle also.

Mineral contents in muscle were analysed during different maturity stages (I to V) and variation were statistically highly significant at 1% level ($p<0.01$) except calcium. The values of minerals analysed in the muscle are given in Table 19a.

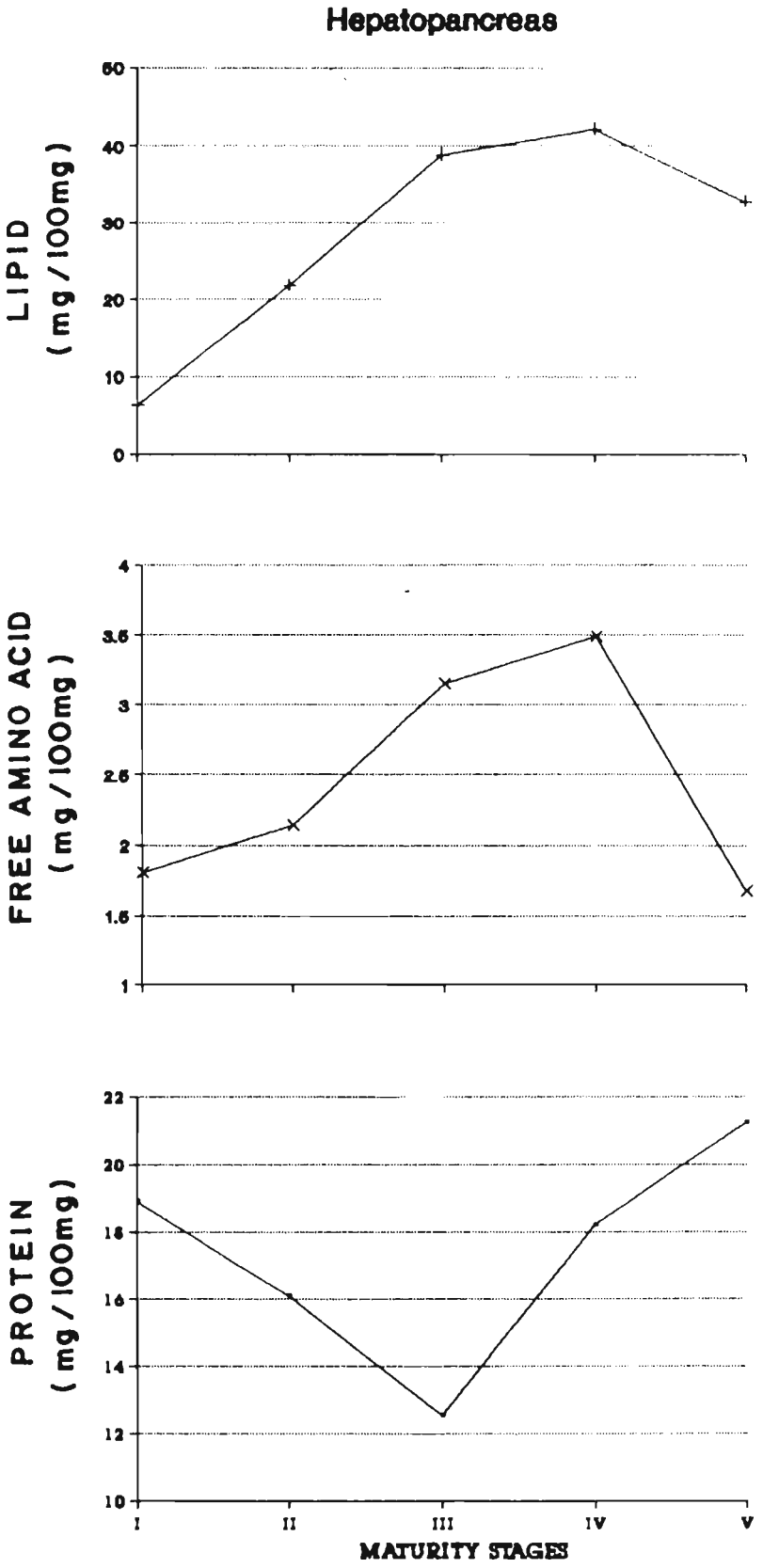


Fig.17 : Trends in biochemical composition of hepatopancreas at different stages of maturity

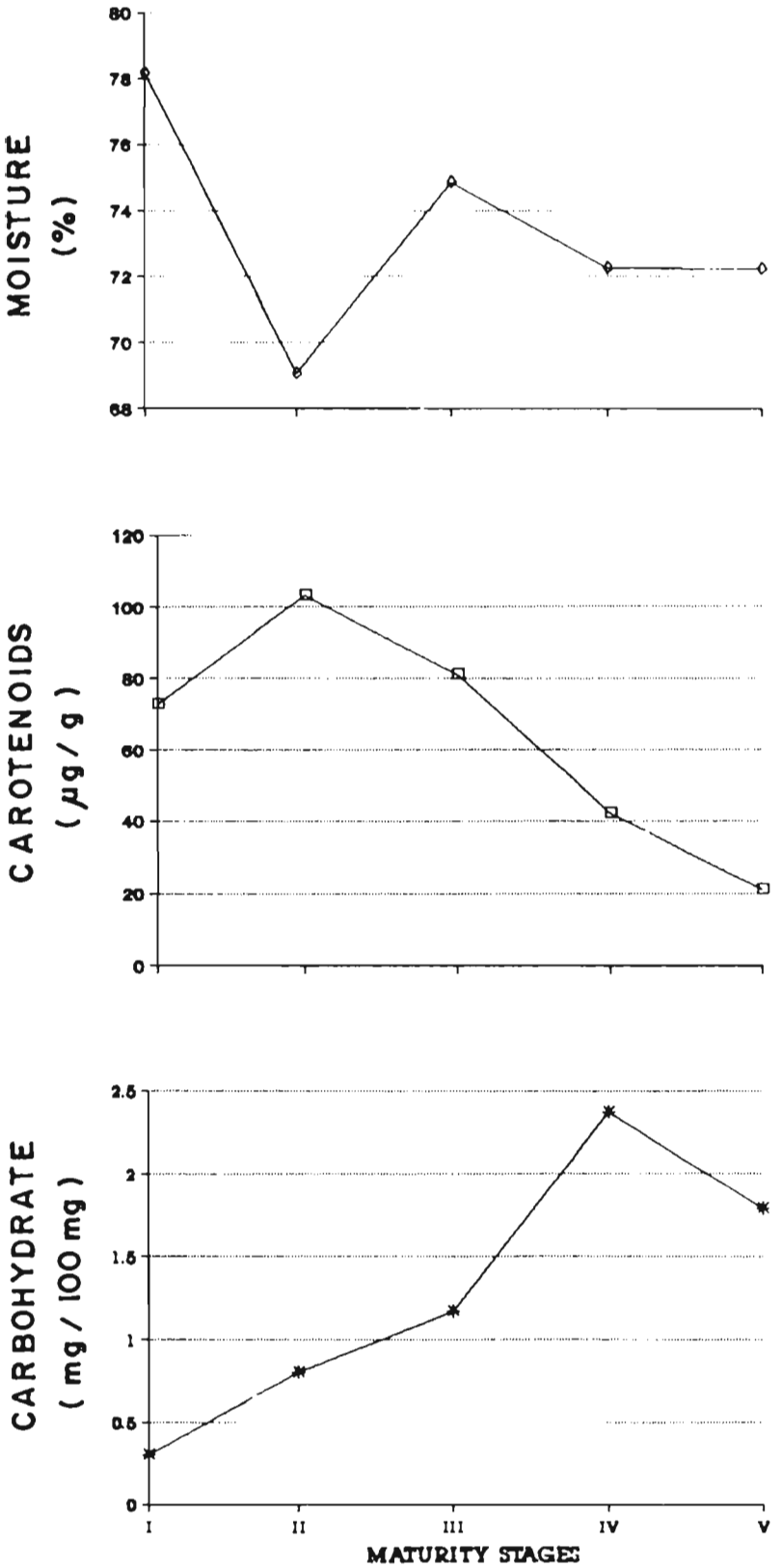


Fig. 18: Trends in biochemical composition of hepatopancreas at different stages of maturity

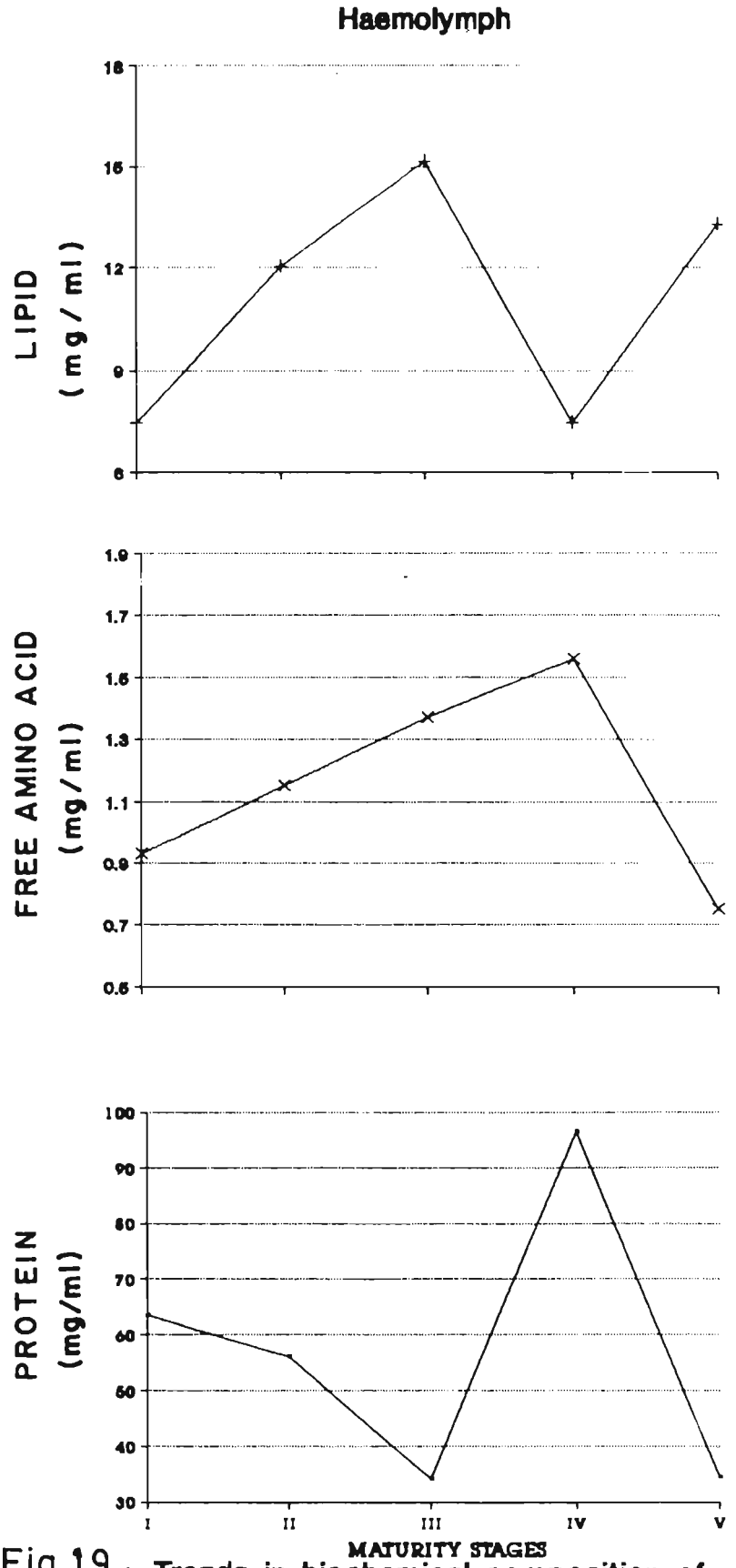


Fig. 19 : Trends in biochemical composition of haemolymph at different stages of maturity

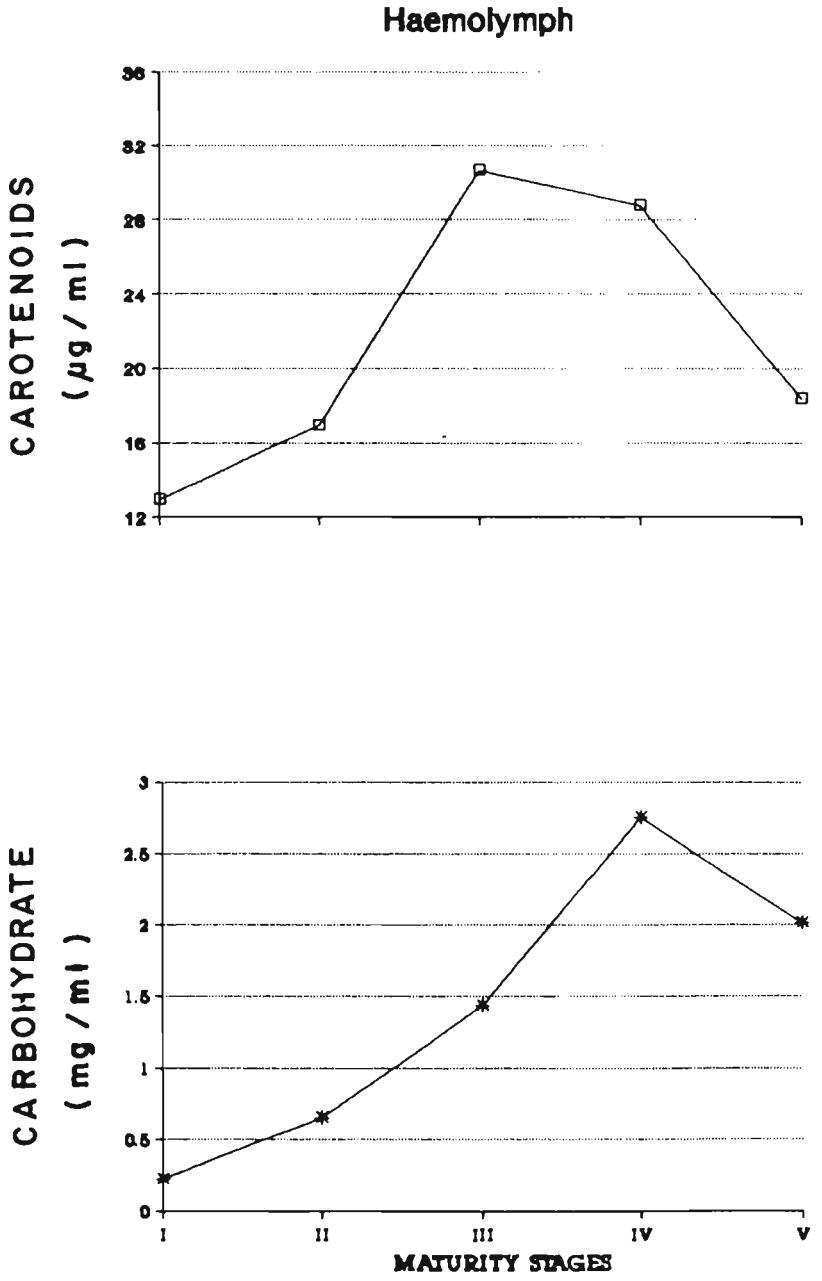


Fig.20: Trends in biochemical composition of haemolymph at different stages of maturity

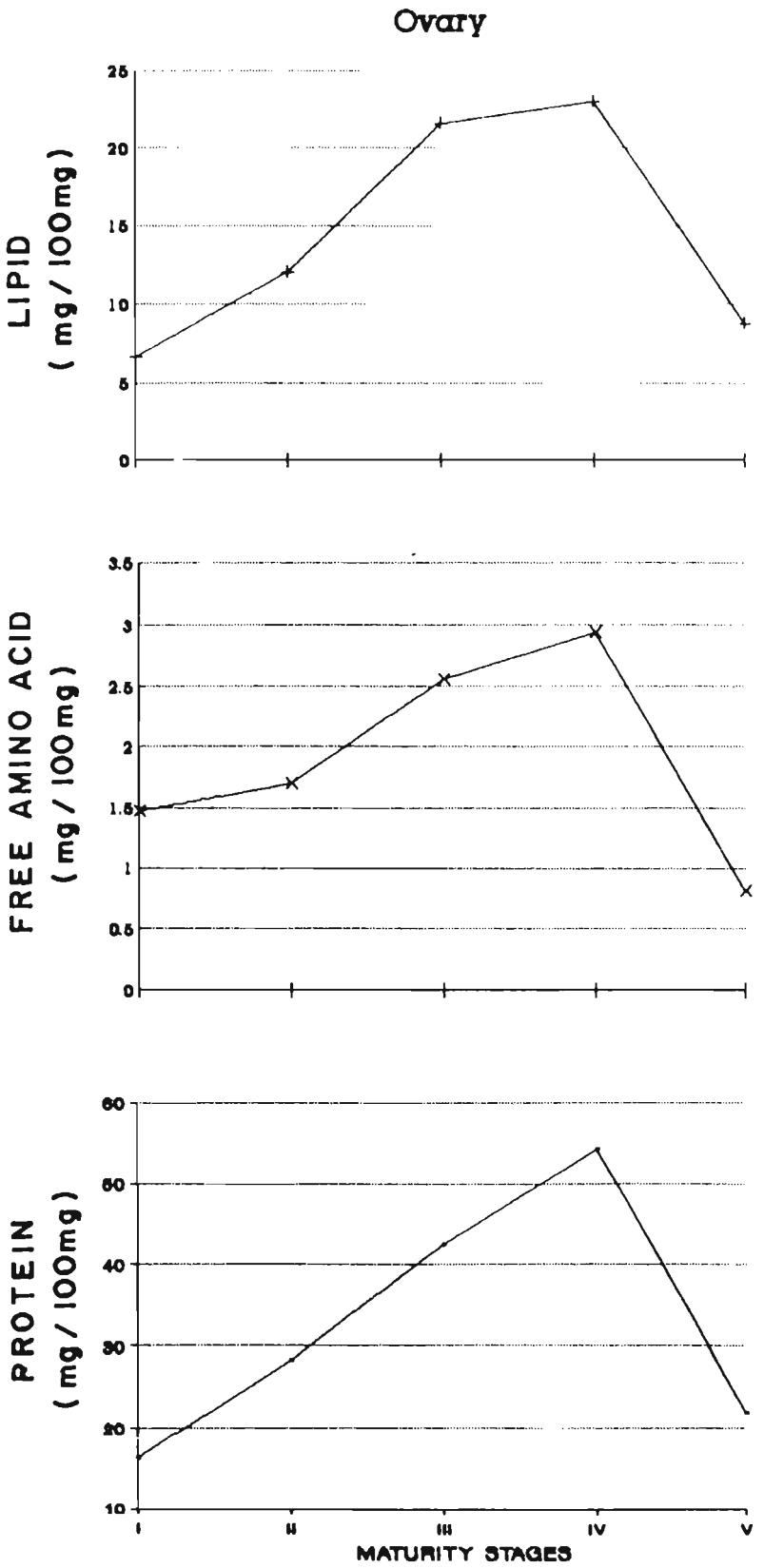


Fig. 21: Trends in biochemical composition of ovary at different stages of maturity

