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INFLUENCE OF A PUTATIVE MALE PHEROMONE ON OVARIAN MATURATION AND OVULATION IN THE FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII* (DE MAN, 1879).

By

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THESIS

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DEPARTMENT OF FISHERY BIOLOGY

COLLEGE OF FISHERIES

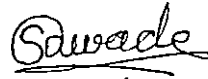
PANANGAD, COCHIN

Dedicated To

My Beloved Parents

DECLARATION

I hereby declare that this thesis entitled **"INFLUENCE OF A PUTATIVE MALE PHEROMONE ON OVARIAN MATURATION AND OVULATION IN THE FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII* (DE MAN, 1879)."** is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship, or other similar title, of any other University or society.



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Introduction

1. INTRODUCTION

Macrobrachium rosenbergii (de Man, 1879) is the largest freshwater prawn ever recorded. The male grows up to 340 mm and female grows up to 286 mm in total length (Jayachandran and Joseph, 1992). Because of its fast growth and market acceptability, this species has been introduced into the water bodies of most countries of the world.

According to a recent report of FAO, the production of freshwater prawns in 2001 from farms worldwide was 213861 mt and from India, it was 24230 mt (New, 2005). There are so many problems met with in the aquaculture of *M. rosenbergii*, viz.,

1. Differential growth
2. Existence of male morphotypes
3. Existence of different ecotypes
4. Aggressive behaviour
5. Peculiarities in reproduction
6. Large number of larval stages
7. Breeding migration
8. Larval migration
9. Cannibalistic and carnivorous feeding habits
10. Complex reproductive peculiarities etc.

During recent years the aquaculture of *M. rosenbergii* has extended in a big way to more areas in India and associated with this a number of hatcheries have started coming up. Most hatcheries depend on the availability of berried females from the wild for their operation. Lack of an assured supply from the natural waters limits the hatchery operation. Knowledge of factors regulating the reproductive events in this species is vital in building up brood stock and its proper utilization for seed production.

In crustaceans there are many studies on hormones controlling reproduction. The X-organ-sinus gland (XO-SG) complex, located in the

eyestalks, contains two distinct hormones, one inhibits moulting (MIH) and the other gonadal development (GIH). Another neurohormone, found in the brain and thoracic ganglia, is the gonad-stimulating hormone (GSH). When MIH and GSH levels in the haemolymph are low, and the levels of GIH and the moult hormone (MH) secreted by the Y organs are high, moulting is induced. Low titres of GIH start the vitellogenic and spermatogenic processes (Adiyodi & Adiyodi, 1970; Adiyodi, 1985; Fingerman, 1987; Ismael & New, 2000; Vincent *et al.*, 2003).

Another factor playing an important role in crustacean reproduction is pheromone. Sex pheromones previously reported to be present in many crustaceans have chemokinetic, chemotaxic and releaser effects (Dunham, 1978). Reviewing the studies on sexual behaviour in *M. rosenbergii*, Ismael and New (2000) concluded that sex pheromones could be playing a major role both in attraction between sexes and in the differential responses of morphotypes. The discovery of a male "ovary stimulating pheromone" with primer effect on ovarian maturation and ovulation added an important external factor to the mechanism regulating female reproduction in the freshwater shrimps/prawns. Studies in *Paratya compressa* (Takayanagi *et. al.*, 1986 b) and *Macrobrachium kistnensis* (Nagabhushanam *et. al.*, 1989) showed that ovaries of females kept isolated from males failed to develop into the vitellogenic stages of ovarian maturation. The extracts of testes and vas deferens of these species were shown to contain the ovary stimulating pheromone which acted through the release of GSH from brain and thoracic ganglia. Patil (2001) reported evidence for a male pheromone in *Macrobrachium idella* secreted from testis/vas deferens which is essential for stimulation of ovarian maturation beyond stage IV, i.e. the beginning of exogenous vitellogenesis. Das *et al.* (1999) based on unpublished study reported that ovarian development was delayed in *M. rosenbergii* and *Macrobrachium malcomsonii* in the absence of male.

The present study will be scientifically useful, as it will help to unravel the role, if any of male and/or male pheromone in ovarian

maturation and ovulation in *M. rosenbergii*. This knowledge may also find application in the aquaculture and husbandry of this species.

Review of Literature

2. REVIEW OF LITERATURE

2.1 REPRODUCTIVE BIOLOGY OF *M. ROSENBERGII*

2.1.1 Sexuality

Palaemonid prawns are dioecious, the sexes being distinguished by a number of external characters (Patwardhan, 1937; Ibrahim, 1962; Ling, 1969; Sandifer and Smith, 1979; Holthuis, 1980; Singh and Roy, 1994; Jayachandran, 2001). In general, the females are smaller than males of the same age. The second chelate legs of males are more elongated, stout and profusely covered with setae. The males are also characterized by the presence of appendix masculina in the endopodite of second pleopod. In females, epimera of the abdominal segments are bigger in size and form deep recess for carrying eggs during breeding season. The male genital apertures are paired, present on the arthrodial membrane above the coxa on the inner side of the last pair of walking legs, covered over by small tongue like flaps of integument. The female genital aperture is also paired, each being situated on a raised papilla on the inner side of the coxa of the third walking legs.

2.1.2 Reproductive System

Jayachandran (2001) reviewed the reproductive system of *M. rosenbergii*. The female reproductive organ system consists of paired ovaries, oviduct, gonopores and an unspecialized spermatophore attachment area. The ovaries lie dorsal to the stomach and hepatopancreas. When the female becomes fully mature, the ovaries occupy the entire carapace cavity and also extend partially into the first abdominal segment. The heart is situated mid dorsally over the somewhat flattened posterior lobes of the ripe ovaries. The ripe ovary is bright orange in colour.