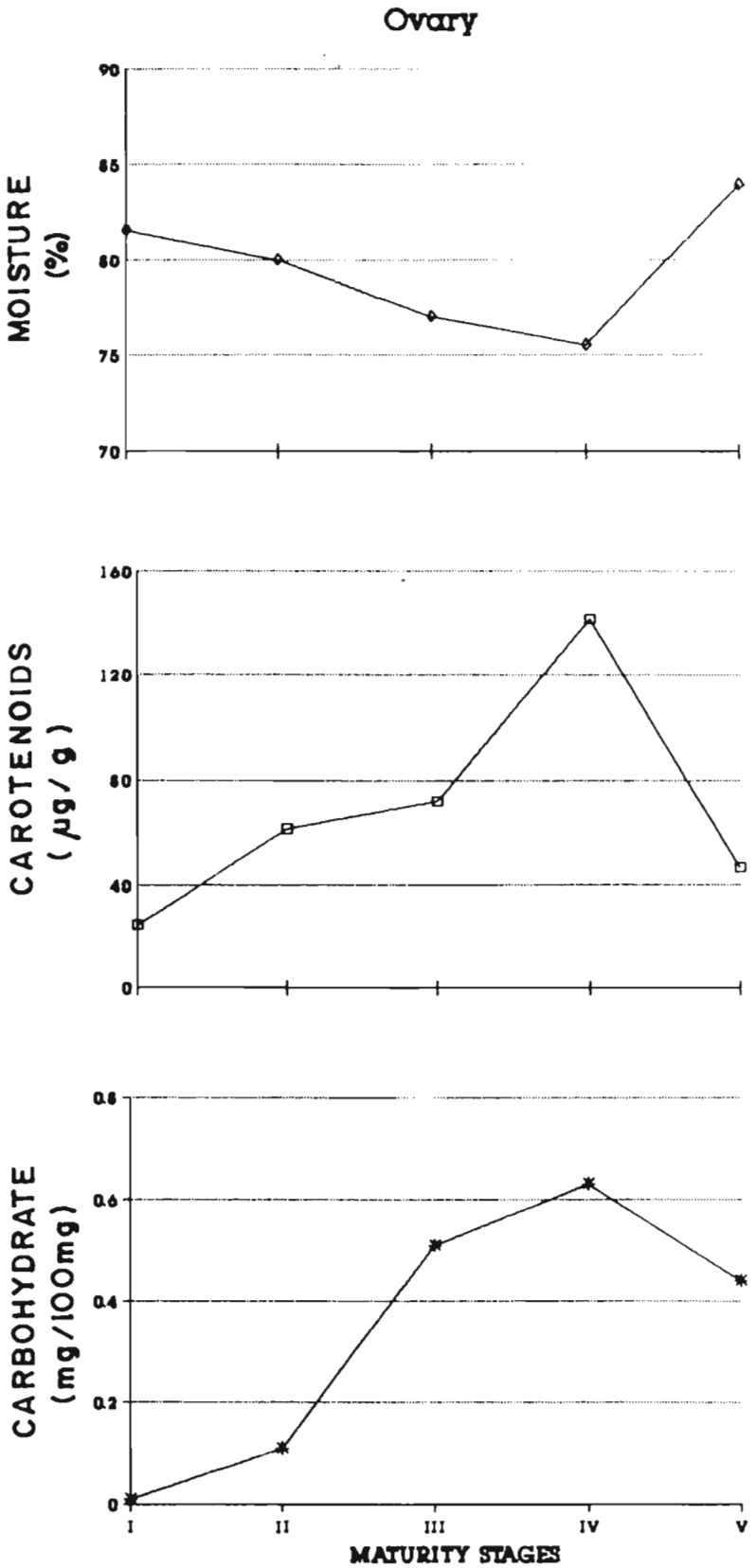


Fig. 22: Trends in biochemical composition of ovary at different stages of maturity

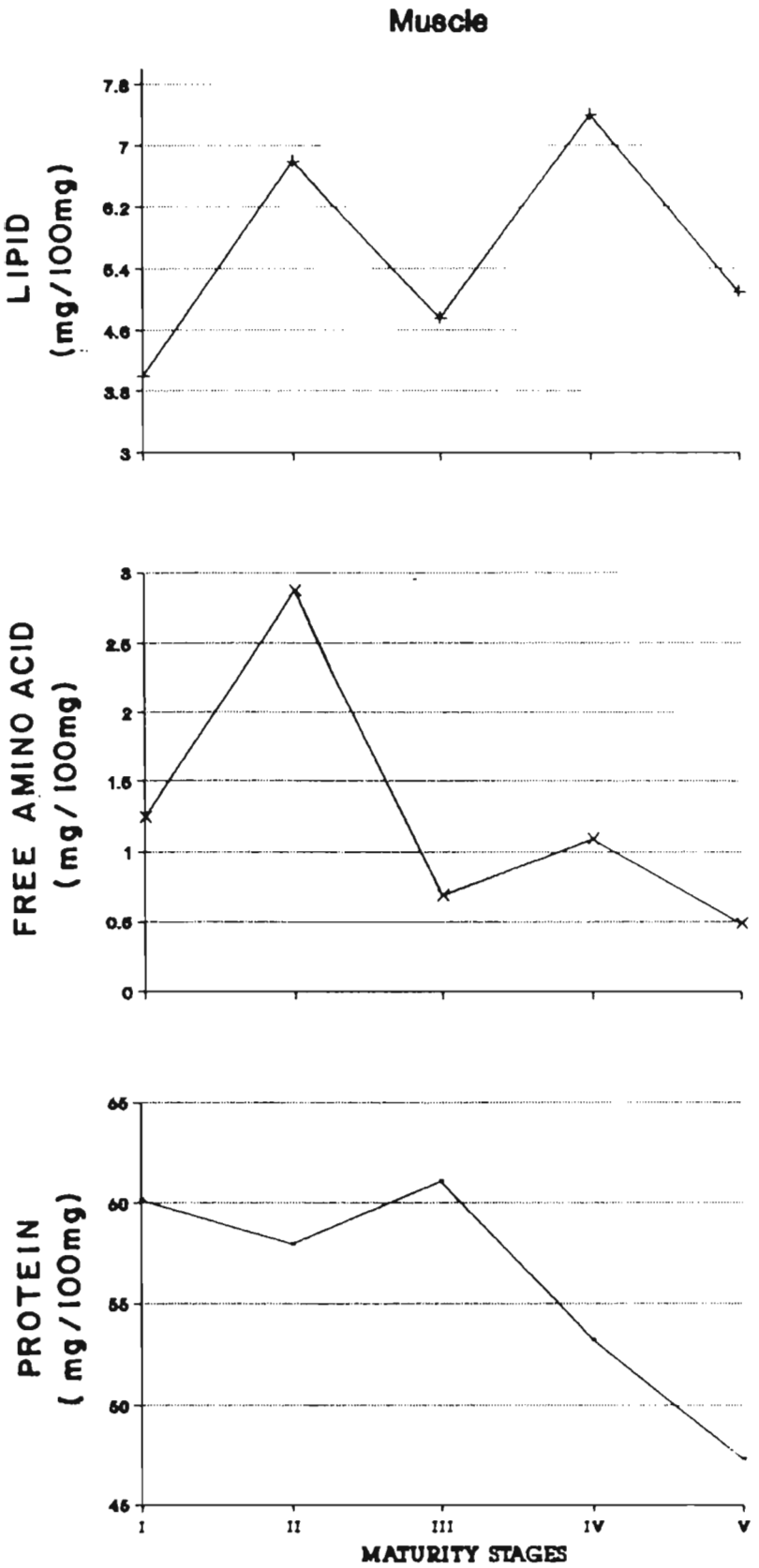


Fig.23: Trends in biochemical composition of muscle at different stages of maturity

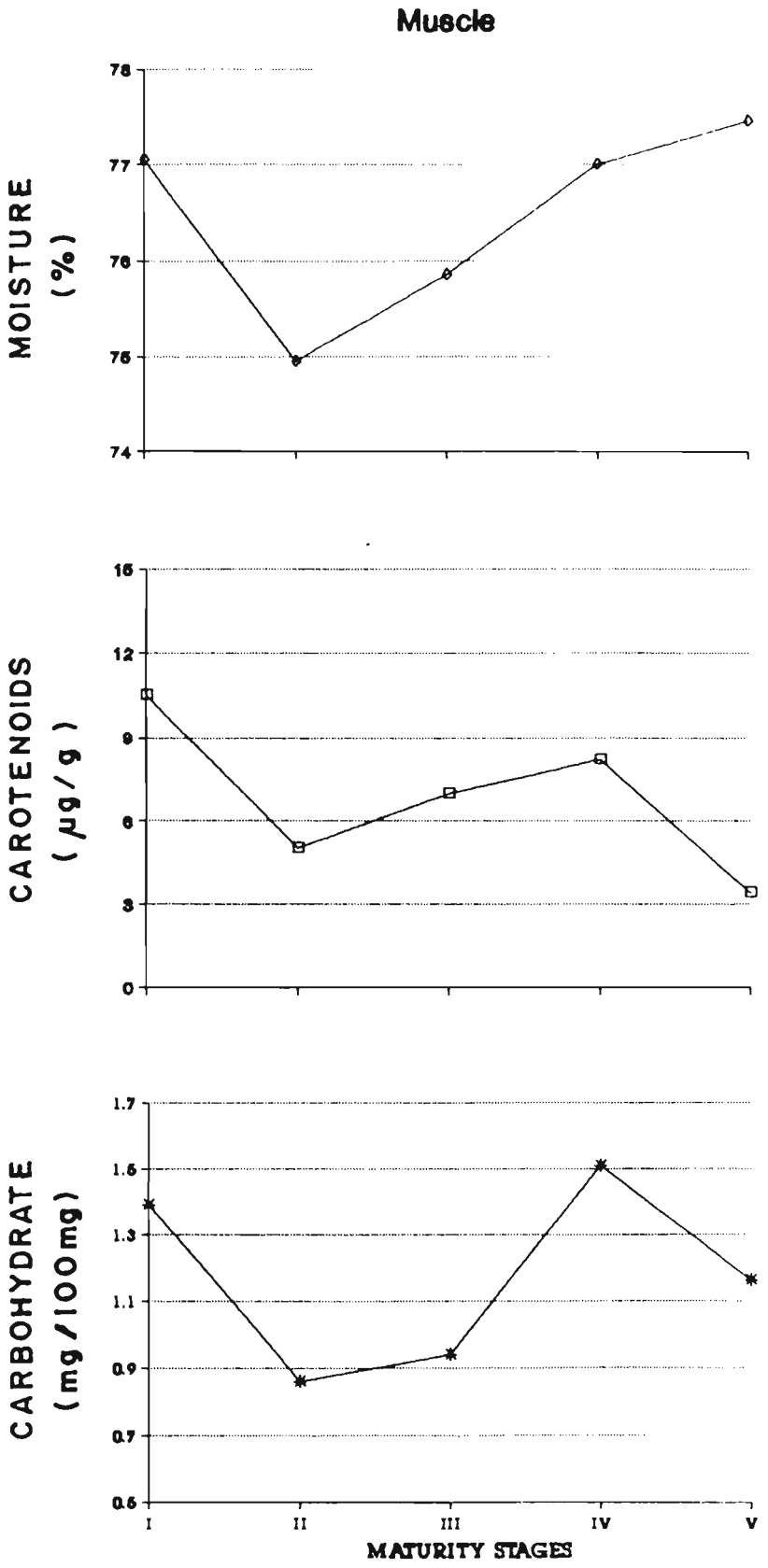


Fig. 24 : Trends in biochemical composition of muscle at different stages of maturity

Composition of mature egg

Based on the analysis conducted, the biochemical constituents and mineral contents of the mature egg of *P.semisulcatus* have been worked out as given below:

Metabolite	Percentage
Moisture	75.49 wet weight
Protein	54.22 dry weight
Free amino acid	2.94 dry weight
Lipid	22.93 dry weight
Carbohydrate	0.63 dry weight
Carotenoids	141.00 wet weight (µg/g)
Macrominerals	**ppm**
Calcium	577.5 dry weight
Magnesium	360.9 dry weight
Sodium	5,479.0 dry weight
Potassium	17,516.6 dry weight
Microminerals	**ppm**
Copper	27.4 dry weight
Manganese	13.0 dry weight
Iron	760.8 dry weight
Zinc	216.0 dry weight
Nickel	164.7 dry weight
Lead	12.1 dry weight
Cadmium	-

Among the various organic reserves, the major component of yolk was found to be water and the next in order of dominance the protein and lipid. The other metabolites were present only in small quantities.

DISCUSSION

Biochemical changes

The analysis of the various biochemical constituents during ovarian maturation showed cyclic changes in the accumulation of organic reserves in hepatopancreas, haemolymph and ovary of *P. semisulcatus*. The variations can be related to vitellogenin synthesis. The biochemical variations in muscle, however did not show any definite pattern.

Protein

In hepatopancreas, the protein content was comparatively high in immature stage (18.91 mg/100 mg) but it decreased slowly in the subsequent stages upto late maturing stage (12.53 mg/100 mg). From late maturing to spent stages, substantial increase in protein contents was noticed. The lowering of protein level during the initial phase of ovarian maturation in the present study can be attributed to mobilization of hepatic protein for vitellogenin synthesis associated with the onset of maturation. However, the substantial increase in protein level during the mature and spent stages (Fig. 17) could be preparatory steps for increased protein utilization in the rematuration process.

In decapod crustacea, the heapatopancreas has been proposed as a logical site for extra-ovarian Vg synthesis (Charniaux-Cotton, 1985; Quackenbush and Keeley 1988). Adiyodi (1969) reported that in *Paratelphusa hydrodromous* the hepatopancreas was not the major source of vitellogenin protein but could well be the source of vitellogenic precursor. Rankin *et al.*, (1989), however, did not detect synthesis of polypeptide in the hepatopancreas although the high molecular weight polypeptide of ~ 175-200 KDa were abundant in the vitellogenic ovaries. Shafir *et al.* (1992), through *in vivo* studies established the intense involvement of hepatopancreas in the

vitellogenic process in *P. semisulcatus*. Fainzilber *et al.* (1992), carrying out *in vitro* studies on *P. semisulcatus* came across Vt specific *de novo* synthesized protein in hepatopancreas from early vitellogenic female, although its abundance was less than 15% of total protein synthesis and concluded that there was a extraovarian contribution to vitellogenesis in the species was low. The present result on protein content in hepatopancreas during mature and spent stages of ovary is in contrast to the trend observed by Dy-penaflorida and Millamena (1990) in *P.monodon* by Mohamed and Diwan (1992) in *P.indicus* and by Vasudevappa (1992) in *M. dobsoni*, in that the protein values have shown a drastic reduction in mature and spent stages. An inverse relationship was observed between hepatopancreas and ovary by Vasudevappa (1992) in *M. dobsoni*. Castille and Lawrence (1989) reported that the protein contents of the digestive glands increased during maturation in both *P. aztecus* and *P. setiferus*, whereas, according to spaargaren and Haefner (1994) the hepatopancreatic protein increased only slightly prior to previtellogenesis and decreased during vitellogenesis in the brown shrimp *Crangon crangon*.

Haemolymph

In haemolymph the protein content showed wide fluctuations in relation to ovarian maturation and no clear trend was discernible (Fig. 19) Higher levels of protein were observed in the immature as well as mature stages. The fluctuations could be related to mobilization of protein for vitellogenin synthesis. In *M. dobsoni*, Vasudevappa (1992) also noticed that the high levels of protein in haemolymph did not show any trend from immature to spent condition. Spaargaren and Haefner (1994) reported the decrease of haemolymph content during vitellogenesis and light increase prior to previtellogenesis in the brown shrimp *Crangon crangon*. The present observation is in contrast to the findings of Barlow and Ridgway (1969), Mohamed and Diwan (1992) and Quinitio and Millamena (1992) in other decapods in which a steady increasing trend in protein levels in haemolymph was noticed from early maturing to fully mature stage and a decrease in spent

stage Quinitio and Millamena (1992) finding higher levels of female specific protein in spent females than in previtellogenic females of *P. indicus*, evolved the hypothesis that the sources of vitellogenin is outside the ovary and the same is trasported there through the haemolymph. Similar trend was also found in the crab *cancer antennarius* (spaizini, 1988), Prawn *P. vannamei* (Quackenbush, 1989) and *Pandalus* Kessleri (Qninitio *et al.*, 1989). Electrophoretic studies on the haemolymph of female crustaceans revealed the presence of a lipoglycoprotein band, immunologically identical to the lipovitellin of the oocyte yolk (Adiyodi, 1968; Kerr, 1969 and Wolin *et al.*, 1973). Chang *et al.* (1994) through electrophoretic study of haemolymph of mature female demonstrated that the vitellogenin is a lipo-glycophosphoprotein with two sub units of polypetides (170 KD and 32 KD). Shafir *et al.* (1992) studying the protein, vitellogenin and vitellin levels in the haemolymph and ovaries of *P. semisulcatus* during ovarian development noticed significant increase in Vt in the ovary (in ovaries with AOD of 150-250 μm) and concluded that the contribution of Vg from haemolymph to the formation of Vt in the ovary was quantitatively insignificant.

Quantitatively the protein content in ovary was much higher than in hepatopancreas and haemolymph at all stages of ovarian development. The protein levels also showed progressive increase (Fig. 21) from immature to mature stages thereby indicating that the ovary is the single major site for synthesis of proteins moiety of vitellin (autosynthesis) than hepatopancreas and haemolymph. This fact is in agreement with the finding of others in prawns including the same species (Pillai and Nair, 1973; Castille and Lawrence, 1989; quackenbush, 1989 a, b; Rankin *et al.*, 1989; Browdy *et al.*, 1990; Dy-Penaflorida and Millamena, 1990; Fainzilber *et al.*, 1992; Mohamed and Diwan, 1992; Quinitio and Millamena, 1992; Shafir *et al.*, 1992; Tom *et al.*, 1992; Vasudevappa, 1992; Khayat *et al.*, 1994; Spaargaren and Haefner, 1994). In other decapod crustaceans also the same pattern has been reported by a number of workers (Diwan and Nagabhushanam 1974; Varadarajan and Subramoniam, 1982; Eastman-Reks and Fingerman, 1984, 1985; Yano and

Chinzei, 1987. This is in contrast to the well characterized extraovarian syntehsis of vitellins in isopods and amphipods (Meusy and Payen, 1988). In *Penaeus indicus*, Mohamed and Diwan (1992) reported that the maximum protein level in the ovary during the mature stage was 39.27 mg/100 mg in *P.indicus* (Mohamed and Diwan, 1992), 73.34 mg/100 mg in *P. monodon* (Dy-Penaflordia and Millamena (1990) and 31.10 mg/100 mg in *M.dobsoni* (Vasudevappa, 1992) as against 54.22 mg/100 mg recorded for *P. semisulcatus* during the present study. Browdy *et al.* (1990) and Shafir *et al.* (1992) studied vitellin synthesis by *in vitro* incubations of several pieces removed from the ovaries of one female prawn and found the most significant increase in Vt in the ovary of *P. semisulcatus*. Tom *et al.* (1992), who studied the ovarian protein synthesis in *P. semisulcatus* and *P. vannamei*, identified high molecular weight cortical crypts protein and amino acid in both the species.

In the present investigation, though high protein values were noticed in muscle, it did not reveal any definite trend in relation to the process of maturation (Fig. 23) and therefore this can not be correlated to the process of vitellogenesis. The irregular pattern observed in the muscle protein content might be due to its involvement in growth and metabolism rather than in reproduction and this is in agreement with the results obtained in *P. indicus* (Mohamed and Diwan, 1992), *M. dobsoni* (Vasudevappa, 1992) and the crustaceans in general (Claybrook, 1983).

Free amino acid

Free amino acid values showed highly significant variations in tissues during the process of maturation. The amino acid contents of ovary, haemolymph and hepatopancreas increased from a low level in immature stage to the highest level in fully mature stage and then sharply declined in spent condition (Fig. 17). In muscle, amino acid content, such a trend was not noticed. The hepatopancreas showed a relatively higher amino acid content than the ovary, muscle and haemolymph. In contrast to this, Dy-Penaflorida

and Millamena (1990) reported higher amino acid profile in muscle than in ovary and hepatopancreas though it could not be related to ovarian maturity in the case of *P. monodon*. Vasudevappa (1992) obtained identical results in *M.dobsoni*. The variation reported was that amino acid content was more in muscle than that of ovary. The increasing trend noticed in hepatopancreas was inverse to the pattern noticed for total protein. This clearly indicated the gradual storage and mobilization of simpler forms of protein in the hepatopancreas. A steady increase of free amino acid in ovary and haemolymph also apparently indicated the mobilisation, storage and utilization of free amino acids during vitellogenin process.

Lipid

The lipid levels analysed in various tissues of *P. semisulcatus* during ovarian maturation showed that the values in hepatopancreas and ovary were higher than in haemolymph and muscle (Figs.17-24). Among hepatopancreas and ovary, the former showed considerably higher values of lipid level in the different stages of maturation than in the latter. The lipid contents in both hepatopancreas and ovary increased from immature to mature stages, while in the spent stage the values suddenly decreased. A perusal of Table 13 would reveal that, in ovary, the lipid contents decreased more drastically from mature stage to spent stage than in hepatopancreas, evidently showing active utilization of lipid for Vg synthesis.

The hepatopancreas has been identified as the principal storage site for lipids in crustaceans (Chang and O' Connor 1983). Sarojini *et al.* (1986) reported that the hepatopancreatic lipid increased in the maturing ovary of *M.affinis* and considered hepatopancreas to be the storage organ for glycogen and lipid in great quantities. She further observed that when the ovarian activity was in peak, there were indications for the mobilization of atleast a part of the lipid from hepatopancreas to ovary. Castille and Lawrence (1989) reported that the lipid content of the hepatopancreas increased during

maturation in *P. aztecus*, but in *P. setiferus* there was a decrease. He suggested that mobilization of stored lipid from the hepatopancreas to ovaries may be more important in *P. setiferus* than in *P. aztecus*. The increase from immature to mature stages and sudden decrease in spent stage during the present study indicate absorption and mobilisation of lipid for subsequent use in vitellogenesis. A scrutiny of the hepatopancreatic lipid level data (Table 13) in comparison with that of ovary would clearly show that decrease from the highest level in mature stage to spent stage is relatively less than the drastic decrease observed from mature stage to spent stage in the ovary. This would point to the fact that although lipid mobilization takes place from hepatopancreas to ovary only a part of lipid content of hepatopancreas is thus mobilized and then substantial quantity of the same stored in the hepatopancreas itself to be mobilized for other metabolic activity. This is in support of the view expressed by Sarojini *et al.* (1986) in *M. azffinis*. Khayat *et al.* (1992), who studied fat transport in *P. semisulcatus*, reported that hepatopancreas contained 70% of triacylglycerol and 30% of phosphatidylcholine indicating its function as a storage organ of absorbed lipids for subsequent use in vitellogenesis. Further they suggested from the additional protein obtained after precipitation with Vt specific antiserum that the additional protein functions as lipid carriers from the hepatopancreas to the ovary. The significant increase in lipid levels of hepatopancreas at stage IV noted during the present study would also suggest additional lipid storage to meet the energy requirement for spawning. An increase in hepatopancreatic lipid during the entire phase of ovarian development was also observed in *P.japonicus* by Teshima and Kanazawa (1983) and Teshima *et al.* (1989), *P.monodon* by Millamena and Pascual (1990) and *M. dobsoni* by Vasudevappa (1992). Variations from the present pattern of lipid concentration in hepatopancreas during different phases of ovarian development have been reported in other species of shrimp. Mohamed and Diwan (1992) reported in *P. indicus* that the lipid level of hepatopancreas increased upto late maturing stage and slowly decreased during mature stage. Spaargaren and Haefner (1994) reported that the total lipid increase was traceable to hepatopancreatic

accumulation prior to vitellogenesis and subsequent decrease during vitellogenesis was associated with increases in haemolymph and ovarian lipid content in the brown shrimp *Crangon crangon*. Wide fluctuations in lipid composition due to mobilization of stored lipid for vitellogenin synthesis have been reported to occur both in hepatopancreas and gonads of prawns by George and Patel (1956), Pillai and Nair (1973) and Gopakumar and Nair (1975).

The haemolymph lipid gradually increased from immature to late maturing stages and after a sharp fall in mature stage its level increased almost near to the level in maturing stage. The value obtained in spent stage was more than the value obtained in early maturing stage. Peak lipid level in haemolymph was found at stage III. The fluctuations in the lipid content of haemolymph could be due to vitellogenin synthesis. This is in agreement with the observation made by Khayat *et al.* (1992) who studied the fat transport in the penaeid prawn *P. semisulcatus*. They reported that the lipoprotein present in the haemolymph contained phosphatidylcholine of high specific radio activity. In the present study, during spent stage, the lipid content in haemolymph was more than that of early maturing stage. Similar trend was reported in *P. japonicus* by Teshima and Kanazawa (1978), *P. indicus* by Adiyodi (1985), Mohamed *et al.* (1981) and Mohamed and Diwan (1992) and *M.dobsoni* by Vasudevappa (1992) through the peak lipid level was observed in stage IV. They reported that the lipid content remained comparatively at a high level in spent stage due to the possible resorption of relicit oocytes and the subsequent retransport of nutrient material from the ovary back to the storage sites and also perhaps due to the rapid rematuration capabilities of these species. Spaargaren and Haefner (1994) recently reported that the lipid level in haemolymph of *Crangon crangon* increased only slightly prior to previtellogenesis and decreased during vitellogenesis. On the contrary, Young *et al.* (1993) observed in *M. rosenbergii* that the free ecdysteroids level in haemolymph increased during vitellogenesis from stage I to stage IV. They further noted in *P. monodon* that the major component of the stage IV haemolymph ecdysteroids was 20-hydroxyecdysone 22-acetate.

In the ovary, Gehring (1974) noticed the changes in total lipids, neutral lipids, phospholipids, triglycerides and sterols in relation to maturation, with peak levels in late maturing stage in the pink shrimp *P.duorarum*. In *P. japonicus*, Guary *et al.* (1975) also related such changes in fatty acids to ovarian maturation. Similar variations in the lipid composition during ovarian maturation were also reported by Middleditch *et al.* (1980) in *P. setiferus* and Teshima and Kanazawa (1983) in *P. japonicus*. In the present study the lipid synthesis in ovary gradually increased during oogenesis. During spent stage, the value suddenly decreased to the lowest. However, it was more than the value observed in the immature stage. Similar trend was reported by Khayat *et al.* (1992) and Shenker *et al.* (1993) in *P. semisulcatus*. Khayat *et al.* (1992) studied fat transport in this species and concluded that the vitellogenesis was associated with the accumulation of large amounts of proteins mainly vitellin (Vt) and lipids mainly triglycerol (TG), phosphatidylcholine (PC) and cholesterol in the ovary. Further, in the female, an additional protein was obtained after precipitation with Vt-specific antiserum and it was suggested that these proteins function as lipid carriers from the hepatopancreas to ovary. Shenker *et al.* (1993) reported from the *in vitro* incubated ovaries that the lipid synthesis gradually increased during oogenesis, and the phosphatidylcholine (50%); triacylglycerol (30 - 35%); diacylglycerol were the relatively important components of the synthesised neutral lipids present in immature ovaries. They could not detect cholesterol and the phosphatidylcholine (50%); triachylglycerol (30-35%); diacylglycerol were the relatively important components of the synthesised netural lipids present in immature ovaries. They could not detect cholesterol and polyunsaturated fatty acid except the presence of saturated and mono unsaturated fatty acids. Further they observed the binding of lipids (Mainly triacylglycerols and phosphatidylcholine) at the commencement of vitellogenesis and concluded that the binding of lipids of Vt and the alteration of the lipid composition while entering the oocyte could be efficient tools for distinguishing genuine lipid uptake from lipoprotein binding to the external

surface of the oocyte plasma membrane when studying lipid transport to the ovary.

The trends in ovarian lipid noticed during the present sutdy as well as those reported by Khayat *et al.* (1992) and Shenker *et al.* (1993) in *P.semisulcatus* are similar to the results reported in other prawns like *P.duorarum* (Gehring, 1974), *P. japonicus* (Guary *et al.*, 1975; Teshima and Kanazawa, 1983; Teshima, *et al.*, 1989), *P. setiferus* (Middleditch *et al.*, 1980), *P. indicus* (Asokan and George 1984; Galois, 1984; Mohamed and Diwan, 1992), *P. aztecus* and *P. setiferus* (Castille and Lawrence, 1989), *Pleoticus muelleri* (Jeckel *et al.*, 1989), *P. kerathurus* (Mourente and Rodriguez, 1991), *M. dobsoni* (Vasudevappa, (1992) and *Crangon crangon* (Spaargaren and Haefner, 1994). Sarojini *et al.* (1986) reported in *M. affinis* that the fluctuation of the lipid was considerably more than that of protein and glycogen. They further added that when the ovarian activity was in peak stage during the breeding period, there was an indication of the mobilization of atleast a part of the lipid from hepatopancreas to ovary. Millamena and Pascual (1990) reported that peak lipid level in *P. monodon* was found at stage III though relative increase could be noticed at stages II and IV. It suggested storage and utilization of lipids for maturation and spawning processes and the phospholipids were mainly responsible for increases in ovarian lipids.

Chang *et al.* (1993) reported eight polypetide subunits and a molecular weight of 492 KDa present in the purified vitellin in *P. monodon* and suggested that the purified vitellin was a lipo-glyco-carotenoprotein. Chang *et al.* (1993) isolated four polypeptide in the vitellin (Yolk protein) and a glycolipoprotein from the same species. In contrast to this, in *P. monodon*, Young *et al.* (1993) reported that the concentration of free ecdysteroid, predominantly 20-hydroxyecdysone in *P. monodon* was maximal in immature ovary (stage O). It decreased gradually during early vitellogenesis (Stage 0 to stage I) and fell sharply during later vitellogenesis (stages I to IV). Komatsu and Ando (1992) studied the isolation of crustacean egg yolk protein by differential density

gradient and reported that high density lipoprotein (HDL) and low density lipoprotein (LDL) possessed phospholipid as a major lipid in the egg yolk protein and HDL, but not LPL, and contained carotenoids. In the present study, the ovarian lipid increased during oogenesis indicating its main role for vitellogenin synthesis next to the major role played by ovarian protein.

Lipid content of muscle in the present study was low showing no relation with ovarian maturation. Similar conclusion was also drawn by Millamena and Pascual (1990) in the case of *P. monodon*. According to Mohamed and Diwan (1992) amd Vasudevappa (1992), however, the level of lipid in muscle increased from immature to mature stages and subsequently decreased in spent stage in *P. indicus* and *M. dobsoni* respectively.

Carbohydrate

The variations in carbohydrate levels of hepatopancreas, haemolymph, ovary and muscle varied significantly in relation to the maturation ($p<.01$). In hepatopancreas though the carbohydrate content was generally lower than the portein and lipid, it showed more than a four fold increase in mature stage and a drastic decrease in spent stage indicating the possible transport of carbohydrate substances from this storage organ to the ovary. Identical conclusions were drawn by Adiyodi and Adiyodi (1970 b) who found that free sugars like glucose, galactose and sucrose in the hepatopancreas of *P.hydrodromous* underwent quantitative and qualitative cyclic fluctuations in relation to the ovarian cycle. Trujillo and Luna (1981) studied on the glucidic metabolism in *P. notialis* and they suggested that during ovarian development glycogen is mobilized from the hepatopancreas to the gonads. Similarly, Castille and Lawrence (1991) noticed that both carbohydrate and protein indices increased in the digestive glands of *P. aztecus* in contrast to *P.setiferus*. The overall contribution of carbohydrate in the haemolymph to maturation was relatively high than that of hepatopancreas, muscle and ovary.

The carbohydrate content of haemolymph also showed more than a four fold increase during mature stage and a drastic decrease in spent stage. Similarly, a hike in glucose level in haemolymph during ovarian maturation was also observed by Nagabhushanam and Kulkarni (1980) in *P. hardwickii*, Mohamed and Diwan (1992) in *P. indicus* and Vasudevappa (1992) in *M.dobsoni*. While Mohamed and Diwan (1992) noticed a four fold increase in haemolymph glucose level. The other authors indicated a three fold increase. The increasing trend of haemolymph carbohydrate content during the entire process of maturation and the sudden decline in spent stage in *P. semisulcatus* would suggest active transport of carbohydrate substances to the ovary.

The carbohydrate content in the ovary also showed a similar trend with a steady increase in values with the progress of maturation and a decrease in the spent stage. However, the value in spent stage was more than that of the late maturing stage. Adiyodi and Adiyodi (1970 b) reported in *P.hydrodromous* that the sugars present in the hepatopancreas and haemolymph were also found in some abundance in the ovary during the early stage of vitellogenesis, but disappeared progressively as the proteins in the ovary become conjugated in the course of yolk formation. In *C. clibanarius*, Varadarajan and Subramoniyam (1982) observed that the carbohydrate levels in the ovary diminished to very low level during the ripe stage. From this these authors inferred the possibility of carbohydrate levels in the ovary remaining constant, and the relative amount of other macromolecules reducing its percentage in later stages. Spaargarren and Haefner (1994) reported that ovary never accumulated large quantities of carbohydrate. The trend noticed for carbohydrate content in ovary during the present study is identical with the observation of Sarojini *et al.* (1986) in *Metapenaeus affinis*, Castille and Lawrence (1989) in *P. aztecus* and *P. setiferus*, Mohamed and Diwan (1992) in *P. indicus* and Vasudevappa (1992) in *M. dobsoni*. Chang *et al.* (1994) proved electrophoretically the presence of carbohydrate and lipid in vitellogenin getting stained with periodic acid schiff reagent and Sudan Black B, respectively.

The colour of the ovary during vitellogenesis is due to the increased presence of carotenoid pigments. Establier (1966) observed in the deep-sea shrimp *Plesiopenaeus edwardsianus* an increase in weight and carotenoid content of the ovary during vitellogenesis. The highest value of carotenoid in hepatopancreas was recorded during early maturing stage and the value suddenly decreased in mature stage. In spent stage, the lowest value was recorded. In haemolymph, the values increased upto late maturing stage and slowly came down during vitellogenesis and spent stage. In ovary on the other hand, the carotenoid levels increased sharply in relation to maturation and decreased to the lowest value in spent stage. According to Castillo *et al.* (1982) the hepatopancreas plays a major role in the absorption of carotenoids from food and its carotenoid content fluctuates during vitellogenesis. In *C. maenas*, Ceccaldi and Martin (1969) reported that the carotenoid pigments were concentrated from the hepatopancreas into the haemolymph during vitellogenesis. The fluctuations in the carotenoid content in haemolymph and hepatopancreas observed during vitellogenesis in the present study is apparently owing to the transfer of pigments from the hepatopancreas to the ovary. The steady build up of carotenoids in the ovary may be used for the formation of the glycolipo-caroteno-protein yolk as also inferred by Mohamed and Diwan (1992) in the case of *P. indicus*. The dark green colouration of the ripe ovary in *P. semisulcatus* is apparently due to the high content of astaxanthin pigment since the green-in-blue complexes are often found to contain astaxanthin as the prosthetic group (Tanaka *et al.*, 1976; Castillo *et al.*, 1982). According to Ishikawa *et al.* (1966), astaxanthin is considered to be the major carotenoid of crustacea, comprising about 90% of the total pigments in *P. japonicus*.

The various functions attributed to carotenoids are, as a cross link between lipid and protein to stabilize the lipoprotein molecule, to protect the protein from catalytic action of the enzyme and to protect the eggs from high illumination and solar radiation (Castillo *et al.*, 1982).

Moisture

Moisture was observed to be the principal component in the ripe ovary of *P. semisulcatus*. Moisture content of ovary and hepatopancreas showed significant variations and a declining trend during vitellogenesis. However, the moisture content of muscle did not show significant variation during vitellogenesis. In both ovary and hepatopancreas, the moisture content indicated an inverse relationship between water content and gonad development (Figs.18,22 & 24). In lobsters, George and Patel (1956) observed an inverse relationship between water and fat content of the gonad, and the increase in fat content was evidently associated with gonadal development. This inverse relationship was also reported by Pillai and Nair (1971) and Sarojini *et al.* (1986) in *M. affinis*, Read and Caulton (1980) in *P. palagicus*, Mohamed and Diwan (1992) in *P. indicus* and Vasudevappa (1992) in *M.dobsoni*. In general, the water content in the ovary tended to decline along with an increase in total mass of organic substances in the ovary. It is possible that the continued deposition of organic materials in ovarian and hepatic tissues results in loss of water content.