An oviduct arises laterally from each ovary at a point just anterior to the position of the heart, extends downwards and opens into gonopores, situated on the coxa of the third pereiopod. The oviducts are generally

transluscent and somewhat difficult to see, but they can be observed easily during spawning when the brightly coloured eggs move through them to the gonopores. The gonopores are simple, posteriorly directed openings on the inner surfaces of the coxae of the third pereiopods. In sexually receptive females, each pore is equipped with a large tuft of long setae. The long setae arrange themselves as a "tube" through which ova pass out. Coxae of the fourth and fifth pereiopods also bear tufts of long setae, which may help to direct the newly spawned eggs over the spermatophore and also to the brood chamber. The tufts of setae that develop on the pleopods after the pre mating, parturial moult are termed the "breeding dress".

The sperm receptacle is a relatively smooth and essentially unspecialized area of the thoracic sternum extending from just in front of the third to fifth pereiopods.

The male reproductive system consists of paired testes, vas deferens and gonopores. The testes lie dorsal to the stomach and hepatopancreas. They give rise to highly coiled vas deferens. From the tightly coiled region of the vas deferens, a more or less straight tube extends down the posterior lateral side of the cephalothorax, ending as a terminal ampulla. The terminal ampulla is the swollen area where the sperm mass is stored prior to ejaculation. Within the vas deferens, thousands of non-motile sperm cells are embedded in a sticky gelatinous matrix, which upon extrusion forms the spermatophore. The male gonopores are situated medially on the coxae of the fifth pereiopods, on the inner side. Each gonopore is covered with a flap, which opens as the spermatophore is extruded, presumably by muscular contractions, from the terminal ampulla.

2.1.3 Size at First Maturity and Sex Ratio

Many of the palaemonids reach sexual maturity within a year. The size at first maturity of *M. rosenbergii* has been investigated by many workers. In rivers of West Bengal it takes two years (Rajyalakshmi, 1961;

1975) and in Kerala one year (Raman, 1967) to attain maturity. Rajyalakshmi (1975) reported that males have been estimated to attain lengths of 107 and 149 mm at the end of first and second years of life and females 82.5, 130.5 and 168.5 mm at the end of first, second and third year respectively. Rao (1967) recorded the mean size of 155 mm as the maturity size in Hoogly estuary. Goorah and Parameshwaran (1983) recorded 118 mm and 20 g (5-7 months old) as the smallest size of berried females in ponds at Mauritius. The largest recorded size of this species is 340 mm for male and 286 mm for female (Jayachandran and Joseph, 1992). Regarding sex ratio, in the Vembanad lake, females dominate in September-December and males during March-June (Kurup *et al.*, 1992). Varghese *et al.* (1992) observed the best results of oviposition by maintaining the ratio of 1 male: 4 females in brood stock rearing of *M. rosenbergii*.

2.1.4 Breeding Season

The appearance of berried females marks the onset of the breeding season, while the time by which majority of prawns appear to have dehisced the brood indicates the end of the period (Rajyalakshmi, 1961).

The breeding season of freshwater prawns shows considerable variation. *M. rosenbergii* breeds from December to July in the Hoogly estuary (Rajyalakshmi, 1961; 1975; Rao, 1967) and in Kerala, it breeds during August to December with a peak in September- November (Raman, 1967; Kurup *et al.*, 1992; Sebastian *et al.*, 1993).

2.1.5 Male Morphotypes

Different male morphotypes are reported to be available in *M. rosenbergii*. Sagi *et al.* (1986) noted three different types of male morphotypes in *M. rosenbergii*, which are small males, orange clawed and blue-clawed males. Each type of male morphotype is said to represent a different reproductive strategy (Sagi *et al.*, 1990; Joseph and Kurup , 1998).

Small males and blue clawed males actively take part in mating while orange clawed males are characterized by fast growth rate (Sagi, 1984; Ra'anan and Sagi, 1985; Sureshkumar and Kurup, 1998).

2.1.6 Eggs and Fecundity

The eggs are slightly elliptical, 0.6 to 0.7 mm along the long axis, bright orange and each has a thin membrane (Jayachandran, 2001). A typical mature female of about 80 g in weight and 14 cm in length can produce about 70,000 eggs (Jayachandran, 2001). Large females may produce up to 100,000 eggs (John, 1957). Mature females may oviposit 3 to 4 times a year under natural conditions. They were found to spawn throughout the year and repeatedly in captivity (Damronghphol *et al.*, 1991)

2.1.7 Spermatophore Formation and Insemination

Sandifer and Smith (1979) described spermatophore formation and insemination in *M. rosenbergii*. They observed that immediately after copulation the spermatophore appears as two fused cords of an opaque gelatinous material lying parallel to the long body axis between the female's last three pairs of pereiopods. As they are extruded, the gelatinous sperm cords are sticky apparently fuse into a double strand spermatophore. As soon as it is formed, the male's first and second pair of pleopods probably transfers the spermatophore to the female thoracic sternum.

2.1.8 Mating

Rao (1967) and Ling (1969) have studied the mating behaviour of this species. They observed that once the male and female get accustomed to each other, mating behaviour is initiated. The male starts its courtship display, lifts its head, raises its body and waves its feelers. It raises and extends its long and powerful chelate legs in an embracing gesture. It is

accompanied by intermittent jerking movements of the body. This display continues for 10-30 minutes before the female is successfully won over.

2.1.9 Fertilization and Spawning

Jayachandran (2001) reviewed the fertilization and spawning in *M. rosenbergii*. Typically, spawning takes place roughly 24 hours after the pre-mating moult. Ling (1969) observed that spawning takes place 6-20 hours after the pre-mating moult depending on the time of the mating. During spawning the female's abdomen is tightly flexed and the pleopods extended to form a protected egg passage. The ova stream down the oviducts and exit the gonopores as separate eggs. Eggs pass out of the gonopores in slow, steady streams and are channeled posteriorly along the medial body line by the 'tubes' formed by the long setae surrounding the genital orifices. The passage may be facilitated by a lubricating fluid secreted by the oviducts as suggested by King (1948) for *Penaeus setiferus*. As the eggs pass across the thoracic sternum, they encounter the spermatophore, which had previously been manipulated by small chelae for fertilization.

The sperm cells are apparently brought into contact with ova by the mechanical action of the ova passing across the spermatophore on the way to brood chamber. As suggested by Descouturelle (1971), movement of the coxae of the fourth and fifth pereiopods with their more or less medially directed tufts of long setae may also help to facilitate egg passage towards abdomen.

During spawning the abdomen is flexed and the pleopods extended as described by Ling (1969) and Sandifer and Smith (1979), so that the first two pairs of pleopods overlap the sperm receptacle area, essentially forming a floor for the egg passage way. After the eggs pass over the spermatophore, they are paired up by the setae on the first two pleopods and moved into abdominal brood chamber. The first two pairs of pleopods are

essential for fertilization of the eggs and also for their arrangement in brood chamber.

As the eggs pass into brood chamber they become attached to each other and to the ovigerous setae of the first four pairs of pleopods by a 'cementing substance' which is produced by tegumental glands present in the pleopods (Yonge, 1955). Sandifer and Smith (1979) have observed that even eggs which do not come into contact with the tegumental glands of the pleopods develop gelatinous outer membrane shortly after release from the oviduct.

2.1.10 Incubation

Jayachandran (2001) reviewed the incubation of eggs in *M. rosenbergii.* In freshwater prawns the eggs are carried underneath the abdomen in a brood pouch cemented to among the setae in the pleopods. The females incubate the egg mass for about 19 days in *M. rosenbergii* (Ling, 1969). The developing eggs are ventilated by the fanning activity of the mother to facilitate the gaseous and ionic exchange for eggs during incubation. From the 12th day of incubation, the bright orange colour of the eggs gradually lightens and becomes light grey, deepening to dark grey by the 16th to 17th day of incubation. By this time the larvae inside the eggs are fully developed. Dead eggs and other foreign materials are carefully removed by the female with her first pereiopods.

Several other investigators reported incubation period of 15-24 days (Rao, 1986), 16 days (Diaz, 1987a) and 17 days (Diaz, 1987b) in *M. rosenbergii.*

Incubation of eggs is reported to be energy demanding process (Mathavan and Murugadass, 1988). The presence of developing eggs in the brood pouch delays moulting in crustacea (Schone, 1961).

2.1.11 Hatching

During hatching the mother prawn creates the powerful water current by beating the pleopods and as a result the eggs hatch (Ling, 1969). During this process mother preens the egg mass with maxillipeds to sever the eggs. The exposure to strong current and preening of the eggs might trigger hatching (Balasundaram and Poyyamoli, 1984). However, Balasundaram (1980) observed that when disturbed, the pleopod beat frequency decreases and the females postpone the process of hatching.

2.1.12 Ovarian Development in *M. rosenbergii*

Rajyalakshmi (1961, 1980) classified the maturity stages in *M. rosenbergii*, *Macrobrachium mirabile* and *Macrobrachium malcomsonii* in the Hooghly estuary. Charles and Subramoniam (1982) identified five histological stages for *M. malcomsonii* and *Macrobrachium lamarrei*. Jayachandran and Joseph (1988) and Patil (2001) reported seven maturity stages in *M. idella*. Sebastian (1993) reported six well-marked maturity stages in *Macrobrachium equidens pillai*.

O'Donovan *et al.* (1984) and Wilder *et al.* (1991) have described ovarian development and histology in *M. rosenbergii*. Chang and Shih (1995) studied the histology of ovarian development of *M. rosenbergii* and classified five stages, based on their size and colour, which can be observed through the carapace.

Histological studies of the ovaries of *M. rosenbergii* revealed the presence of oocytes in various stages of development. Five stages of development could be identified viz. previtellogenic, early vitellogenic, vitellogenic, late vitellogenic and mature oocytes. The distinction of these stages depended upon their cytoplasmic content and the size of the oocytes.

2.2 HORMONAL AND PHEROMONAL REGULATION OF REPRODUCTION IN SHRIMPS/PRAWNS AND OTHER DECAPOD CRUSTACEANS

A crustacean gears its reproductive activity in such a way that the liberation of young takes place when conditions are optimal for the survival of young. This adaptive synchrony is part of the individual's genetic endowment, which expresses itself via nervous, endocrine and neuroendocrine channels. Various extrinsic cues, such as changes in day length, availability of food, fluctuations in temperature and proximity of prospective mating partners are noted by the sensory receptors and the resulting afferent nervous impulses converge on the central nervous system (CNS), which in turn send directional message to the concerned organs, thereby eliciting specific response such as promotion or suppression of gametogenesis, uptake of vitellogenin or sexual receptivity (Adiyodi, 1985).

The reproductive biology is cardinal to biological science and is under endocrine control in both invertebrates and vertebrates (Adiyodi, 1980). The eyes in decapods are generally stalked which are known to contain a variety of hormones or factors apparently governing such diverse functions as growth, moulting, metabolic rate, heart rate, metabolism of sugars and proteins, water balance, dispersion of pigments and sexual activity (Lockwood, 1968). The X-organ-sinus gland (XO-SG) complex in the eye stalk is believed to produce a hormone controlling both reproduction and moulting (Adiyodi and Adiyodi, 1970). Two hormones have been later postulated to be involved in the control of moult, growth and development and reproduction jointly (Adiyodi, 1980).