A general characteristic of crustacean reproduction is the production of yolk laden eggs. The yolk is a combination of protein, lipid, sugars, and some steroid hormones (Wallace *et al.*, 1967; Couch and Hagino, 1983 and Adiyodi, 1985). The purpose of the yolk is primarily nutrition for the developing embryo. After hatching, it may serve as the sole source of nutrition until the functional mouth parts are developed in the larvae (Anderson *et al.*, 1949; Cook and Murphy, 1969). The quality and quantity of egg yolk proteins (vitellin) the females incorporate into the eggs may have a direct and measurable effect on larval survival (Quackenbush, 1991). In the present study, the biochemical composition of the mature ovary of *P. semisulcatus* revealed that the water formed the most significant portion of the yolk while proteins and lipids constituted the major organic reserves than carbohydrate. It is generally accepted that the yolk proteins provide the basic structural material needed

for tissue buildup during embryonic development, while lipids serve as the major fuel (Adiyodi and Subramoniam, 1983). The present study has revealed that protein is the largest organic constituent of the ovary and lipid the second largest. Carbohydrate is present at a much lower level than protein and lipid. The protein content analysed in all the tissues like hepatopancreas, haemolymph and ovary showed more accumulation of protein in the ovary. This clearly indicates that the ovary is the major single site for synthesis of the protein moiety of vitellin by the process of autosynthesis than the heterosynthetic source from hepatopancreas through haemolymph. In addition to this, some amount of lipoglyco-carotenoprotein is also mobilised from hepatopancreas through haemolmph to the ovary.

Minerals changes

Analysis of various minerals in hepatopancreas, ovary and muscle from immature to mature stages and in the spent recovering stage in *P.semisulcatus* has revealed the presence of all the tested minerals in varying levels except Cd which was totally absent. Studying the mineral changes during ovarian maturation in *P. indicus* and in spiny lobster *Panulirus homarus*, Vijayakumaran (1990) came across, Cd content in hepatopancreas, ovary and muscle in both the animals in the immature and mature stages. Though this trace element is generally considered as non-essential and toxic, he noticed substantial increase of Cd in the hepatopancreas at maturity in both *P.indicus* and *P. homarus* and significant reduction of the same in *P. homarus* after spawning. Nimmo *et al.* (1977) showed that shrimps contained highest residues of Cd in hepatopancreas, followed by gill, exoskeleton, muscle and serum. In *Carcinus maenas*, Jennings *et al.* (1979) encountered in the midgut gland the greatest concentration of Cd and at least 4 Cd-binding proteins could be isolated. Among the various minerals Na, K, Ca, Mg, Cu, Mn, Fe, Zn, Ni and Pb whose presence was detected, K, Na and Ca were the most dominant items in all the tissues examined. In general, most of the elements were seen in the highest concentration in the immature stage, while in the different maturing

and maturity stages their concentration was relatively low. A perusal of the distribution of mineral concentration values given in tables 17-19 would reveal no clear trend in any of the tissues examined.

Hepatopancreas

In hepatopancreas, while the dominant elements K, Na and Ca recorded the highest values in the immature stage, Fe, Ni, Mg and Mn in late maturing stage and Cu and Pb in the spent stage. The concentration of Cu, Zn and Fe was relatively high in the late maturing and mature stages. This finding is in close agreement with the observation of Vijayakumaran (1990), in which the concentration of these elements as well as Cd showed substantial increase at maturity in *P. indicus* and *P. homarus*. Davis and Lawrence (1993) concluded from their study on evaluation of the dietary zinc requirement of *P.vannamei* that hepatopancreatic zinc appeared to be a sensitive indicator of dietary zinc status, whereas carapace mineralisation and growth were not affected by dietary zinc intake or the presence of phytic acid. Gopalakrishnan (1991) reported from *Mugil cephalus* that as liver being the site of yolk synthesis, the estimation of calcium and iron in this gland might give a more clear picture of incorporation of these metals into Vtg in teleosts.

Some reports are available on the role of minerals content in hepatopancreas of prawns. Ahearn (1974) and Brick and Ahearn (1978) reported that glycine and lysine transport by the midgut of *P. marginatus* is an active, carrier mediated process with a sodium requirement. Similarly, Ahearn *et al.* (1977) showed that glucose transport by the midgut of *M.rosenbergii* was an active, sodium dependent process. In *P. longipes*, Malley (1977 b) observed the gut to uptake and absorption small amounts of Na, K, Cl, SO_4 and water. In the present study, Na content in hepatopancreas was relatively more in immature, early maturing and spent stages and low in late mature and mature stages. Few reports are available on the iron and lead contents of hepatopancreas. Iron was shown to be associated with soluble

proteins in the hepatopancreas and haemolymph of *Cancer pagurus*. In hepatopancreas, iron was generally associated with high molecular weight proteins Ca 450,000 which might be the crustacean ferritin, an iron storing protein (Guary and Negrel, 1980). The highest Pb-210 concentration factors were found in shrimp, *Sergestes* spp. ranging from a low of 10 in muscle to as high as 1,000,000 in hepatopancreas (Heyraud and Cherry, 1979). The crustacean midgut serves as an organ for accumulation and metabolism of Ca and Cu and for the inactivation of a number of other potentially toxic metals. Calcium is stored as small concentrically structured granules (Becker *et al.*, 1974, 1976; Chen *et al.*, 1974; Hopkin and Nott, 1979; Icely and Nott, 1980). These granules appear to have the additional role of immobilizing Zn and Pb (Hopkin and Nott, 1979). Martin *et al.* (1977) reported that the hepatopancreas is a major storage and regulating organ for Cu in crustaceans, with the result that Cu residues are almost always highest at that site. However, sites of copper accumulation, storage and action vary widely among crustaceans.

Such a storage of mineral contents corresponds, in small part, to the metabolic requirements necessitating Ca for skeleton constitution,Zn for enzymatic catalysis, and Cu for haemocyanin synthesis (Gibson and Barker, 1979; Durfort, 1981; Al-Mohanna and Nott, 1982, 1989). In decapods, this function is principally devoted to R-cells that are able to store several metals (i.e Cu, Zn, Cd, Fe, Pb) during the digestive cycle and in greater quantities when their environmental concentration is high (Hopkin and Nott, 1979, Icely and Nott, 1980; Chassard - Bouchaud, 1981, 1982; Al-Mohanna and Nott, 1982; Brown, 1982; Lyon and Sinkiss, 1984). Organic protein ligands, such as metallothionins, play an essential role during the accumulation process (Jennings *et al.*, 1979). It was suggested (Al-Mohanna and Nott, 1986) in *Penaeus* that B-cells could assume a detoxification function with respect to minerals and an excretion function for several organic and sulphated residual substrates.

Ovary

In the ovary almost all minerals except Ni registered maximum values in the ovary. All of them were noted in the highest level in immature stage. Among the major elements K, Na, Ca, Mg and Fe whose values remained comparatively low during various stages of maturation, K and Na showed marked increase during spent stage. In contrast to the present investigation, Vijayakumaran (1990) reported that all the minerals increased in mature stage and subsequently decreased in spent stage in both *P. indicus* and *P. homarus*. Gopalakrishnan (1991) reported pronounced rise of Ca and Fe content of ovary in stage III and presumed it to be associated with the presence of appreciable quantities of vitellogenin at this stage. According to Bjornsson *et al.* (1986), Ca and Mg are bound to vitellogenin at a ratio of 9:1 in *Salmo gairdneri*.

In the present study, all the minerals (Na, K, Ca, Mg, Mn, Cu, Fe, Zn, Ni and Pb) analysed in various tissues like hepatopancreas, ovary and muscle, registered higher values in ovary than other tissues except potassium which was uniformly high in muscle. All these minerals recorded the highest values in immature stage. K was high in the spent stage. The values of all minerals decreased at maturity stage. In terms of quantity, K and Na values were relatively higher than Ca and Mg. Values of Fe and Ni were found to be higher than Zn and Pb. Cu and Mn values were the lowest in ovary. Vijakumaran (1990) studied the presence of various minerals like Na, K, Ca, P, Mg, Fe, Cu, Zn, Cd, Co, Mn and Pb in the ovary of *P. indicus* and *P. homarus* and reported that all the minerals increased during mature stage, which is in contrast to the present results. Increase in values of minerals in ovary could be associated with vitellogenin synthesis during ovarian maturation, and the inorganic reserves are probably mobilized from hepatopancreas to ovary.

Mn is a constituent of pyruvate carboxlase (Nair, 1984) and is essential along with Fe, Cu, and Zn for normal biochemical process in

invertebrates (Harrison and Hoare, 1980). As observed in the present study, Pillai and Subramoniam (1985) reported in *P. hydrodromous* that Mn increased 5 times in early stages of development and reduced in later stage. K and Na are the two major important macro minerals associated with vitellogenesis. The Na values in ovary decreased from immature to mature stages and subsequently increased in spent stage. Similarly, the K content of ovary increased in immature stage and suddenly decreased in early maturing stage and again increased in late maturing stage. During mature stage, it decreased slowly and subsequently increased in spent stage. This behaviour would probably indicate the involvement of these macro minerals, more in immature stage and also being used subsequently in all the vitellogenin phases as these minerals have been known to be associated with protein and lipid. The increase in K content of ovary in spent stage showed perhpas the storage and subsequent use in resorbing oocytes. Regarding these minerals Na and K, other than the study of Vijayakumaran (1990) in *P. indicus*, no other report is available in relation to maturation in penaeid prawn. Next to K and Na, Ca and Mg were in substantial quantities in immature stage and they decreased upto mature stage. During spent stage, Ca content increased slightly while Mg content decreased. In general, Ca is being used for calcification of exoskeleton and activation of enzymes and is believed to be essential for the absorption of vitamin B_{12}. Mg is found in many enzymes and is essential for several enzymatic processes including protein, lipid and carbohydrate metabolism. In the present study, Ca and Mg secured to be associated with vitellogenin synthesis.

Apart from the above minerals Fe, Ni and Zn values were more in terms of quantity in ovary. Similar to the above minerals, these minerals were also at the maximum level in immature stage and the level increased relatively in early maturing stage. During mature stage, the values of these mineral decreased substantially. The pronounced rise in the concentration of these minerals in immature stage would indicate subsequent use of these inorganic reserves during all the maturity stages.

In general, Fe has been found to be a prominent element in haemoglobin and is more a store house of protein for energy than a respiratory pigment in crustaceans. Fe is also utilised in various enzymes, catalases, peroxidases and dehydrogenases. Zn is a component in more than 80 metalloenzymes and a cofactor in enzyme systems. These metabolic processes include protein, nucleic acid, lipid, carbohydrate and mucopolysaccharide metabolism. In the present study, Cu, Mn and Pb contents recorded the lowest value in ovary compared to other minerals. Cu, which comprised 93% of weight of haemocyanin in the blood of crab *Carcinus maenas* (Martin *et al.*, 1977) is undoubtedly one of the most essential trace elements in crustaceans. Cu also is a component of cytochrome oxidase (Nair, 1984). Mn serves as a cofactor for a number of enzymes including phosphate transferases and dehydrogenases, alkaline phosphatase, arginase and hexokinase (Akiyama *et al.*, 1992). Pb, which is considered as non-essential and toxic was at the lowest in different maturity stages. It will be worth studying whether the process of detoxification is taking place.

Muscle

In muscle, K was the most dominant element at all stages of maturation although its concentration in immature stage was minimal. All the other mineral values were at the lowest. The values of Mn and Pb were below the detectable limit in different stages of maturity. Among the values recorded for Ca, Cu, Mn and Zn in muscle, higher values were recorded in immature, mature and spent stages. Other minerals viz. Na, K, Mg, Fe, Ni and Pb showed maximum values in early maturing stage. In total, the trend of minerals content in relation to maturity stages fluctuated. The variation indicated that the muscle contributes to the inorganic matter build up in the ovary as well as the involvement of metabolic activity in all the stages of *P.semisulcatus*. In agreement with the present study, Vijayakumaran (1990) reported that in *P indicus* and *P. homarus* marked decrease could be noticed in most of the major elements (N, K, P) and trace elements (Cu, Co and Mn)

Table 17. Changes in Ca, Mg, Na, K, Cu, Mn, Fe, Zn, Ni, Pb and Cd in the hepatopancreas during different maturity stages of *P. semisulcatus* (ppm dry weight)

Trace elements	Maturity stages				
	I	II	III	IV	V
Ca (ppm)	3213.3 ± 3.4	2532.8 ± 0.2	2950.5 ± 1.8	2409.3 ± 0,8	2345.2 ± 1.6
Mg (ppm)	1666.3 ± 0.6	1658.3 ± 0.6	1673.8 ± 0.6	1573.3 ± 1.9	1582.8 ± 0.5
Na (ppm)	16872.6 ± 3.4	16872.4 ± 3.7	10622.8 ± 3.6	10006.2 ± 5.9	12505.4 ± 5.9
K (ppm)	18749.8 ± 3.2	12504.6 ± 4.6	10003.6 ± 5.0	11873.6 ± 2.2	17504.6 ± 4.6
Cu (ppm)	97.9 ± 0.8	70.9 ± 0.3	396.8 ± 1.0	119.0 ± 0.4	418.1 ± 0.9
Mn (ppm)	86.7 ± 0.2	45.8 ± 0.2	162.9 ± 1.0	71.6 ± 0.2	51.1 ± 0.1
Fe (ppm)	1678.9 ± 133.7	1421.0 ± 0.7	13604.8 ± 5.0	1079.8 ± 0.7	979.5 ± 1.4
Zn (ppm)	6977.1 ± 1.4	656.7 ± 0.7	504.9 ± 0.4	534.5 ± 1.0	430.7 ± 1.0
Ni (ppm)	651.4 ± 1.4	803.2 ± 4.1	21798.6 ± 2.2	902.5 ± 2.4	702.5 ± 2.3
Pb (ppm)	45.2 ± 0.7	30.6 ± 0.1	30.5 ± 0.2	51.1 ± 0.2	51.1 ± 0.1
Cd (ppm)	Nil	Nil	Nil	Nil	Nil

All values are mean ± standard deviation

Table 17a. Analysis of variance (Hepatopancreas)

Trace elements	Source	D.F.	Mean SQRS ($x10^6$)	F-Value
Ca (ppm)	Treatment	4	1666073776	1.0000
	error	24	1666102441	
Mg (ppm)	Treatment	4	0.0118	11608.8140**
	error	24	0.000001	
Na (ppm)	Treatment	4	55.1808	2559405.79**
	error	24	0.0000003	
K (ppm)	Treatment	4	71.8636	4414226.21**
	error	24	0.000016	
Cu (ppm)	Treatment	4	0.1471	262791.276**
	error	24	0.0000005	
Mn (ppm)	Treatment	4	0.01116	43851.7532**
	error	24	0.0000002	
Fe (ppm)	Treatment	4	152.04521	42477.2616**
	error	24	0.0036	
Zn (ppm)	Treatment	4	0.06056	66244.2286**
	error	24	0.0000009	
Ni (ppm)	Treatment	4	442.4630	63716917.2**
	error	24	0.0000069	
Pb (ppm)	Treatment	4	0.00055	4174.4673**
	error	24	0.0000001	

'* Highly significant at 1% level ($p < 0.01$)

' Significant at 5% level ($p \leq 0.05$)

NS Non significant

Table 18 **Changes in Ca, Mg, Na, K, Cu, Mn, Fe, Zn, Ni, Pb and Cd in the ovary during different maturity stages of *P. semisulcatus* (ppm dry weight)**

Trace elements	Maturity stages				
	I	II	III	IV	V
Ca (ppm)	46230.4 ± 1.1	4771.7 ± 2.0	631.2 ± 1.0	577.5 ± 0.4	581.7 ± 2.2
Mg (ppm)	32618.8 ± 0.7	3261.8 ± 0.2	361.1 ± 0.5	360.9 ± 0.6	351.2 ± 0.7
Na (ppm)	62499.8 ± 6.3	15200.0 ± 4.5	6753.6 ± 6.1	5479.0 ± 31.2	6749.6 ± 3.6
K (ppm)	75000.6 ± 4.7	13749.6 ± 2.9	19250.0 ± 3.5	17516.6 ± 3.0	22004.6 ± 4.6
Cu (ppm)	760.0 ± 1.2	97.8 ± 0.5	36.8 ± 0.2	27.4 ± 0.4	57.3 ± 0.4
Mn (ppm)	816.2 ± 0.7	61.1 ± 0.2	13.3 ± 0.2	13.0 ± 0.3	14.3 ± 0.1
Fe (ppm)	13636.2 ± 0.6	1676.3 ± 0.4	392.5 ± 10.1	760.8 ± 1.7	448.6 ± 0.9
Zn (ppm)	5120.1 ± 1.5	494.0 ± 2.5	123.8 ± 1.5	216.0 ± 0.7	211.3 ± 0.5
Ni (ppm)	12004.4 ± 4.4	1398.3 ± 2.0	130.7 ± 4.0	164.7 ± 10.0	180.4 ± 0.4
Pb (ppm)	1121.8 ± 1.0	131.7 ± 1.1	11.3 ± 0.1	12.1 ± 0.3	9.2 ± 0.1
Cd (ppm)	Nil	Nil	Nil	Nil	Nil

All values are mean ± standard deviation

Table 18a. Analysis of variance (Ovary)

Trace elements	Source	D.F.	Mean SQRS ($x10^6$)	F-Value	F Prob.
Ca (ppm)	Treatment	4	2004.6000	911505008**	0.0000
	error	24	0.0000022		
Mg (ppm)	Treatment	4	1002.3661	2952181747**	0.0000
	error	24	0.000034		
Na (ppm)	Treatment	4	2986.2135	75162.7622**	0.0000
	error	24	0.0397		
K (ppm)	Treatment	4	3279.0313	16968698.3**	0.0000
	error	24	0.0002		
Cu (ppm)	Treatment	4	0.5010	1219899.57**	0.0000
	error	24	0.0000004		
Mn (ppm)	Treatment	4	0.6275	4133466.13**	0.0000
	error	24	0.0000001		
Fe (ppm)	Treatment	4	165.5890	7746890.67**	0.0000
	error	24	0.0000213		
Zn (ppm)	Treatment	4	23.7054	9691826.13**	0.0000
	error	24	0.0000024		
Ni (ppm)	Treatment	4	134.5193	25328693.1**	0.0000
	error	24	0.0000053		
Pb (ppm)	Treatment	4	1.1817	2472910.27**	0.0000
	error	24	0.0000004		

* Highly significant at 1% level ($p < 0.01$)

Significant at 5% level ($p \leq 0.05$)

NS Non significant

Table 19 Changes in Ca, Mg, Na, K, Cu, Mn, Fe, Zn, Ni, Pb and Cd in the muscle during different maturity stages of *P. semisulcatus* (ppm dry weight)