Although many observations have been made on the inhibition of reproductive maturation by eyestalk hormone(s), recent research has focused mostly on brain, thoracic ganglion, ovary and mandibular organ and their functions which are closely related with the release of gonad stimulatory factors or hormone(s) (Yano, 1992a). As with ovarian

maturation, it has long been suspected that vitellogenesis in crustaceans is controlled by two antagonistic hormones. In penaeids, gonad inhibiting hormone (GIH) secreted from the XO-SG complex inhibits vitellogenesis and gonad stimulating hormone (GSH) secreted from the thoracic ganglion and brain stimulates vitellogenesis (Yano, 1992a).

2.2.1 Gonadal Maturation

There is still much speculation and divergence of opinion about the process of crustacean gonad maturation and the model of Adiyodi and Adiyodi (1970) is still valid (Adiyodi, 1985). This scheme proposes that the actions of moult inhibiting hormone (MIH) and GIH are antagonistic and also that there is GSH, produced by brain and thoracic ganglion. Moulting occurs when the titres of MIH and GSH are low and those of GIH and moulting hormone (MH) are high. Gonad maturation occurs in the converse situation. This model applies primarily to the females, but there is evidence that in male crabs, GIH acts via the androgenic gland by inhibiting its secretion and in its absence maturation takes place (Payen *et al.*, 1971). Moulting and reproduction are generally considered as antagonistic events in malacostracan crustaceans since both require large amounts of energy and are mechanically incompatible (Anilkumar and Adiyodi, 1981; Quackenbush and Herrnkind, 1981; Chang, 1984; 1992).

During the reproductive period the mature crustacean ovary may weigh as much as 10 % of the total body weight (Quakenbush and Herrnkind, 1981; 1983) and during the secondary vitellogenesis the decapod ovary usually increases in weight by 300 % or more depending on the species (Adiyodi, 1985; Quakenbush, 1986).

2.2.2 Inhibitory Factors of Gonad Maturation

2.2.2.1 Gonad inhibiting hormone (GIH)

In decapods, it is known that the removal of eyestalk induces ovarian activity (Adiyodi and Adiyodi, 1970). The existence of gonad inhibiting principle in the eyestalk of decapod crustacean was first demonstrated in

Palaemon serratus by Panouse (1943). When the eye stalk is removed, precocious gonadal development ensues. This was later confirmed in many decapod crustaceans, in cray fish *Cambarus* (Stephens, 1952); in *Uca pugilator* (Brown and Jones, 1949); in *Lysmata seticaudata*, (Carlisle, 1953) and in *Carcinus maenas* (De'meusy and Veillet, 1952). Panouse (1944; 1946) further found that the removal of the sinus gland alone leads to some increase in size of ovary, but not nearly so great an increase as after eyestalk ablation. In intact animal the normal increase in ovarian size which precedes the breeding season may be inhibited by injection of extracts of whole eyestalk or sinus gland or medulla terminalis ganglionic X organ (MTGX) (Carlisle, 1953). Knowles and Carlisle (1956) took these results as evidence for existence of an ovary inhibiting hormone (OIH). Eyestalk ablation according to them removes an inhibition, which is preventing ovarian growth, thus leading to rapid uninhibited proliferation of the ovarian tissue, which may increase several folds in a month. Conversely, injection of eyestalk extract supplies the inhibitor which keeps the ovary in check.

Eyestalk ablation in freshwater prawns have not given as much dramatic acceleration of reproduction as seen in penaeids. Kumari and Pandian (1987) reported that unilateral eyestalk ablation in juvenile *Macrobrachium nobilii* advanced the onset of sexual maturity. Bijulal (1994) observed that in female *M. equidens* destalking does not stimulate growth but there is better response for reproduction, where as in males there is better response to growth. Karplus and Hulata (1995) found the differential effect of eyestalk ablation on laggards and jumpers of *M. rosenbergii*. A marked enhancement of growth rate in laggards via shortening of moult cycle interval and increasing the size increment per moult is in contrast with a lack of an effect on growth rate in male jumpers. Soundarapandian *et al.* (1995) successfully carried out induced maturation through eyestalk ablation and cross breeding of *M. malcolmsonii* and *M. rosenbergii*. Sherine (1998) conducted an experiment on *M. idella* and

found that destalking brings about a positive change in the development of androgenic gland and sex characters. Okumura and Aida (2001) reported that in bilaterally destalked males and females, ecdysteroid level increased rapidly, and moult intervals were significantly shortened in comparison with control non-destalked prawns of *M. rosenbergii.* These observations suggest the usefulness of eyestalk ablation in rematuration of spent female of *M. rosenbergii.*

Though Carlisle (1954) proposed sex specificity of GIH, Adiyodi and Adiyodi (1970), quoting Otsu (1963), Payen *et al.* (1967) Juchault and Legrand (1967) argued that OIH and testis inhibiting hormone (TIH) are not different from one another and termed the inhibitory hormone in both sexes as GIH.

Quackenbush (1991) agrees to this argument and Chang (1992) finds little reason to suspect that OIH and vitellogenesis inhibiting hormone (VIH) mentioned by various workers are different from that of GIH. GIH appears to be present not only in adults, but also in immature stages as well. In *Potamon dehaani*, eyestalks of immature crabs of both sexes have been stated to contain the hormone (Otsu, 1963). When vitellogenesis is already in full swing, eyestalk ablation does not perceptibly accelerate ovarian growth, suggesting that during this period the synthesis of GIH and/or its release from eyestalk into general circulation may be very low (Adiyodi and Adiyodi, 1970).

Many workers suggest that GIH is produced by the XO-SG complex in alternation with MIH (Laufer and Landau, 1991; Yano, 1992 a). In adult female of several species of decapods eyestalk ablation results not in moulting, as in juveniles and some adults, but in premature yolk deposition in the ovary, both during the non-breeding season and breeding season in certain species like *Paratelphusa hydrodromous* (Gomez, 1965) and *Scylla serrata* (Rangneker and Deshmukh, 1968) even in prepubertal stages. Adiyodi (1980) suspects that MIH and GIH represent a single hormone say

growth restraining hormone (GRH) that exercises its influence in two target processes namely, growth and reproduction. Crustacean eyestalk contains a hormone that inhibits moult and reproduction, but the course of events initiated by eyestalk ablation varies with species, age of individual, season and other factors (Adiyodi, 1985; Quackenbush, 1986; Fingerman, 1987). The production of GIH has been shown to be seasonal and is responsible for the period of ovarian rest during the non-breeding season (Adiyodi and Adiyodi, 1970; Bomirski and Kelk, 1974; Kelk-Kawinska and Bomirski, 1975).

Van Deijuen (cited by Subramonium and Keller, 1993) demonstrated the inhibition of oocyte growth in the shrimp, *Atytephra desmaresti*, by administration of sinus gland extract from lobster, *Homarus americanus*. Quackenbush and Keeley (1987) showed that partially purified eyestalk extract from the shrimp *Penaeus vannamei* could inhibit ovarian synthesis of vitellogenin of the crab, *U. pugilator in vitro*, while Eastman-Reks and Fingerman (1984) found inhibition of vitellogenin production in cultured ovaries of this crab. Interestingly, almost all the GIH bioassays thus far found have been heterologous, implying lack of species specificity of this peptide (Subramonium and Keller, 1993).

GIH is thought to exert its effect directly on the ovary and hepatopancreas *in vivo* since eyestalk extract inhibits protein synthesis by cultured ovaries (Paulus and Laufer, 1987; Quackenbush, 1989; Yano, 1992 a). The fact that cyclic adenosine monophosphate (cAMP) can mimic this inhibition suggests its function as an intermediate (Eastman-Reks and Fingerman, 1984). The putative target tissue of the GIH probably responds to eyestalk ablation by rapid increase in biosynthetic activity of yolk proteins (Quackenbush, 1989).

Primary action of GIH in females apparently occurs during the secondary vitellogenesis, the time when ovary increases dramatically in size due to synthesis and uptake of yolk proteins produced in either follicle cells

or extra ovarian sites (Quackenbush, 1991; Chang, 1992). But in *P. hydrodromous* there is evidence to show that GIH principally inhibits the primary vitellogenesis in these crabs, however, its effects on secondary vitellogenesis is far from impressive (Kurup and Adiyodi, 1980). Alternatively GIH may have non-ovarian target or in fact there may be more than one eyestalk factor which inhibit ovarian growth (Laufer *et al.*, 1992).

It was found that water soluble, heat stable eyestalk factor(s) inhibit mandibular organ synthesis of methyl farnesoate and because of a role that mandibular organ seems to play in reproduction, the eyestalk factor(s) may be considered as GIH. It may affect hepatopancreas, the ovary as well as the mandibular organ. It is termed Mandibular organ-inhibiting hormone (MO-IH) (Laufer *et al.*, 1986; 1987 a, b; Laufer and Landau, 1991) and preliminary reports suggest that eyestalk factor(s) may work through a cyclic guanosine monophosphate (cGMP) intermediate (Tsukimura *et al.*, 1986). Tsukimura *et al.* (1989) also found an eyestalk factor inhibiting methyl farnesoate synthesis in *H. americanus* and *Orconectes virilis*.

Kallen and Meusy (1989) have advanced the theory that GIH is similar in structure and not different from crustacean hyperglycemic hormone (CHH). There are indications that moult inhibition and hyperglycemic activity are associated with the same peptide as demonstrated in the lobster *H. americanus* (see Chang *et al.*, 1990; Soyez *et al.*, 1991) and in the shore crab, *C. maenas* (see Webster and Keller, 1986).

2.2.2.2 Other inhibitory factors

The androgenic gland which is responsible for the masculinization of the animal seems to produce a number of compounds including farnesylacetone, a molecule similar in structure to methyl farnesoate (Ferzou *et al.*, 1978) and this will inhibit ovarian lipovitellin synthesis *in vitro* (Berreur- Bonnenfant and Lawrence, 1984).

It is well known that biogenic amines release peptide neurohormones from neuroendocrine structures in several crustaceans (Fingerman, 1985).

Certain biogenic amines (Octopamine and Serotonin) inhibited methyl farnesoate synthesis in *Libinia emarginata* (Homola *et al.*, 1989). Serotonin has been found to induce the release of GIH from isolated eyestalk of crab (Mattson and Spaziani, 1985). These biogenic amines may stimulate release of GIH from XO-SG complex in crustaceans (Yano, 1992a). Landau *et al.* (1989) found that pigment dispersing hormone (PDH) significantly inhibits mandibular organ synthesis of methyl farnesoate in *Procambarus clarkii.* Quackenbush and Herrnkind (1983) reported that partially purified GIH could not be separated from PDH. Thus in some cases the functions of pigment dispersal and gonad inhibition may be performed by the same or similar molecules (Laufer *et al.*, 1987 b).