Trace elements	Maturity stages				
	I	II	III	IV	V
Ca (ppm)	393.0 ± 0.5	369.7 ± 1.1	303.3 ± 0.2	381.7 ± 0.4	295.8 ± 1.1
Mg (ppm)	185.6 ± 0.3	186.4 ± 0.5	183.6 ± 0.4	183.0 ± 0.4	183.5 ± 0.5
Na (ppm)	311.6 ± 1.0	2003.6 ± 5.0	311.9 ± 0.7	124.3 ± 1.0	312.9 ± 1.2
K (ppm)	28123.4 ± 2.3	60001.6 ± 5.0	54685.1 ± 3.2	43752.6 ± 3.7	46873.6 ± 2.2
Cu (ppm)	16.2 ± 0.3	17.9 ± 0.2	17.4 ± 0.4	24.7 ± 0.6	24.4 ± 0.4
Mn (ppm)	8.0 ± 0.3	8.7 ± 0.02	8.7 ± 0.02	8.1 ± 0.01	9.2 ± 0.008
Fe (ppm)	181.7 ± 0.4	467.8 ± 1.0	136.3 ± 0.2	188.6 ± 0.5	251.8 ± 3.5
Zn (ppm)	116.4 ± 0.6	94.2 ± 00.7	91.7 ± 0.5	115.3 ± 0.5	86.8 ± 0.6
Ni (ppm)	77.7 ± 1.0	84.8 ± 0.6	70.6 ± 1.0	87.0 ± 5.2	80.1 ± 0.4
Pb (ppm)	3.5 ± 0.0002	4.1 ± 0.0003	3.1 ± 0.0001	4.1 ± 0.0003	3.6 ± 0.0002
Cd (ppm)	Nil	Nil	Nil	Nil	Nil

All values are mean ± standard deviation

Table 19a. Analysis of variance (muscle)

Trace elements	Source	D.F.	Mean SQRS ($x10^6$)	F-Value
Ca (ppm)	Treatment	4	0.0104	17740.4436**
	error	24	0.0000005	
Mg (ppm)	Treatment	4	0.0000	59.4557**
	error	24	0.0000	
Na (ppm)	Treatment	4	3.0552	5331193.528**
	error	24	0.0000005	
K (ppm)	Treatment	4	743.1262	62711070.1**
	error	24	0.00001	
Cu (ppm)	Treatment	4	0.0000841	515.8552**
	error	24	0.0000001	
Mn (ppm)	Treatment	4	0.0000011	48.3208**
	error	24	0.0000	
Fe (ppm)	Treatment	4	0.0859	30817.4167**
	error	24	0.0000027	
Zn (ppm)	Treatment	4	0.0010	2722.9809**
	error	24	0.0000003	
Ni (ppm)	Treatment	4	0.0002	35.7720**
	error	24	0.0000058	
Pb (ppm)	Treatment	4	0.0000009	18212814.6**
	error	24	0.0000	

** Highly significant at 1% level ($p < 0.01$)

* Significant at 5% level ($p \leq 0.05$)

NS Non significant

in the muscle through immature to mature stages and suggested that it might possibly indicate the role of muscle in the inorganic matter built up. Bryan and Ward (1965) reported from the body of the lobster that possible absorption and release of Mn by the shell can take place which may assist in maintaining the blood and tissue Mn concentrations. It would apparently indicate that there is no accumulation of Cd in the tissues.

CHAPTER VI

HISTOCHEMISTRY OF OOCYTES, TESTIS AND SPERMATOPHORE

RESULTS

Histochemistry of oocytes

Previtellogenic oocyte

The histochemical results on immature ovary showed that the oogonial cells and previtellogenic oocyte displayed intense positivity to proteins of both basic and acidic nature, but slightly deviated in the accumulation of the two groups of protein in the cytoplasm, nucleus and nucleolar material. In oogonial cells, amino groups, -SH groups and tyrosine showed positivity to the cytoplasm and negativity to the N and NUL, while other groups like -SS groups and tryptophan showed negativity to CY, N & NUL (Table 20). In previtellogenic oocytes, the protein and basic protein showed positivity to CY and more positivity to NU and NUL. In total protein, basic protein and acidic groups showed more positivity and intense positivity to oogonial cells and previtellogenic oocytes denoting abundant presence of these groups.

Among the oogonial cells and previtellogenic oocytes analysed for the presence of carbohydrate (Table 22), the nucleus was devoid of carbohydrate. PAS showed negative or mild reaction to the oogonial cells and previtellogenic oocytes, while the Best carmine for glycogen was positive, mild and more positive to CY, N and NUL in oogonial cell and more positive, mild and intense positive to CY, N and NUL, respectively. CEC Method for acid mucopolysaccharide at 0.1 M showed more positivity to CY, N, NUL in oogonial cell and more positivity to CY and NUL and positivity to N, in previtellogenic oocyte, for the abundant presence of sulphated mucosubstances. The concentration at 0.2 M, 0.5 M and 0.6 M was shown to be negative or of mild reaction to the oogonial cells and PVO except the presence of positive reaction at 0.5 M and 0.6 M to N in PVO. At the concentration of 0.8 M, it was stained positively to N, NUL in oogonial cell and positively to CY and NUL

and negatively to N in PVO. At 1.0 M, it was stained intense positively and positively to CY and N and NUL in oogonial cell and intense positively and negatively to CY and N and NUL in PVO, respectively. Bracco-curtis-sulphated acid mucopolysaccharide results showed that there was a negative reaction in oogonial cells and PVO except the presence of positive reaction to NU in PVO. Toluidine blue-acid mucopolysaccharides at different pH (1.09, 1.99, 3.09, 4.19 and 7.00) tests showed more positive and positive to CY and NU, NUL in oogonial cell and PVO, respectively. These results denoted the presence of glycogen, more sulphated mucosubstances at 0.1 M, loss of alcianophilia in sulphated mucosubstances at 1.0 M and sulphated or phosphated AMP and carboxylated AMP at pH 1.99 and 7.00 positively or more positively to the oogonial and previtellogenic oocytes. Generally, the lipid was very poor in oogonial and previtellogenic oocytes (Table 24) except the phospholipid.

Early vitellogenic oocytes

Histochemically the basic protein was present abundantly in N, NUL and follicle cells and was doubtful in cytoplasm, while the acidic groups were present more abundantly in cytoplasm and follicle cells and in moderate amounts in N and NUL. The protein, amino groups (Plate 16b) and tryptophan gave positive reaction to CY, N and NUL, while -SH groups, -SS groups and tyrosine gave negative reaction or were of doubtful nature to N, NUL and follicle cells except in the cytoplasm (Table 21).

Best carmine was stained more positively in cytoplasm and nucleoli and positively in nucleus and intense positively in follicle cells indicating the presence of glycogen. Alcian CEC stained at 0.1 M positively to CY, N, NUL and FC for the presence of more sulphated mucosubstances and also stained positively at 0.8 M indicating that sulphated mucosubstances lost its alcianophilias. Toluidine blue at different pH stained intense positively showing the abundance of both sulphated and phosphated acid mucopolysaccharide (Plate 15a). Bracco-curti's methods for sulphated

mucopolysaccharides and PAS test for glycogen did not give the positive reaction to this oocyte (Table 23). Lipid, neutral and acidic lipids, phospholipids and cholesterol showed positivity to this oocytes indicating the presence of the above metabolites in moderate quantity (Plate 16a). Oil red 'O' for neutral lipids and UV schiff reaction for unsaturated lipid indicated their absence in these oocytes (Table 25).

Late vitellogenic oocyte

The Mercuric bromophenol was positive to CY, N and NUL showing the presence of simple protein. Basic protein gave more positive reaction to N, NUL and FC showing its abundant presence except the CY where it was doubtful in nature. Acidic groups stained intense positively to FC, more positively to NUL and positively to N for the abundant availability of this group. The -SS, -SH and Tyrosine (Plate 17b) were positively present in yolk spheres, yolk platelets and FC and absent or doubtful in N and NUL. Tryptophan was positively present in YP, N, NUL and FC. Among the protein groups (Table 21) analysed in the oocyte, basic protein, amino groups (Plate 17a), acidic groups and tryptophan were present abundantly (Table 21). Yolk platelets were positive to all classes of protein tested. Toluidine blue pH_7 stained intense positively showed the abundant presence of carboxylated AMP. Phospholipid and neutral and acidic lipids were present in more quantity.

Vitellogenic oocytes

The protein (Plate 20a), basic protein and acidic group were stained intense positively indicating their abundant presence in vitellogenic oocyte (Table 21). The protein and basic protein were more positive to N and FC. The basic protein was more positive in cortical rods (CR). The amino groups (Plate 20b), -SH and tryptophan showed mild reaction in this oocyte, while the -SS groups and tyrosine were present positively. Acidic group was present intense positively in CY, FC and CR and in the nucleus it was present moderately.

Table 20. Histochemical responses of the ovary for proteins during pre-vitellogenic phase in *P. semisulcatus*

Histochemical Tests	Epithelial tissue	Connective tissue	Oogonial cells			Pre-vitellogenic oocytes			Follicle cells
			CY	N	NUL	CY	N	NUL	
Mercuric Bromophenol Blue (Protein)	++	++	++	++	++	+	++	++	++
Aqu. Bromophenol Blue Test (Basic Proteins)	++	+	++	+++	++	+	++	++	++
Deamination	-	-	-	-	-	-	-	-	-
Ninhydrin-Schiff Test (Amino groups)	-	±	+	-	-	+	±	-	±
Deamination	-	-	-	-	-	-	-	-	-
Toluidine Blue Test (Acidic groups)	+	+++	+++	±	+	+++	++	++	+++
Methylation	-	-	-	-	-	-	-	-	-
Ferric-Ferricyanide Method (-SH groups)	±	±	+	-	-	+	+	+	±
Mercaptide	-	-	-	-	-	-	-	-	-
Thioglycollate Ferric-Ferricyanide Method (SS groups)	±	-	-	-	-	+	-	-	+
Thioglycollate reduction	-	-	-	-	-	-	-	-	-
Million's Test (Tyrosine)	±	±	+	±	-	+	±	-	+
Iodination	-	-	-	-	-	-	-	-	-
DMAB-Nitrite Method (Tryptophan)	±	-	±	±	-	+	±	±	-
Formaldehyde	-	-	-	-	-	-	-	-	-

\+ = Positive ++ = More positive +++ = Intense positive

Table 21. Histochemical responses of the ovary for proteins during vitellogenic phases in *P. semisulcatus*

Histochemical Tests	Early vitellogenic oocytes				Late vitellogenic oocytes				Vitellogenic oocytes				Spent oocytes			
	CY	N	NUL	FC	CY	N	NYL	FC	CY	N	FC	CB	CY	N	NUL	FC
Mercuric Bromophenol Blue (Protein)	+	+	+	±	+	+	++	±	+	+++	+++	±	+	+	+	+
Aq. Bromophenol Blue Test (Basic proteins)	±	++	++	++	±	++	+++	++	±	++	+++	++	±	++	+	++
Deamination	++	±	±	-	-	-	±	±	-	-	-	-	-	-	-	-
Ninhydrin-Schiff Test (Amino groups)	++	±	+	++	+	±	+	+	++	++	±	±	+	±	±	+
Deamination																
Toluidine Blue Test (Acidic groups)	+++	+	++	+++	++	+	++	+++	++	±	+++	+++	+++	+	++	+++
Methylation	-	-	-	-	-	-	-	-	-	±	±	-	-	-	-	±
Ferric-Ferricyanide Method (-SH groups)	+	-	±	±	±	-	±	±	±	±	-	-	±	-	-	-
Mercaptide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thioglycollate Ferric-Ferricyanaide Method (SS groups)	+	-	-	+	++	-	-	++	+	±	++	+	±	-	-	±
Thioglycollate reduction	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	-
Millon's Test (Tyrosine)	+	±	±	+	+	-	-	+	±	+	+	++	+	-	±	+
Iodination	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DMAB-Nitrite Method (Tryptophan)	+	+	++	+	+	+	±	+	+	±	±	±	±	-	-	±
Formaldehyde	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 22. Histochemical responses of the ovary for carbohydrates during pre-vitellogenic phase in *P. semisulcatus*

Histochemical Tests	Epithelial tissue	Connective tissue	Oogonial cells			Pre-vitellogenic oocytes			Follicle cells
			CY	N	NUL	CY	N	NUL	
Schiff alone	±	±	±	±	±	+	-	-	±
PAS-Technique (Periodic Acid-Schiff) (Glycogen & Mucosubstances)	+	±	±	-	-	±	-	-	±
Deamination	±	-	-	-	-	-	-	-	±
Acetylation									
Deacetylation	±	-	-	-	-	-	-	-	+
Delipidation	±	-	-	-	-	±	-	-	±
Diastase digestion	-	-	-	-	-	-	-	-	±
Best's Carmine Test (Glycogen)	+	++	+	±	++	++	±	+++	+++
Diastase digestion	-	-	-	-	-	-	-	-	±
Alcian CEC Method (Acid Mucopolysaccharides) Molar concentrations									
0.1 M	++	+	++	++	++	++	+	++	++
0.2 M	±	-	-	-	-	±	-	±	±
0.5 M	±	±	-	±	±	-	+	±	+
0.6 M	±	±	-	-	±	-	+	±	±
0.8 M	±	±	-	+	+	+	-	+	±
1.0 M	++	+	+++	+	+	+++	-	-	++
Bracco-Curti's Test (Sulphated Acid Mucopolysaccharides)	±	±	-	±	±	-	+	±	+
Toluidine Blue at different pH (Acid Mucopolysaccharides)									
pH 1.09	++	++	+++	+	+	++	-	-	++
pH 1.99	+	+	++	-	++	++	-	++	++
pH 3.09	+	+	++	-	+	++	+	+	++
pH 4.19	+	+	++	+	+	++	+	+	++
pH 7.00	+	+	++	+	+	++	+	+	++

Table 23. Histochemical responses of the ovary for carbohydrates during vitellogenic phases in *P. semisulcatus*

Histochemical Tests	Early vitellogenic oocytes				Late vitellogenic oocytes				Vitellogenic oocytes				Spent oocytes			
	CY	N	NUL	FC	CY	N	NUL	FC	CY	N	FC	CB	CY	N	NUL	FC
Schiff alone	±	-	±	±	+	+	-	+	±	±	++	++	++	+	±	±
PAS-Technique (Glycogen and Mucosubstances)	-	-	±	-	-	-	-	-	-	±	±	-	±	-	-	±
Deamination	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acetylation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deacetylation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Delipidation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diastase digestion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Best's Carmine Test (Glycogen)	++	+	++	+++	+	++	+++	+++	±	-	+	+	±	-	+	+++
Diastase digestion	+	-	±	±	-	-	±	±	-	-	-	-	-	-	-	±
Alcian CEC Method (Acid Mucopolysaccharides)																
Molar concentrations																
0.1 M	+	+	+	++	+	-	+	+	+	±	+	-	++	±	++	++
0.2 M																
0.5 M	+	-	±	±	±	-	-	±	±	-	±	-	±	-	-	-
0.6 M	±	-	±	-	+	±	±	±	+	-	±	±	+	-	±	±
0.8 M	+	-	+	+	±	-	±	±	+	±	±	±	+	-	±	±
1.0 M	+	±	±	+	+	±	±	++	+	-	+	-	+	-	±	±
Bracco-Curti's Test Sulphated Acid Mucopolysaccharides)	±			-	+	++	++	±	++	-	-	+	±	-	-	-
Toluidine Blue at different pH(Acid Mucopolysaccharides)																
pH 1.09	++	++	++	+++	+	-	-	+++	+	±	+++	-	+	++	++	+++
pH 1.99	++	-	++	+++	+	±	±	++	+	-	+++	±	++	++	++	+++
pH 3.09	++	+	++	+++	±	-	-	++	+	±	++	+	+++	++	++	+++
pH 4.19	++	+	++	++	±	-	-	++	±	+	++	+	+++	++	++	+++
pH 7.00	+++	++	++	+++	++	-	+	+++	++	++	+++	++	+++	++	++	+++

Table 24. Histochemical responses of the ovary for lipids during pre-vitellogenic phase in *P. semisulcatus*

Histochemical Tests	Epithelial tissue	Connective tissue	Oogonial cells			Pre-vitellogenic oocytes			Follicle cells
			CY	N	NUL	CY	N	NUL	
Sudan Black B (Lipid)	+	±	-	±	±	±	+	++	±
Delipidation	+	-	-	±	-	±	-	-	-
Nile Blue Method (Neutral & Acidic Lipids)	±	-	+	-	±	+	±	-	+
Delipidation	-	-	-	-	-	±	-	-	-
Nile Blue Sulphate Method (Phospholipids)	++	++	++	+	+	++	++	++	++
Delipidation	±	±	±	-	-	-	-	-	±
Oil red 'O' Method (Neutral lipids)	±	-	-	-	-	-	-	-	±
Delipidation	-	-	-	-	-	-	-	-	-
U.V. Schiff reaction (Unsaturated lipids)	+	+	+	±	-	+	±	+	-
Pyridine extraction	+	±	+	-	-	±	-	±	±
Cholesterol	±	±	+	±	±	+	±	±	±

Table 25. Histochemical responses of the ovary for lipids during vitellogenic phases in *P. semisulcatus*

Histochemical Tests	Early vitellogenic oocytes				Late vitellogenic oocytes				Vitellogenic oocytes				Spent oocytes			
	CY	N	NUL	FC	CY	N	NUL	FC	CY	N	FC	CB	CY	N	NUL	FC
Sudan Black B (Lipid)	+++	±	+	±	+++	+	-	±	+++	+	±	±	++	+	-	-
Delipidation	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
Nile Blue Method (Neutral & Acidic lipids)	+	+	+	+	±	±	±	+	+	+	+	+	+	+	+	+
Delipidation	-	-	-	±	-	-	-	±	-	-	-	-	±	-	-	-
Nile Blue Sulphate Method (Phospholipids)	±	+	+	+	+	+	++	++	++	++	+	±	+	±	±	±
Delipidation	±	-	-	-	±	-	-	-	+	-	-	-	±	-	-	++
Oil Red 'O' Method (Neutral lipids)	+	-	-	±	++	±	±	±	++	-	±	±	+	±	±	±
Delipidation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
U.V. Shiff Reaction (Unsaturated lipids)	±	-	-	-	±	-	-	±	+	-	-	-	-	-	-	-
Pyridine extraction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cholesterol	+	+	+	+	++	+	+	±	++	+	±	±	+	±	±	±

Tyrosine (Plate 21a) was found to have a positive reaction for the presence of this group moderately. The vitellogenic oocyte had a doubtful PAS positivity, while the best's carmine showed positivity in FC, CR and CY except NU. All the results showed apparently that the carbohydrate groups (Table 23) were very poor in yolk platelets. Since the sulphated mucosubstances were positively stained at 0.1 M and above (0.2, 0.5, 0.6, 0.8 and 1.0 M), most sulphated mucosubstances were found to loose their alcinophilia. Carboxylated and sulphated AMP (Plates 18a & 21b) were present in the ooplasm as well as in yolk platelets. Lipid, neutral and acidic lipids were present positively in the N, FC and CR and intense positively in the cytoplasm. The phospholipid was present more positivity in yolk platelets and N than in FC and CR while the neutral lipid by oil red 'O' was present more positively than N, FC and CR. Unsaturated fatty acid was present positively in the yolk platelet and was absent in the N, FC and CR. The cholesterol content was present more positively in the cytoplasm and positively in N, FC and CR (Table 25).