2.2.3 Stimulatory Factors for Control of Reproduction

2.2.3.1 Gonad stimulating hormone (GSH)

A second decapod reproductive neurohormone is found in the brain and thoracic ganglion (TG) which acts to stimulate ovarian development in shrimps, crabs and lobsters. The concept of 'bihormonal system' was first proposed by Otsu (1960; 1963). Otsu (1963) after working with *Potamon dehaani* and Yano and Wyban (1992) with *P. vannamei* suggested its existence because eyestalk ablation caused precocious ovarian growth in adult, but not in juvenile. This led them to reason that not only was the absence of GIH required for ovarian growth, but the presence of stimulatory hormone was also necessary. Otsu (1963) also observed that implantation of adult thoracic ganglion was effective in triggering maturation of ovary in eyestalk ablated juveniles. The experiments of Hinsch and Bennett (1979) using *Libinia emarginata*, Gomez (1965) using *P. hydrodromous* with both brain and thoracic ganglion and Takayanagi *et al.* (1986a) using the shrimp *P. compressa* also proved that GSH from thoracic ganglion has got a role to play in ovarian maturation. Extract of thoracic ganglion of reproductive *U. pugilator* stimulates ovarian growth in adult crabs (both intact and ablated) while thoracic ganglion extract from non reproductive crabs has no effect on

normal crabs and actually inhibited ovarian growth in ablated crabs (Eastman-Reks and Fingerman, 1984). Nagabhushanam *et al.* (1988) found that GSH from brain was more effective than that from the thoracic ganglion in stimulating ovarian growth in *M. kistnensis*. Yano and Wyban (1992) propose a GSH – releasing hormone (GSH-RH) from brain of the lobster *H. americanus* which stimulated ovarian maturation in *P. vannamei*. Yano (1992b) speculates that in immature females the ovarian stimulating principle is absent or not yet functioning. Yano (1992b) found that thoracic ganglion extract prepared from maturing females is effective in increasing serum vitellogenin in *Penaeus japonicus* and suggested that GSH also stimulates vitellogenin synthesis and/or secretion into the blood in penaeid shrimps. Yano further noted that brain extract prepared from maturing females induced vitellogenin secretion in *P. japonicus* suggesting a brain hormone which stimulates release of GSH in penaeid shrimps.

Implantation of brain and thoracic ganglion into the male *P. hydrodromus* results in precocious maturation of testes and even hypertrophy of the vas deferens (Gomez, 1965). This observation together with the finding of Otsu (1963) suggests that the thoracic ganglion effectively accelerate ovarian development in young female of *P. dehaani* and the experiment of Yano *et al.* (1988) where the thoracic ganglion implantation of mature female *H. americanus* into *P. vannamei* induced ovarian growth, suggests that GSH which is effective in both species and different genera is present in thoracic ganglion and perhaps also in the brain of crabs and shrimps. Yano (1993) reported that GSH from the brains of maturing female shrimp, *P. japonicus* is a peptide that has molecular weight of 1000-2000 Da.

The role of GSH appears to be dual in that it promotes oocyte growth and prevents Y-organ (YO) activity; the latter is accomplished either directly or indirectly by raising the level of MIH and/or lowering the level of GIH (Adiyodi and Adiyodi, 1970).

2.2.3.2 Juvenile hormone (JH)

The role of terpenoid hormones unique to arthropods and collectively known as JHs or Juveniods has been established in insect reproduction (Raabe, 1982; Laufer *et al.*, 1992). Downer and Laufer (1983) proposed that the JHs appear not only in the development of insect larval stages, but also in the regulation of reproduction. In recent years, attention has been focused on another gland, the mandibular organ as a source of gonad stimulating factor in decapod crustaceans (Subramoniam and Keller, 1993). Since both arthropod sub-phyla, the Insecta and Crustacea, are already known to regulate moulting with identical hormone, 20-hydroxy ecdysone (Karlson, 1956; Hampshire and Horn, 1966; Laufer *et al.*, 1987b), it is speculated that the crustaceans might also have a functional JH for development and reproduction (Laufer *et al.*, 1992; Chang *et al.*, 1992). There are reports of insect JH or related compounds having biological activity in Crustacea and of crustacean tissue having JH activity in insects. Schneiderman and Gilbert (1958) detected some JH activity in the eyestalk of the Crustacea. Laufer *et al.*, (1987a) identified a sequiterpenoid compound methyl farnesoate in the mandibular organ as well as in the heamolymph of the spider crab. The methyl farnesoate, the immediate precursor of the insect JH III, has been shown to be present in several decapod crustacean species (Laufer *et al.*, 1986). In addition, the mandibular organ of decapod crustacea is structurally similar to the corpora allata of insects (Chaudenneret, 1956; Le Roux, 1968; Byard *et al.*, 1975). After critically reviewing the literature in this field Subramoniam and Keller (1993) proposed methyl farnesoate as the crustacean juvenoid probably involved in the stimulation of vitellogenesis and farnesoic acid as a pre-hormone which could undergo conversion to methyl farnesoate or even JH III in the target tissues.

Landau *et al.* (1989) noticed that red pigment concentrating hormone (RPCH) significantly stimulates the rate of inhibited synthesis of methyl

farnesoate by the mandibular organ of the crayfish, *P. clarkii*. Experiments of Laufer and Landau (1991) also indicated that RPCH has mandibular organ stimulating activity in *P. clakii* and *L. emarginata.*

2.2.3.3 Steroid hormones

Steroid hormones have been localized by several methods in many crustacean tissues (Skinner, 1985; Fingerman, 1987). Steroid hormones other than the ecdysone have been found in crustacean eggs, ovarian tissue and the mandibular organ (Adiyodi, 1985; Couch and Hagino, 1983). The location of these steroid hormones, progesterone and estradiol suggests that they may have a role in regulation of reproduction in crustaceans (Fairs *et al.*, 1989; 1990; Quackenbush, 1991; Quinitio *et al.*, 1991; Young *et al.*, 1992).