Spent oocytes

The spent oocyte was intense positive to aqueous toluidine blue denoting the abundant presence of acidic proteins. The basic protein was more positive in N and FC denoting the moderate presence of basic protein (Plate 22). Amino group, -SS, tyrosine and tryptophan were palely present in CY and FC and were doubtful in N and NUL. The -SH group could not be detected in the spent oocyte stage. When the carbohydrates (Table 23) were detected in this oocyte, the toluidine blue at different pH (1.09, 1.99, 3.09, 4.19 and 7.00) was intense positive to CY and FC and more positive to N and NUL indicating the abundant presence of sulphated or phosphated and carboxylated AMP, and more sulphated mucosubstances were detected by the presence of deeply alcinophilic at 0.1 M level (Table 21). PAS was negative to this oocyte or of mild reaction in FC. The best carmine was intense positive to FC denoting the abundant presence of glycogen. Spent oocyte was found to be negative or of mild reaction to N, NUL

and FC except CY which was found to have positive reaction. Neutral and acidic lipid were positively present in this oocyte, while sudanophilic lipid was moderately present in CY and N (Table 25).

Histochemical composition of testis

The histochemical examination of testis (Tables 26-28) revealed the complex chemical nature of the sperm cells of *P. semisulcatus*. The testis contained spermatogonial cells, spermatocytes, spermatids, spermatozoa, epithelial and nurse cells which were more positive to the aqueous Bromophenol blue showing the abundant presence of basic protein, while the acidic groups were moderately present in these cells except in spermatid nucleus where the presence was abundant. The cytoplasm of the spermatogonial cells was more positive to the ferric-ferricyanide test vouching for abundant presence of -SH groups, while the nuclei of spermatogonial cells, spermatocytes, spermatids, spermatozoa and epithelial and nurse cells were positive for -SH groups. Tyrosine could not be detected in the cytoplasm of spermatogonial cells, epithelial and nurse cells except the doubtful presence of tyrosine groups in spermatozoa, while the moderate presence of tyrosine could be detected in the nuclei of spermatogonial cells, spermatocytes and spermatids. Tryptophan was very poor in spermatogonial cells, spermatocytes and spermatids and it was absent in sperms, epithelial and nurse cells. In general, the basic protein was present abundantly in the testis as compared to other groups (Table 26).

The cytoplasm of spermatogonial cells, nucleus of spermatozoa, epithelial and nurse cells were positive to the PAS test showing the moderate presence of glycogen and mucosubstances while the negative reaction could be noticed to the Best's carmine test for the presence of glycogen. However, the nuclei of spermatogonial cells, spermatocytes and spermatids showed moderate positivity to the PAS reaction due to the presence of 1,2 glycol groups. The luxuriant presence of glycogen in the above cells was also confirmed by its

more positivity to Best's carmine. Toluidine blue test at different pH applied to the spermatogonial cells, spermatocytes, spermatids and spermatozoa, nurse and epithelial cells showed the abundance of sulphated and carboxylated AMP. The toluidine blue stained more positively to violet colour at pH 3.09 in these cells showing the presence of dimeric beta form which is known as ß-metachromasia, while the abundant presence of monomeric alpha form known as orthochromasia was found at pH 1.09 showing more positivity of blue colour in these cells. In spermatozoa, the form of ß-metachromasia was more abundant as compared to orthochromaşia formed in spermatozoa at pH 1.09 (Table 27). Among the lipids analysed in the spermatogonial cells, spermatocytes, spermatids and spermatozoa, phospholipid showed more abundance (Table 24) than other groups. The cholesterol (Table 24) was detected less in spermatogonial cells while its abundant presence was detected in spermatocytes, spermatids and spermatozoa. The neutral and unsaturated lipids were very poor in these cells. The spermatids and spermatozoa were positive to the Sudan black B test (Plate 32b) showing the abundant presence of lipids, while the spermatogonial cells and spermatocytes were doubtful for their presence. The epithelial and nurse cells were positive to Sudan Black B showing moderate presence.

Histochemical compositions of spermatophore

The histochemical tests applied to the spermatophore (Tables 29-31) revealed the chemical nature of the spermatophore layers as well as the sperm matrix and wing. The spermatophore layers I & II and adhesive globules were positive to mercuric bromophenol blue showing moderate presence of protein, while the spermatophore matrix and spermatophore wing were more positive indicating abundant presence of this group. The spermatophore layers, SPH matrix and SPH wing were positive to the ferric ferricyanide reaction showing moderate presence of -SH groups. The tyrosine was moderately present in the spermatophore layers, whereas, its presence was doubtful in SPH matrix, SPH wing and adhesive globules. Compared with other protein groups, the basic

Table 26. Histochemical responses for proteins in the testis of *P. semisulcatus*

Histochemical Tests	Epithelial tissue	Spermatogonial cells		Spermatocytes	Spermatids	Spermatozoa	Nurse cells
		Cytoplasm	Nucleus	Nucleus	Nucleus		
Mercuric Bromophenol Blue (Protein)	+	±	±	±	++	++	±
Aqu. Bromophenol Blue Test (Basic proteins)	++	++	++	++	+++	++	++
Deamination	-	-	-	-	±	-	-
Toluidine Blue Test (Acidic groups)	+	+	+	+	++	+	+
Methylation	-	-	-	-	-	-	-
Feric-Ferricyanaide Method (-SH groups)	±	++	+	+	+	+	±
Mercaptide	-	-	-	-	-	-	-
Million's Test (Tyrosine)	-	-	+	+	+	±	-
Iodination	-	-	-	-	-	-	-
DMAB-Nitrite Method (Tryptophan)	-	±	±	±	±	-	-
Formaldehyde	-	-	-	-	-	-	-

Table 27. Histochemical responses for carbohydrates in the testis of *P. semisulcatus*

Histochemical Tests	Epithelial tissue	Spermatogonial cells		Spermatocytes	Spermatids	Spermatozoa	Nurse cells
		Cytoplasm	Nucleus	Nucleus	Nucleus		
Schiff alone	++	++	++	++	++	+	+
	indicating mucous substances than deep magenta				(Glycoprotein)		
PAS-Technique (Periodic Acid-Schiff) (Glycogen & Mucosubstances)	+	+	++	++	++	+	+
Deamination Acetylation	-	-	-	-	-	-	-
Deacetylation	-	-	-	-	-	-	-
Delipidation	-	-	-	-	-		-
Diastase digestion	-	-	-	-	-	-	-
Best's Carmine Test for Glycogen	-	-	+	++	++	++	+
Diastase Digestion	-	-	-	+	+	+	-
Bracco Curti's Test (Sulphated AMP)	-	±	-	±	+	±	±
Toluidine Blue at different pH (AMP)							
	++	++	++	++	+	+	±
pH 1.09	+	+	+	+	+	+	+
pH 1.99	++	++	++	++	++	++	++
pH 3.09	++	++	++	++	+	+	+
pH 4.19	++	++	++	++	+	+	+
pH 7.00							

3.09 Stained with violet (ß-Metachromasia)
1.09 Orthochromasia

Table 28. Histochemical responses for lipids in the testis of *P. semisulcatus*

Histochemical Tests	Epithelial tissue	Spermatogonial cells		Spermatocytes	Spermatids	Spermatozoa	Nurse cells
		Cytoplasm	Nucleus	Nucleus	Nucleus		
Sudan Black B (Lipids)	+	-	±	±	++	+++	+
Delipidation	-	-	-	-	-	-	-
Nile Blue Method (Neutral & Acidic lipids)	-	-	-	+	+	+	-
Delipidation	-	-	-	-	-	-	-
Oil red 'O' Method (Neutral lipids)	±	±	±	±	±	±	-
Delipidation	-	-	-	-	-	-	-
U.V. Schiff reaction (Unsaturated lipids)	+	±	-	-	±	±	-
Delipidation	-	-	-	-	-	-	-
Nile Blue Sulphate Method (Phospholipids)	+	+++	+++	+++	+++	+++	±
Delipidation	-	±	-	-	-	-	-
Cholesterol	±	+	+	++	++	++	±

Table 29. Histochemical responses for proteins in extruded spermatophore (SPH) in *P. semisulcatus*

Histochemical tests	SPH layer I	SPH layer II	SPH matrix	SPH wing	Adhesive globules
Mercuric Bromophenol Blue (Protein)	+	+	++	++	+
Aqu. Bromophenol Blue Test (Basic protein)	++	++	+	++	++
Deamination	-	-	-	-	-
Ninhydrin-Schiff Test (Aminogroups)	+	-	-	-	±
Deamination	-	-	-	-	-
Toluidine Blue (Acidic groups)	±	±	-	-	-
Methylation	-	-	-	-	-
Ferric-ferricyanide Method (-SH groups)	++	+	+	+	+
Mercaptide	-	-	-	-	-
Million's Test (Tyrosine)	+	+	±	±	±
Iodination	-	-	-	-	-
DMAB-Nitrite Method (Tryptophan)	-	-	-	-	-
Formaldehyde	-	-	-	-	-

Table 30. Histochemical responses for carbohydrates in extruded spermatophores (SPH) in *P. semisulcatus*

Histochemical tests	SPH layer I	SPH layer II	SPH matrix	SPH wing	Adhesive globules
Schiff alone	++	++	++	++	++
	(Mucous substance is dominant)				
PAS-Technique (Periodic Acid-Schiff) (Glycogen & mucosubstances)	+	++	++	±	+
Deamination	±	+	+	-	+
Acetylation	-	-	-	-	-
Deacetylation	+	+	+.	±	+
Delipidation	±	+	+	±	+
Diastase digestion	±	+	+	-	-
Best's Carmine Test (Glycogen)	-	-	±	±	-
Diastase digestion	-	-	-	-	-
Bracco Curti's Test (Sulphated AMP)	±	±	+	±	±
Toluidine Blue at different pH (AMP)					
pH 1.09	+	+	+	±	±
pH 1.99	-	-	-	-	-
pH 3.09	-	-	-	-	-
pH 4.19	±	±	±	-	-
pH 7.00	+	+	+	±	+

Table 31. Histochemical responses for lipids in extruded spermatophores (SPH) in *P. semisulcatus*

Histochemical tests	SPH layer I	SPH layer II	SPH matrix	SPH wing	Adhesive globules
Sudan Black B (Lipids)	++	++	+	+	+
Delipidation	-	-	-	-	-
Nile Blue Method (Neutral & Acidic lipids)	-	±	±	-	-
Delipidation	-	-	-	-	-
Oil red 'O' Method (Neutral lipids)	±	±	-	-	-
Delipidation	-	-	-	-	-
U.V. Schiff reaction (Unsaturated lipids)	+++	++	-	-	-
Delipidation	-	-	-	-	-
Nile Blue Sulphate Method (Phospholipids)	++	+++	+++	+++	+
Delipidation	-	+	+	+	±
Cholesterol	+	+	+	+	+

protein was abundantly present in the spermatophore layers, SPH matrix, SPH wing and adhesive globules (Table 29). Aminogroups, acidic groups and tryptophan were absent in spermatophore layers and its matrix and adhesive globules.

The spermatophore layer I and adhesive globules were positive to PAS reaction showing moderate presence of glycoprotein, while the SPH II and SPH matrix were more positive indicating abundant presence. The spermatophore layers and their adhesive globules showed positivity to the toluidine blue at 7.00 pH vouching for the moderate presence of carboxylated acid AMP except the SPH wing where its presence was doubtful. The sulphated acid AMP was present moderately in spermatophore layers and its matrix, while it was doubtful in SPH wing and adhesive globules (Table 30). The spermatophore layers had abundant lipids, and the SPH matrix, SPH wing and adhesive globules had moderate lipids. Neutral and acidic lipids were very poor. Spermatophore layers I and II were intense positive and more positive to UV Schiff reaction showing more abundant and abundant presence of unsaturated lipid, respectively. The unsaturated lipid was absent in SPH matrix, wing and adhesive globules. Spermatophore layers, SPH matrix and wing except adhesive globules were intense positive to Nile Blue sulphate reaction indicating the presence of more abundant phospholipid, while cholesterol was moderately present (Table 31).

DISCUSSION

Histochemical studies have been carried out in *P. semisulcatus* to find out the accumulation of organic reserves at cellular level and the changes associated with the vitellogenin synthesis in the ovary. The studies showed that the oogonial cells (Tables 20,22 & 24) are rich in basic protein, moderately rich in acid mucopolysaccharide and poor in lipid. From this investigation, it is known that the oogonial cells are of glycoprotein in nature and this agrees with the observation of Mohamed (1989) and Quinitio and Millamena (1992) in *P. indicus*. In the present study, the previtellogenic oocytes (Plates 20,22 & 24) showed abundant presence of acidic groups than basic proteins, moderate quantity of glycogen in cytoplasm and nucleoli, moderate presence of sulphated or carboxylated acid mucopolysaccharides, and poor presence of lipid except phospholipid which is present in moderate quantity. In follicle cells the same quantity of protein, carbohydrate and lipid was present as noticed in previtellogenic oocyte indicating that the glycoprotein is also being supplied to the ooplasm through this source in addition to the glycoprotein synthesis taking place in the ovary itself. The present study on previtellogenic oocyte is in agreement with the results furnished by Browdy *et al.* (1990) for the same species. They reported that the previtellogenic oocytes stained faintly by PAS for the presence of glycogen and by Sudan Black-B for the presence of lipid. Identically, Joshi *et al.* (1982) in *P. stylifera* reported that the previtellogenic oocyte is basophilic and shows positive reaction to PAS and alcian blue indicating the presence of carbohydrate and mucopolysaccharides in the ooplasm and contrary to the present study, there is lipid content in ooplasm. Mohamed (1989) reported that the follicle cells in *P. indicus*, apart from protein synthesis, may be involved in synthesis and mobilization of carbohydrate substances into the ooplasm for incorporation as the carbohydrate moiety of the yolk as observed in the present study. Contrary to the present results, King (1948) reported that there is no fat in undeveloped ovary in *P. setiferus*, Tan-Fermin and Pudadera (1989) reported that

glycoproteins and lipids are absent in the primary oocytes in *P. monodon* and Quinitio and Millamena (1992) reported that the glycoprotein and lipid are absent in oogonia and primary oocytes in *P. indicus*.

The early and late vitellogenic oocytes of *P. semisulcatus* (Tables 21,23 & 25) showed that the acidic groups of protein are abundantly present in the ovary when compared to the moderate presence of basic protein in nucleus and nucleoli except the cytoplasm which is of doubtful nature. The presence of -SH, -SS and Tyrosine is poor in cytoplasm and nucleus, and nucleoli are devoid of this group. The tryptophan is poorly present in the CY, N and NUL. The follicle cells are seen actively with the presence of acidic and basic groups of protein. The Best's carmine stained more positivity with cytoplasm and nucleoli indicating the moderate presence of glycogen in early vitellogenic oocyte, while the abundant presence of glycogen is noticed in late vitellogenic oocytes. In early vitellogenic oocyte, the acid mucopolysaccharides showed sulphated or carboxylated nature. The ooplasm of late vitellogenic oocytes shows abundant presence of total lipid, neutral lipid and cholesterol, while early vitellogenic oocyte shows poor presence of neutral lipid and cholesterol except the total lipid which is abundantly present. The present study closely agrees with that of Browdy *et al.* (1990) in the same species and as the oocyte enlarge, the PAS positive reaction weakens and Sudan Black B positively stained globuli become prominent in the cytoplasm. Similar trend has also been reported by King (1948) in *P. setiferus*, Munuswamy and Subramoniam (1986) in the fairy shrimp *Streptocephalus dichotomous*, Mohamed (1989) and Quinitio and Millamena (1992) in *P.indicus* and Tan-Fermin and Pudadera (1989) in *P. monodon*. It is concluded from the present study that in the early and late vitellogenic stages the ovary of *P. semisulcatus* is almost made of glycolipoprotein. The histochemical reactions of the developing oocytes in this species are mostly in agreement with the findings in other crustaceans (Fautrez-Firelefyn, 1957; Raven, 1961; Bonina, 1974; Fyhn and Costlow, 1974 and Varadarajan and Subramoniam, 1980).

The fully formed yolk in *P. semisulcatus* is a glycolipoprotein complex as in most other crustaceans. The present investigation (Tables 21,23 & 25) indicated that proteins (basic proteins: histidine, arginine and lysine, acidic groups, less amount of -SS groups and tyrosine), carbohydrates (sulphated or carboxylated acid mucopolysaccharides) and lipids (abundant presence of total lipids, moderate amount of phospholipid and cholesterol, less amount of unsaturated lipid and neutral lipid) are present in the fully formed yolk. This is similar to the observations made by King (1948) in *P. setiferus*, Munuswamy and Subramoniam (1986), Mohamed (1989) and Quinitio and Millamena (1992) in *P. indicus*, Tan-Fermin and Pudadera (1989) in *P. monodon*, Browdy *et al.* (1990) in *P. semisulcatus* and Quinitio *et al.* (1990) in *P. monodon*. The present study thus clearly makes out that the fully formed yolk is made of glicolipoprotein. Histochemically, the cortical rods formed in the cortex of the oocytes of *P. semisulcatus* are rich in acidic groups of proteins, basic proteins and Tyrosine and low in -SS groups. Among carbohydrate contents, it showed sulphated or carboxylated acid mucopolysaccharide. The cortical rods are known to be devoid of lipid except the neutral and acidic lipid present in poor quantity. According to Meyer (1947), non-sulphated AMP controls the permeation of water (through gel forming properties) and protects the egg against bacterial attack. Thus the AMP of the jelly layer may have these role plus the conventional roles of cell construction during morphogenesis. The cortical granules eventually fuse with egg oolemma and contribute to an egg jelly which surrounds a fertilized egg (Anderson *et al.*, 1984 and Clark *et al.*, 1984). With regards to formation and role of cortical rods, the present study agrees with Browdy *et al.* (1990) who made similar observation in *P.semisulcatus*. The histochemical nature of spent oocyte of *P. semisulcatus* is almost similar to previtellogenic and early vitellogenic oocytes of the species. The changing histochemical property of the yolk components has also been reported in many decapod oocytes (Blinski, 1979; Zerbib, 1980).