2.2.3.3.1 Ecdysteroids (ECDs)

The MH, ecdysone is known to play a role in insect reproduction and therefore may act in a similar fashion in crustaceans (Laufer and Landau, 1991; Laufer *et al.*, 1992).

Crustaceans resemble insects in that MH secreted by the Y-organ is not required for the maintenance of the gonad once puberty is attained (Adiyodi, 1969). There is now a growing body of evidence to suggest that in insects and crustaceans, ECDs which are primarily MH (Adiyodi and Adiyodi, 1970) are also there in adult life to stimulate the ovarian growth (Adiyodi, 1980). Prepubertal growth and development of gonads appear to be part of the normal genetically determined growth process and ECDs may have a role in post pubertal development (Adiyodi, 1985). Lachaise and Hoffman (1977) were successful in detecting several ECDs, especially ecdysone of ovaries of crab, *C. maenas* whose titre in ovary registered a gradual increase with the progress of vitellogenesis, with peak levels detected on termination of the process. ECDs may be synthesized or get accumulated in the gonads (Young *et al.*, 1993). Experiments have shown that Y-organ removal can result into in either stimulatory or inhibitory

effect on vitellogenesis depending on species, age and stage in the moult stage and reproductive cycle (Chang, 1992). Thus though ECDs are primarily moulting hormones, they may also have secondary effect on reproduction (Young *et al.*, 1993).

2.2.3.3.2 Other Steroids

Evidence is accumulating nonetheless, from scattered works suggesting that the crustacean ovary might play a role in the biosynthesis of steroid hormone(s). Lisk (1961) confirmed the estrogenic compound reported by Donahue (1957) in *H. americanus* to be 17-β estradiol. Subsequently, Teshima and Kanazawa (1971) have found that the ovaries of *Portunus triterberculatus* possess the enzymes involved in the conversion of progesterone to 17α-hydroxyprogesterone, testosterone and deoxycorticosterone. Kanazawa and Teshima (1971) detected progesterone and testosterone in the ovaries of *Panulirus japonicus*. Estrogen was detected in the ovaries of the shrimp *Parapenaeus fissurus* (Jeng *et al.*, 1987), *Nephros norvegicus* and *P. monodon* (Fairs *et al.*, 1989; 1990) and *Pandalus kessleri* (Quinitio *et al.*, 1991). A number of steroids including testosterone, progesterone and pregnenolone have been identified in the gonads and serum of the crayfish *Astacus leptodactylus*, the lobster *H. americanus* (Burns *et al.*, 1984; Ollivier *et al.*, 1986), the shrimps, *P. monodon* and *P. kessleri* (Fairs *et al.*, 1989; Young *et al.*, 1992; Quinitio *et al.*, 1991) and the crab *C. maenas* (Hazel, 1986). Couch *et al.* (1987) detected significant levels of estradiol and progesterone in the mandibular organ of *H. americanus*.

2.2.3.4 Other factors

Tensen *et al.* (1989) found a stimulatory effect on oocyte growth of the shrimp, *Palaemonetes varians* by a peptide of *H. americanus* which is similar to that of Crustacean Hyperglycaemic Hormone (CHH). With regard to the possible existence of such an ovary stimulating hormone in sinus gland, it will be interesting to know whether this peptide works

antagonistically to GIH or synergistically with the putative GSH (Subramoniam and Keller, 1993).

From the investigations of Richardson *et al.* (1991) on the effect of 5-hydroxy tryptamine on ovary development in the fiddler crab, *U. pugilator*, it is speculated that this biogenic amine might release the GSH from brain/ thoracic ganglion (Subramoniam and Keller, 1993).

Octopamine and serotonin not only affect the mating behaviour of lobster, *H. americanus* (Beltz, 1988), an external manifestation of reproductive activity, but also affect MH production which may be an internal manifestation of gonad maturation. According to Laufer and Landau (1991), the same may play a role in the shrimp reproduction.

The interesting possibility of the involvement of prostaglandins in the penaeid shrimp reproduction has been suggested by Middleditch *et al.*, (1979) and supported by D' Croz *et al.* (1988).

2.3 SEX PHEROMONES IN CRUSTACEA

2.3.1 Releaser Pheromones

Pheromones are substances, released by an organism, that influence a definite behaviour or developmental process in other organisms of the same species (Karlson and Luscher, 1959). The existing literature on crustacean sex pheromones has accepted three different behavioural assays as admissible evidence for a sex pheromone. These are best described as: (a) chemokinetic reactions (b) chemotaxic reactions; and (c) releaser reactions. Chemokinetic reactions are defined as changes in the speed of movement (orthokinesis) and/or rate of turning (klinokinesis) produced by chemical stimulation. Chemotaxic reactions are defined as movements oriented directly towards or away from the source of stimulation; and finally, releaser reactions are defined as stereotyped patterns of motor movement (e.g. courtship behaviour) elicited by a chemical stimulus alone or in combination with other stimuli (Dunham, 1978). Among decapods, studies

on sex pheromones have been extensively done on *H. americanus*, *P. homarus*, *Jasus lalandii* and *P. clarkii*. Two general assumptions emerged from these. Firstly, mating is a moult-related phenomenon. Secondly, the post-moult female releases a pheromone to attract males and elicit the appropriate courtship and mating behaviour (see Dunham, 1978).