The spermatozoa of decapod crustaceans are aflagellate and non-motile except perhaps in *M. dobsoni* (Vasudevappa, 1992). In general, sperm

transfer occurs by means of spermatophores (Subramoniam, 1990). The histochemical study (Tables 26-28) on spermatogenesis in *P. semisulcatus* revealed an abundance of basic proteins (lysine, histidine and arginine), acid mucopolysaccharide and phospholipid. The histochemical investigation on testis containing spermatogonial cells, spermatocytes, spermatids and spermatozoa showed moderate presence of basic protein and poor presence of acidic groups, cysteine and tyrosine. The carbohydrate content of testis showed moderate presence of glycogen and sulphated acid mucopolysaccharids. The lipid content of testis, revealed an abundant presence of phospholipid and moderate presence of cholesterol. This investigation would indicate that basic protein, sulphated mucopolysaccharide and phospholipid are the principal reserve nutrient material for the spermatozoa in *P. semisulcatus*. This almost agrees with the results furnished by Mohamed (1989) in *P. indicus*. Jacob *et al.* (1988) in the freshwater crab *Gecarcinucus steniops* reported that the stored glycogen in spermatozoa could serve as the endogenous source of energy under aerobic and anaerobic conditions.

Spermatophores play a major role in sperm transfer and storage in decapod crustaceans. They not only protect the spermatozoa during transmission to females but also serve the function of providing energy rich substrates for prolonged sperm storage in the female (Subramoniam, 1990). Histochemical investigation (Tables 29-31) on spermatophore in *P.semisulcatus* showed the predominance of basic protein and presence of cysteine and tyrosine (hydroxyl-phenyl group). The spermatophore matrix, wing and adhesive globules contain basic protein and -SH groups. Regarding carbohydrate, spermatophore layers (SPL I, II), SPH matrix, SPH wing and adhesive globules contain glycogen and sulphated mucopolysaccharides except in wing where the substance is of doubtful nature. The spermatophore layers, SPH matrix and wing in *P. semisulcatus* contain an abundance of phospholipid and presence of cholesterol. Agreeing with the present study, Jeyalectumie and Subramoniam (1987) reported that seminal secretions containing spermatophores of *P. paratelphusa* have large quantities of free carbohydrates,

proteins and lipids. With regards to histochemical composition of spermatophore, some prominent works have been carried out in penaeid prawns and other decapod crustaceans. King (1948) in *P. setiferus* reported that a chitinous layer form an outer covering to the sheath in which the sperms are enclosed and this slightly deviates from this study. The presence of tyrosine-rich proteins is taken as an indirect evidence for phenolic tanning in the spermatophore of *P. trisulcatus* (Malek and Bawab, 1971). Subramoniam (1984) also detected tyrosyl groups and the enzyme phenolase in anomurans in support of this view. Similarly, the spermatophore layers noticed in the present study contain tyrosine, while Mohamed (1989) encountered in *P.indicus* the presence of tyrosine in spermatophore layers. In agreement with the present study, Malek and Bawab (1974) in *P. kerathurus* reported abundance of lipid in spermatophore layers I-V, while spermatophore layers I and III incorporate protein and carbohydrate in their composition. The gel forming antimicrobial properties of AMPs are well known (Itow and Sekiguchi, 1984) and hence the spermatophore layers may have an adhesive and protective role. Based on biochemical investigation, Sasikala and Subramoniam (1987) reported the predominance of AMP like chondroitin sulfate and hyaluronic acid in the spermatophore of *P. indicus*. Such a mucopolysaccharide heterogeneity has been reported in crustacean by Subramoniam (1984) in the anomuran crabs *Albunea symnista* and *Emerita asiatica*. The spermatophore matrix in *P. semisulcatus* is composed of basic protein in little quantity, abundance of glycogen with sulphated AMP and phospholipid. In addition to these, cholesterol is present in little quantity. In *A.symnista* and *E.asiatica* Subramoniam (1984) reported the sperm mass substance or sperm matrix to be an AMP, and the gelatinous cord to be a neutral mucopolysaccharide. The glycogen and sulphated AMP observed in the spermatophore layers in the present study, have a nutritive function beside giving protection from bacterial attack as revealed by Subramoniam (1984) and Mohamed (1989). Martin *et al.* (1987) reported bacterial colonization on the hardened spermatophore surface. But bacteria are not found to penetrate into the deeper layers of the spermatophores, probably because of the antibacterial activity of the phenolic

substances present in it. Agarose gel electrophoresis of the isolated and purified AMPs of spermatophores of both prawns *P. indicus* and *M. dobsoni* revealed two fractions corresponding to chondroitin sulphate and hyaluronic acid (Sasikala and Subramoniam, 1987). Further, the sperm sac contained the maximum quantity of AMPs, while the "wing" contained the least in *P. indicus*. In *M. monoceros*, the AMP content of the sperm mass and crystalline structure is similar. High AMP content in the sperm sac may have functional implication, enabling sperm release by inhibition of water (Uma and Subramoniam, 1979). Chow *et al.* (1991) reported in *P. setiferus* and *P.vannamei* the abundance of glycogen in spermatophore layer, accessary layer, middle vas deferens and wing. Agreeing with the present study, Subramoniam (1990) while reviewing the chemical composition of spermatophores in decapod crustaceans reported that the occurrence of large quantities of AMPs in spermatophores might keep the spermatozoa viable in a fluid medium until fertilization. Again, the antimicrobial activity of sulphated AMPs such as hyaluronic acid and chondroitin sulphate may protect the delicate sperm cells when exposed to seawater, both during their transfer to the thelycum and during their release for fertilization. Similar to the present study, Jeyalectumie and Subramoniam (1987) reported that seminal secretions containing spermatophores of *Paratelpusa* have large quantities of free carbohydrates, proteins and lipids.

CHAPTER VII

INDUCED MATURATION

RESULTS

Induced maturation experiments

Reproduction in crustacea is perceived to be hormonally controlled (Highnam and Hill, 1977). Hormones are chemical messages synthesized and secreted by an endocrine gland and released to circulating blood. Blood carries the hormone to a distant target cell(s) where the hormone alters that cell's physiology (Chang, 1992). Knowledge about the control of reproduction by neuroendocrine hormones is necessary to successfully manipulate the breeding cycle of the commercially important prawns in aquaculture. Neuroendocrine hormones produced by the neuroendocrine organs (neurosecretory cells) are invariably associated with the blood vascular system which are termed as neurohemal organs. These identified neuroendocrine areas are eyestalk, brain and thoracic ganglia (Adiyodi and Adiyodi, 1970; Fingerman, 1970, 1973; Diwan and Nagabhushanam, 1975; Mohamed, 1989; Subramoniam and Keller, 1993). The methylfarnesoate produced by Y organ located in mandibular organ is believed to be mainly responsible for ovarian maturation, whereas the egg maturation is negatively controlled by inhibitory neuropeptide from x-organ/sinus gland complex (Fingerman, 1987; Laufer and Landau, 1991 and Vijayan *et al.*, 1993). To study the role of eyestalk neurosecretory hormone on gonadal maturation, eyestalk ablation, eyestalk and CNS extract injection and application of UV rays, were attempted on *P. semisulcatus* (Plates 43-45) and the results obtained are summarized in tables 32-34.

The study was carried out using five experimental groups of immature female prawns. Each group comprising of five prawns in the size range 130-158 mm TL. Each of the experimental groups was tried for a particular type of experiment and the duration of experiment lasted for 10 days.

PLATE 43

A. A view of experimental set up for induced maturation study.

B. A view of experimental set up for studying the effect of UV rays on maturation.

PLATE 43

PLATE 44

A. A closeup dorsal view of experimental animal prior to unilateral eye stalk ablation.

B. A closeup dorsal view of experimental animal after ablation of one eye.

PLATE 44

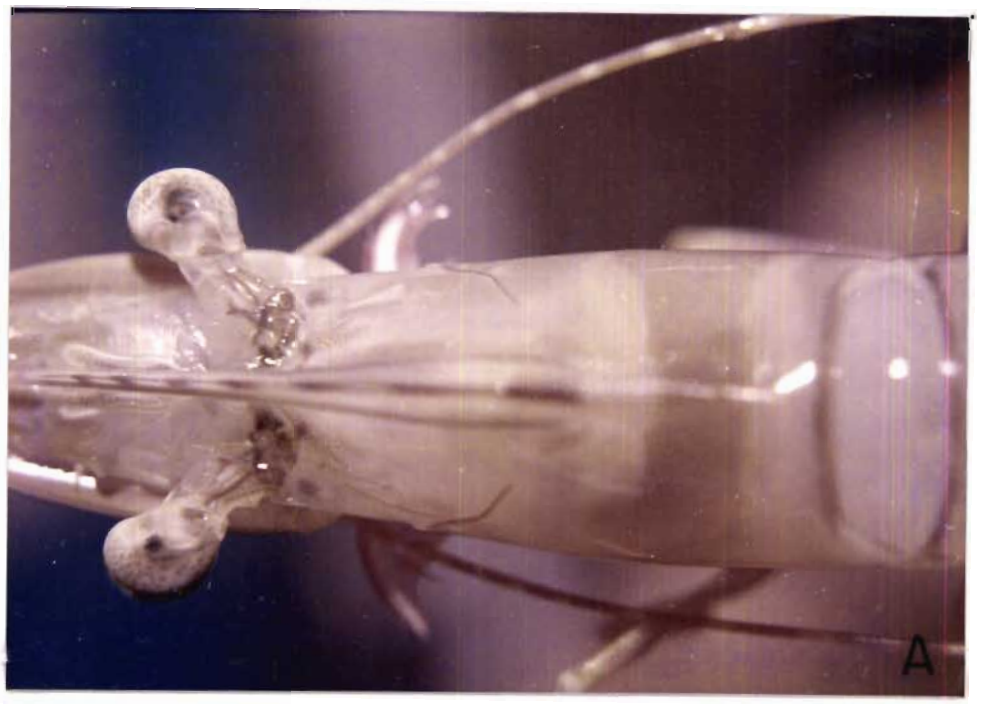
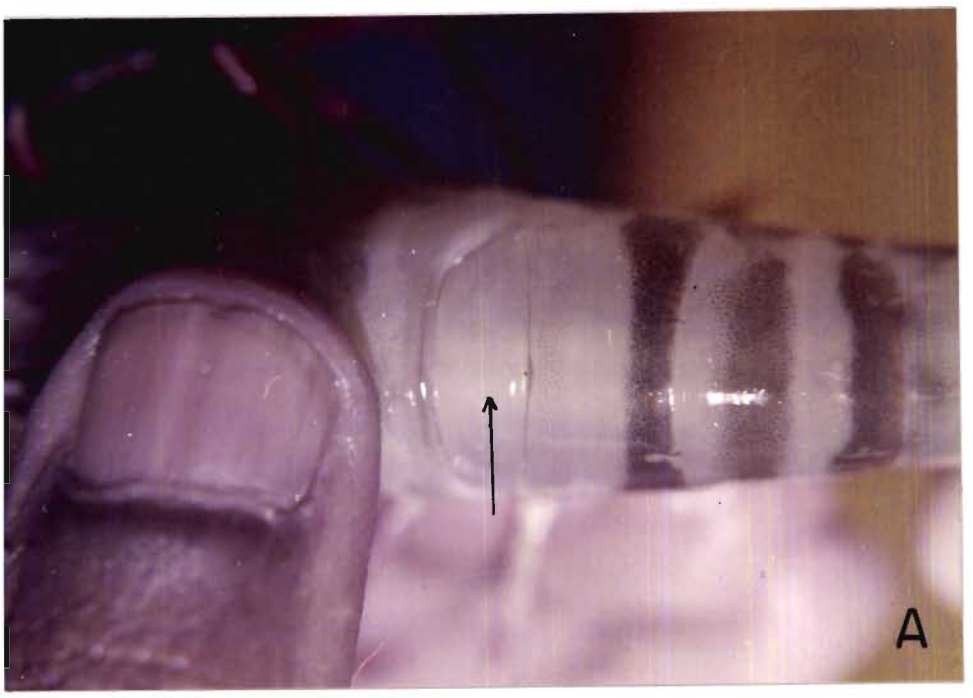


PLATE 45

A. Dorsal view of abdomen of eye ablated female prawn indicating sign of ovarian maturation.

B. A close up dorsal view of experimental animal subjected to bilateral eyestalk ablation.

PLATE 45



An attempt is also made to study the effect of UV rays (Plate 43b) on gonadal maturation in immature prawn of *P. semisulcatus*. As the container in which the experimental animal was subjected to UV rays was small, only a single prawn was used at a time. First, three advanced immature female prawns measuring 140-156 mm and weighing 28-37 g were introduced into the UV container. These animals jumped out of the container and died within two or three days. Secondly, two fully mature prawns measuring 155-210 mm and weighing 30.2-79 g were introduced to observe the spawning process. As experienced in the previous case the animals did not survive in that condition for long, and died after two days. It was, however noticed that the pigmentation on the uropods was more pronounced after the animal was exposed to UV irradiance than in the normal condition.

In the experimental group I, five unablated female prawns in the size range 130-150mm served as control. In 10 days, all these animals which were initially in intermoult (C) and/or early premoult (DO) stages advanced to premoult (D1', D1" and D1"') and late premoult (D2-D3) stages. Out of the 5 animals, two moulted as they were in the late premoult stage. There was no visible indication of gonadal development although the gonadosomatic indices ranged from 0.48-1.23 and ova diameter from 32 to 110 μm as furnished in table 32. Swimming behaviour of the prawns was normal.

In the experimental group II, the female prawns 135-156mm were subjected to unilateral eyestalk ablation experiments (Plate 44). Due to handling stress, two of the prawns died after 2-3 hours. After the prawns were acclimatised for 48 hrs. the unistalk eye ablation experiment was started on 3.12.1993 with the remaining three animals weighing 28,31 and 37 g. On 8.12.1993, after 6 days, the 37 g size prawn became fully mature and the next day spawned 1,55,500 eggs during night at 23.00 hrs. The same prawn again matured and spawned 54,568 eggs on 13.12.1993 after 5 days of life in captivity. The 31 g size prawn matured and spawned 1,19,680 eggs on 9.12.1993 at 23.30 hrs. after 7 days of stocking. The eggs thus released showed

a hatching rate of about 70-75%. The oocyte diameter of spawned eggs ranged from 300-415 µm. The 28 g prawn attained III stage of ovarian development on the 10th day of experiment, by which time the diameter of ova grew to 252-280 µm. Swimming behaviour of all these prawns was normal. The food intake was substantially increased with voracious feeding on clam meat when offered during the period of experimentation.

In group III, the prawns were in premoult, late moult and intermoult stages and used for bilateral eyestalk ablation (Plate 45b). With size 21.8, 27.5, 30.0, 34.4 and 35.0 g the five prawns weighed individually when reared after eyestalk ablation none of them spawned within ten days although ovarian maturation took place in all. Their GSI ranged from 3.44 to 5.61. Of the five prawns used for experiment two died due to handling stress and one moulted in between. The diameter of oocytes ranged from 210 to 370 µm as shown in the Table 33. Immediately after the extirpation of both the eyestalks, the prawns were found to swim in circles with abnormal speed on the surface of the pool. The prawns were unable to detect the clam food introduced into the tank and the feeding was rather slow. Often the food supplied was not completely eaten and hence some left-over meat was observed on the clam shells.

In group IV, the prawns in the size range 135-146 mm were used for eyestalk extract injection experiments. After the eyestalk extract was administered into the prawns, the animals died within two or three hours.

In group V, the five prawns in the size range 136-156 mm were injected with CNS extract. Two of the prawns died immediately after the administration of CNS extract. The ovaries of the rest of the prawns showed sign of maturation nearly upto III stage. Within the period of ten days, appreciable increase in the GSI could be noticed, which ranged from 1.78 to 2.68 (Table 34). The ova diameter of the oocytes ranged from 150 to 200 µm. Feeding and swimming were normal.

Table 32. Experimental details of eyestalk ablation experiments in relation to gonadal maturation in *P. semisulcatus*

Group I: Control-unablated

Total length mm (Total wt.,g)	Latency period (days)	Moult stage (initial)	Moult stage (final)	GSI	Ova diameter (µm)
(1)	(2)	(3)	(4)	(5)	(6)
130 (18.0)	10	C	D_0	0.48	32-40
136 (21.8)	10	C	D_0	0.53	35-50
140 (28.0)	10	D_0	D_3	0.71	70-90
144 (29.5)	10	C	D_1'	0.94	75-100
150 (31.0)	10	C	D'	1.23	70-110
Mean	10	-	-	0.79	56-78

Group II: Unilateral eyestalk ablated

Total length mm (Total wt.,g)	Latency period (days)	Moult stage (initial)	Moult stage (final)	Egg spawned	Ova dimaeter (µm)
156 37)	6	C (3.12.1993)	C (8.12.1993)	1,55,000	350-400
156 (37)	5	C (8.12.1993)	D_1' (13.12.1993)	54,568	340-380
148 (31)	7	D_0 (3.12.1993)	D_1" (9.12.1993)	1,19,680	350-400
144 (28)	10	D_3	A	GSI 4.38	252-280
135 (21)	-	-	*	-	-
141 (26)	-	-	*	-	-
Mean	7	-	-	-	323-365

* Prawns died during the course of experiments.

Table 33

Group III: Bilateral eyestalk ablated

Total length mm (Total wt.,g)	Latency period (days)	Moult stage (initial)	Moult stage (final)	GSI	Ova diameter (µm)
147 (30.0)	10	D_1'	D_3	6.38	290-360
142 (27.5)	10	D_1'	*		
154 (34.4)	10	D_3	A	4.91	280-300
136 (21.8)	10	C	C	3.44	250-280
158 (15.0)	10	D_3	*		
Mean	10	-	-	4.91	273-313
Group IV: Eyestalk extract injected					
146 (28.5)			*		
142 (27.5)			*		
135 (21.3)			*		
140 (28.0)			*		
145 (28.0)			*		

* Prawns died during the course of experiments.

Table 34. Experimental details of CNS extract administered female *P. semisulcatus*

Total length mm (Total wt.g)	Latency period (Days)	GSI	Ova diameter range (µm)
Group I: Placebo Injected (Control)			
137 (22.0)	10	0.55	34-52
142 (28.0)	10	0.88	70-100
144 (28.5)	10	0.86	70-90
148 (29.5)	10	1.30	70-100
148 (30.0)	10	1.35	70-110
Mean	10	0.99	63-92
Group II: CNS extract Injected			
153 (32.8)	10	2.45	170-200
149 (32.3)	10	1.90	150-160
156 (37.0)	10	2.68	170-190
144 (28.0)	10	1.78	150-160
136 (21.0)		*	
Mean	10	2.20	160-177

* Prawns died during the course of experiments

The present work was carried out on *P. semisulcatus* at CMFRI laboratory, Mandapam Camp. Due to the small container under which the UV rays passed, the single prawn was introduced. As a first step, an advanced immature prawn was introduced. Within two or three days, it jumped outside the container and died. Eventhough more efforts were taken up, successful result could not be obtained. As a second step, fully matured prawn was introduced to observe the spawning condition. Due to conditions present as noticed in the first step, successful result were not obtained. In general, the formation of pigmentation was found more in uropods of the prawns exposed to UV irradiance.

DISCUSSION

The technique of unilateral eyestalk ablation is used in numerous research and commercial industries for the purpose of stimulating the female shrimps to develop mature ovaries and then spawning. The eyestalk ablation in *P. semisulcatus* has led to precocious maturation of the ovary. The relationship between removal of one (or both) eyestalks of a female decapod crustacean and ensuing gonadal development was first discovered by Panouse (1943) in the shrimp *Palaemon serratus*. Among penaeids, eyestalk ablation has so far been synonymous with unilateral eyestalk ablation. Arnstein and Beard (1975), Santiago (1977) and Mohamed (1989) observed that ablation of single eyestalk was sufficient to induce maturation in *P. orientalis* and *P.monodon*, respectively. Similarly in *P. indicus*, Muthu and Laxminarayana (1977) also reported that unilateral eyestalk ablation was sufficient to induce precocious maturation as was observed during the present investigation. Induced maturation has been successfully utilised to promote shrimp gonadal maturation, obtaining more rapid and more frequent spawnings (Aquacop, 1975; Brown *et al.*, 1979; Lawrence *et al.*, 1980; Chamberlain and Lawrence, 1981 b; Emmerson, 1983; Chamberlain and Gervais, 1984; Oyamma *et al.*, 1989; Ottogalli, 1989). The effect of unilateral eyestalk ablation on spawn size and spawn quality is poorly understood. Primavera *et al.* (1979) found no difference in spawn size from ablated and nonablated pond-reared *P. indicus*. Emmerson (1980) described lower egg numbers from ablated females when spawns of domestic ablated and nonablated *P. indicus* were compared. Chamberlain and Lawrence (1981) observed that ablated *P. stylirostris* produced singificantly more eggs per spawn than non-ablated ones.

In the present study, the prawn *P. semisulcatus* matured within five days and spawned 1,55,500 eggs and the same prawn rematured and spawned with less number of eggs per spawn numbering 54,568 eggs under unilateral eyestalk ablation, while bistalk ablated prawn has not matured and spawned like unilateral ablated prawn, even ten days time was given to them. But, the

increase of GSI could be noticed during the course of time. Similarly, Browdy and Samocha (1985 a) reported in the same species *P. semisulcatus* that the average numbers of spawns and eggs per ablated females were more than double those produced by unablated females, although fewer eggs per spawn were produced by ablated females and by reporting 77605 ± 36045 for first spawn, 57563 ± 29690 for second spawn and 60898 ± 31284 for third spawn. Usually, somewhat larger individual spawns were reported for unablated females, but frequency of spawning was considerably higher in eyestalk ablated animals (Emmerson 1980; Browdy and Samocha, 1985, a b; Browdy, *et al.*, 1986). There is not a strong trend toward diminishing spawn size overtime (Poernomo and Hamami, 1983). Browdy and Samocha (1985 a) found decline in eggs/spawn in both ablated females and unablated females in 80 and 110 days respectively. Further, Browdy *et al.* (1986) reported the unilateral eyestalk ablation on *P.semisulcatus*, a slower growth rate (g/day); a trend towards shortening of the molt cycle; a significant smaller number of eggs and nauplii per spawn; significantly increased spawn frequency and increased rate of egg and nauplii production. Lim, *et al.* (1987) reported from *P. merguiensis* that ablated spawners of 25-40 g each, the mean fecundity and hatching rate have been found to be 57,000 eggs and 78% respectively in the early spawners, compared to only 16,000 eggs and 46% in the late spawners. In *P. monodon*, the presence of bigger oocytes in the ovaries of ablated prawns sampled after spawning provided histological evidence for lower number of eggs per spawn and faster rematuration (Tan-Fermin, 1993). Menasveta, *et al.* (1993) in *P.monodon* from Andaman sea reported that eyestalk ablated wild caught prawn has got matured into IVth stage (mature) in the percentage of 89.2 within 9.4 days with the spawning percentage of 78.2. The most commonly accepted theory is that a gonad inhibitory hormone (GIH) is produced in the neurosecretory complexes in the eyestalk. This hormone apparently occurs in nature in the non-breeding season and is absent or present only in low levels during the breeding season (Bomirski and Klek, 1974, Kulkarni and Nagabhushanam, 1980). The increase in spawning and egg production observed for unilaterally ablated females was presumably due to the decrease in the

level of gonad inhibitory hormone in the haemolymph of ablated females (Kulkarni and Nagabhushanam, 1980). Most penaeids develop mature ovaries in captivity is a function of elevated levels of GIH, and eyestalk ablation lowers the high haemolymph titer of GIH. Eyestalk ablation in crustaceans has been reviewed by Adiyodi and Adiyodi (1970), Fingerman (1970), Highnam (1978), Kleinholz and Keller (1979) and Charniaux-Cotton (1985). To prevent the precocious maturation of the ovary, eyestalk extract administrated into unilateral eyestalk ablated prawns *P. semisulcatus* for the present study. But, data could not be collected as the prawns died immediately within two or three hours after the administration of eyestalk extract into unilateral eyestalk ablated prawns perhaps due to handling stress or slight increase in dosage. In *P. indicus*, precocious maturation of the ovary was prevented effectively by administration of an eyestalk extract into unilateral eyestalk ablated prawns. These findings confirmed that the principle factors contained in the eyestalk are indeed gonad inhibitory.

In the present investigation, brain and thoracic ganglion extracts injected into the prawns show that there was a stimulatory effect on the ovary as noticed by Mohamed (1989) in *P. indicus*. Increased ovarian growth has been obtained with implants of thoracic ganglia or injection of ganglionic extracts in a stomatopod as well as in several other brachyuran species (Otsu, 1963; Gomez, 1965; Nagabhushanam and Diwan, 1974; Hinsch and Bennett, 1979; Deecaraman and Subramoniam, 1983; Eastman-Reks and Fingerman, 1984). In the prawns paratya and Parapenaeopsis, extracts of cerebral as well as thoracic ganlia stimulated the ovarian growth, but brain extracts were more effective than thoracic ganglia in *Paratya* (Kulkarni *et al.*, 1981; Takayanagi *et al.*, 1986). Kulkarni *et al.* (1981) inferred from his study on *Parapenaeopsis hardwickii* that the brain and thoracic ganglia produce a factor which accelerates ovarian growth in this prawn. *Chan Hooi Har* (1991) studied the effect of eyestalk ablation, thoracic ganglion extract from 'Spent spawners' on ovarian maturation in pond-reared shrimp, *P. monodon*. But, he could not find much of significant differences between treatment groups and non-treatment

groups and further reported that the ovaries of the larger-sized shrimps gave better GSI and GI responses following eyestalk ablation. Nagabhushanam *et al.* (1993) reported in female prawns injected with eyestalk extract that there was a decrease in ovarian indices and oocyte diameters over controls, whereas in the group injected with extract of brain and thoracic ganglion, ovarian indices and oocyte diameters increased.

In the present study, attempts were made for the application of UV rays on female prawn *P. semisulcatus* to find out the effect either to inhibit or to accelerate the gonadal maturation. Though much efforts were taken for the application of UV rays on this species, the data could not be collected as the prawns jumped outside the container and died. However, it was observed that the prawns exposed to the UV-irradiation were found to contain pigmentation in their exoskeleton. Ijiri (1980) also found the pigmentation while studying the effect of UV on the development of fish and amphibian embryos. But, there is no such information available regarding effect of UV rays on the development of crustacean embryos. However, it is reported that some aminoacid such as tyrosine,tryptophan and phenylalanine accumulate more and absorb the UV-radiation in the UV-exposed organisms (Lehninger *et al.*, 1982 and 1993). These amino acids form the precursor for the synthesis of pigments like melanin. Hence, it is perhaps the UV-induced the synthesis of those aminoacids and then that of pigments.

SUMMARY

SUMMARY

1. The reproductive physiology encompassing the structure of reproductive system, maturation process, fecundity, spawning, gametogenesis, biochemical and mineral changes in relation to ovarian maturation, histochemical composition of ovary, testis and spermatophore and the results of experiment on induced maturation of the green tiger prawn *P. semisulcatus* de Haan are presented based on studies conducted at CMFRI, Mandapam camp.

2. The internal male reproductive system include a testes consisting of a long and thick anterior lobe and seven pairs of lateral lobes, paired vas deferens demarcated into proximal, middle and distal regions and paired terminal ampoules. The female reproductive system include the ovary consisting of a pair of anterior lobes, a middle lobe with eight pairs of lateral lobules and a pair of long posterior lobes and a pair of oviducts originating from the tip of the sixth lateral lobules.

3. Based on the fusion and hardening of petasmal endopodites, structural changes of the internal reproductive organs, size of the terminal ampoule and the presence of spermatophore at the base of the fifth walking leg, four maturity stages have been recognised in male, namely, stage I (immature), stage II (early maturing), stage III (late maturing) and stage IV (mature). Based on the size, shape and colour changes of ovary, microscopic details of ova and gonadosomatic indices, five maturity stages have been recognised in female, namely, stage I (immature), stage II (early maturing), stage III (late maturing), stage IV (mature) and stage V (spent).

4. The smallest mature male measured 115 mm TL. The minimum size at maturity of female at 50% level has been calculated as 145.07 mm TL.

5. The fecundity ranged from 86,580 at 143 mm TL to 7,12,595 at 192 mm TL. The fecundity and gonad weight showed a positive relationship.

6. *P. semisulcatus* is a continuous breeder with distinct peak spawning seasons. The major spawning peaks lasted from March to June in the Palk Bay and May to September in the Gulf of Mannar.

7. The process and events leading to maturation of ovary and testis have been investigated using histological and electron microscopic methods. The oogenesis involved oocyte development and yolk accumulation in a graded manner. Based on the ovarian changes manifested in the cytoplasm and nucleus of oocyte, the maturation process was classified into pre-vitellogenic, early vitellogenic, late vitellogenic, vitellogenic and spent oocyte stages.

8. In the pre-vitellogenic oocyte, the cytoplasm was basophilic and the darkly stained nucleoli numbering 10-15 accumulated in the nucleus. Ultrastructurally, euchromatin and heterochromatin regions were distinguished in the nucleus and the micropinocytosis was found to be taking place in the oocyte by heterosynthetic process. The oocytes and oocyte atresia were surrounded by follicle cells.

9. Early vitellogenic oocyte was less basophilic, but had more granular cytoplasm due to the formation of the primary yolk vesicles. A perinuclear halo of nucleolar material was observed with the basophilic nucleoli which were arranged in the periphery of the nucleus. The investment of the follicle cells around the oocytes was intensified during this phase. As in the previous stage, smooth endoplasmic reticulum was noticed in this stage.

10. In the late vitellogenic oocyte, the cytoplasm was acidophilic and granular due to the formation of yolk globules and yolk platelets. The nucleus was palely stained with haematoxylin and the nucleoli number greatly reduced. The follicle cells become elongated and appeared as a narrow band of flattened cells surrounding the oocytes. Presence of rough endoplasmic reticulum and active exchange of materials between cytoplasm and nucleus were noticed at this stage.

11. Vitellogenic oocytes were characterized by the appearance of specialised elongated cortical rods on the peripheral margin of ooplasm and abundance of yolk globules and yolk platelets.

12. The spent ovary displayed empty follicles, sites of oocyte resorption (atretic oocytes) and areas of proliferative oocyte growth. Zone of proliferation was observed as in all previous stages. Partially spawned oocyte showed rapid breaking down process and resorption due to unsystematic orientation of cortical rods.

13. In male, sperms were formed in the seminiferous tubules of testes by mitotic division of spermatogonial cells and meiotic divisions of spermatocytes and spermatids. The fully formed spermatozoa was tack-shaped with a spherical body and a long spike. The formation of spermatophore was initiated in the middle vas deferens and completed in the terminal ampoule from the secretions of the glandular epithelial cells lining the duct. The spermatophore had an oval-shaped body with a parachute-like wing attached to the middle of the body by a short stalk. Scanning electron micrograph study showed that it had three chambers and five spermatophoric layers.

14. The metabolic components like, protein, free amino acid, lipid, carbohydrate, carotenoid and moisture in the hepatopancreas, haemolymph, ovary and muscle of *P. semisulcatus* female during the

five maturity stages were estimated and the trend of their levels compared and discussed. Highly significant variations in the biochemical parameters in relation to maturity stages except protein and moisture in muscle were observed in the different tissues. Moisture was the principal component of the fully formed yolk followed by protein and lipid in the order of abundance, while the other metabolites occurred only in smaller proportions.

15. While the protein content in hepatopancreas and haemolymph showed a marked decline in the early phases of maturation, the protein levels in the ovary progressively increased from immature to mature stages. This has been attributed to active mobilization of protein from hepatopancreas and haemolymph to ovary for vitellogenin synthesis. In the ovary, the protein content was quantitatively much higher than in hepatopancreas and haemolymph at all stages of ovarian development, which suggested that ovary was the single major site for synthesis of protein moiety of vitellin (autosynthesis) than hepatopancreas and haemolymph. The protein values in muscle did not reveal any definite trend in relation to maturation and therefore could not be correlated with the process of vitellogenesis.

16. The amino acid content was relatively higher in hepatopancreas than in ovary, haemolymph and muscle. Its values in ovary, haemolymph and hepatopancreas progressively increased from immature to mature stages and suddenly dropped in spent stage. The steady increase of free amino acid level in ovary and haemolymph suggested its mobilization, storage and utilization for vitellogenin synthesis.

17. The values of lipid content were comparatively higher in hepatopancreas and ovary than in haemolymph and muscle. In both hepatopancreas and ovary the lipid value showed a steady increasing trend from immature to mature stages and decreased suddenly in

spent stage. The decrease in lipid content was more drastic in the ovary than in hepatopancreas, which indicated the possibility of active utilization of lipid for Vg synthesis. The lesser degree of decline in lipid level in hepatopancreas at spent stage also pointed to the fact that, though lipid mobilization took place from hepatopancreas to ovary, only a part of lipid content was mobilized into the ovary and substantial quantity of this lipid content stored in the hepatopancreas itself for other metabolic purposes.

18. The carbohydrate content showed more than a four-fold increase in mature stage and a drastic decrease in spent stage in hepatopancreas and haemolymph and a similar trend in lesser intensity in ovary, indicating the possible transport of carbohydrate substances to ovary during maturation.

19. The carotenoid levels showed peaks in early maturing stage in hepatopancreas, late-maturing stage in haemolymph and mature stage in the ovary, thereby showing gradual mobilization of the pigment from hepatopancreas to ovary.

20. Moisture was recognised as the principal component of the fully formed yolk in *P. semisulcatus*. Protein and lipids formed the next major organic compounds, while the other metabolites were formed only in small quantities.

21. Analysis of the various macro and micro minerals in hepatopancreas, ovary and muscle indicated that K, Na and Ca were the most dominant ones in these tissues. Ovary proved to be largest storage organ for most of the minerals. Generally, the highest concentration of majority of the minerals was observed in the immature stage. However, substantial increase in the concentration of Cu, Zn and Fe was noticed at maturity in the hepatopancreas. Cd was totally absent

in any of the tissues. The fluctuation noticed in the mineral contents indicated the possibility of their utilization in the vitellogenin phases.

22. Histochemical studies in relation to oogenesis have shown that the basic and acidic groups of protein were more positive and intense positive to oogonial cells and previtellogenic oocytes respectively, denoting abundant presence of these groups. The oogonial and previtellogenic oocytes contained abundant presence of more sulphated mucosubstances. Generally, the lipid was poorly represented in oogonial and previtellogenic oocytes, except the phospholipid.

23. In early vitellogenic oocytes, the basic protein was present abundantly in N, NUL and follicle cells and doubtfully in cytoplasm, while the acidic group was present more abundantly in cytoplasm and follicle cells and moderately in N and NUL. The acidic group of protein was relatively more abundant than the basic protein. Toluidine blue at different pH stained intense positively showed the abundant presence of both sulphated and phosphated mucopolysaccharides. Lipid content was present in moderate quantities.

24. In late vitellogenic oocyte, basic protein, aminogroups, acidic groups and tryptophan were present abundantly. Yolk platelets and yolk globules were positive to all classes of protein tested. Toluidine blue at pH 7 stained intense positively showed the abundant presence of carboxylated AMP. Phospholipid and neutral and acidic lipids were present in more quantity.

25. The fully formed yolk contained basic proteins (histidine, arginine and lysine), acidic groups (less amount of -SS groups and tyrosine), carbohydrates (sulphated or carboxylated AMP) and lipid (Abundant total lipid, moderate phospholipid and cholesterol and poor

unsaturated and neutral lipids) thereby indicating a glycolipoprotein nature.

26. In spent oocyte, the presence of acidic protein was more abundant than basic protein. Toluidine blue at different pH showed the abundant presence of sulphated or phosphated and carboxylated AMP. Neutral and acidic lipid were positively present in this oocyte.

27. Histochemical analysis of testes and spermatophore revealed the abundance of basic protein, acid mucopolysaccharide and phospholipid. The glycogen and sulphated AMP observed in the spermatophore layers has been assigned nutritive function and a role in the protection of sperms from bacterial attack.

28. Prawns (31-37g) when subjected to unilateral eyestalk ablation matured within 5-7 days and spawned 1.20-1.55 lakh eggs/spawn and rematured within 5 days and spawned a lesser number of 0.55 lakh eggs/spawn. The eggs thus released showed a hatching rate of about 70-75% under laboratory condition with pH ranging 8.2-8.5, water temperature ranging 29.5-29.8°C and salinity ranging 28.5-30‰.

29. Prawns when subjected to bilateral eyestalk ablation did not spawn even after 10 days, but appreciable GSI could be noticed in the ranges 3.44-6.38.

30. When prawns were administered CNS extract, they showed an improvement in GSI with the values ranging from 1.78-2.68. However, these values were found to be lower than those obtained in bilateral eyestalk ablation experiment.

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