Kittredge and Takahashi (1972) suggested that crustecdysone, the crustacean moulting hormone also functions as a sex pheromone which attracts a mate and elicits the appropriate patterns of sexual behaviour. They proposed an evolutionary model according to which the primordial Crustacea evolved the capacity to release the moulting hormone into the external environment and externalized the receptor site of the target organ at approximately the same time. The simultaneous development of these two capacities permitted pheromone communication, and because of reproductive advantage, these two events were eventually fixed in the genome. They further suggested that the development of the crustecdysone as a sex pheromone in Crustacea serves as an evolutionary model for the development of all arthropod pheromones. They based this model on evidences from experiments on brachyuran crabs (Kittredge *et al.*, 1971) where mating typically follows female moult. This hypothesis could not be proved by experiments on lobster, *H. americanus* (Atema and Gagosian, 1973).

Regarding the source of sex pheromone, in crabs, urine produced by antennal gland has been found to contain the active substances (Christofferson, 1970; Eales, 1974). In freshwater prawns like *Palaemon paucidens* (Kamiguchi, 1972) and *M. kistnensis* (Sarojini *et al.*, 1982), sternal gland has been found to secrete the sex attractant along with the parturial moult. Antennular chemoreceptors receive these stimuli.

Regarding the chemical nature, Christofferson's work revealed that the active component in the urine of *Portunus sanguinolentus* was a heat stable substance extractable with organic solvents like ether and alcohol (see

Dunham, 1978). Chemical nature of releaser pheromones have been reported by various workers. In *P. hydrodromus,* Sundara Rajulu *et al.* (1973) reported it to have the nature of 5- hydroxy tryptamine and in *P. clarkii,* Ameyaw-Akumfi and Hazlett (1975) recorded it to be a carbohydrate. Sarojini *et al.* (1982) reported the sternal gland pheromone in *M. kistnensis* to have histochemical properties of a glycoprotein. The situation here has some similarity to that in fishes. The spawning pheromone in the herring *Clupea harengus pallasi* is present in the milt and mature male testes extracts and chemically have properties similar to that of polar steroids, prostaglandins or their conjugated forms (sulphates/glucuronide) (Sherwood *et al.*, 1991). In the African catfish *Clarius gariepinus* seminal vesicle fluid contains the male sex pheromone and steroid glucuronides have been detected. 17 α – hydroxy progesterone glucuronide has been found to be the most potent odourant (Lambert and Resink, 1991). Ismael and New (2000) reviewed the work on chemical cues affecting sexual attraction in *M. rosenbergii.* Small males are attracted only to females with ripe ovaries. Females with ripe ovaries are attracted only to blue claw males. Fighting behaviour of some blue claw males are observed only in the presence of females with ripe ovaries while others fight always. They suggested further studies in this line.

2.3.2 Primer Pheromones

Takayanagi *et al.*, (1986 b), reported that in the female shrimp, *Paratya compressa,* reared in the absence of males, ovarian development was usually delayed, whereas it occurred normally in the females reared with males. Ovarian development also occurred in the shrimps reared in the water that contained an extract of testis or vas deferens, which suggested that male shrimps of this species secreted an ovary stimulating pheromone which accelerated ovarian development. This was the first report of a primer sex pheromone in Crustacea. Similar results were also obtained by

Nagabhushanam *et al.* (1989) in *M. kistnensis*. This pheromone is probably synthesized by testis and transported to vas deferens. Boiling did not affect its activity in *P. compressa*, but not so in *M. kistnensis*. In these two species, this is essential for normal ovarian maturation (for initiation of exogenous vitellogenesis). Jayachandran and Jose (1993) reported that in females of *M. idella* reared in isolation, ovaries failed to develop beyond maturation stage IV (exogenous vitellogenesis). The females also suffered from severe hypophagia. Unilateral eyestalk ablation did not help to overcome the ill effects of male deprivation. These results pointed towards a similar male pheromone in *M. idella*, which was essential for normal ovarian maturation and survival during the post breeding season. Further Patil (2001) showed that the male pheromonal activity in *M. idella* was present in testis and vas deferens and indicated that it could be of steroidal nature.

Ovarian maturation in crustaceans reported to be under dual control mechanism (Adiyodi and Subramoniam, 1983). Accordingly, a gonad inhibiting hormone (GIH) is secreted by the X-organ-sinus gland complex and a gonad stimulating hormone (GSH) is secreted by brain/thoracic gland. The ovarian maturation is under the control of these two antagonistic factors and the relative importance of these two mechanisms varies from species to species (Subramoniam *et al.*, 1999). The GIH is now rechristened as Vitellogenesis Inhibiting Hormone (VIH) and in *Homarus americanus* its complete amino acid sequence has been worked out (Subramoniam *et al.*, 1999). The characterization of GSH is not yet materialised. The male pheromone may be affecting this neuroendocrine control mechanism. Brain and thoracic ganglion extracts (containing GSH) were able to induce vitellogenesis in isolated females of *P. compressa* and *M. kistnensis*, showing that the male pheromone is operating through a neurohormone (Takayanagi *et al.*, 1986 b; Nagabhushanam *et al.*, 1988).

Materials and Methods

3. MATERIALS AND METHODS

The experiments were conducted during January to October 2005.

3.1 EXPERIMENTAL LABORATORY

Two sets of experiments were conducted. The first experiment was conducted in the wet laboratory of the Department of Aquaculture and the second was conducted in the laboratory of the Department of Fishery Biology, College of Fisheries in natural day light condition.

3.2 EXPERIMENTAL ANIMALS

Adult specimens of *M. rosenbergii* (both male and female) were collected from wild from Cochin backwaters at Murinjapuzha, 20 Km south east of Kochi. All animals were acclimated in freshwater in circular big fibreglass tanks for at least one week before experiment. The size range (total length) of the animals for the first experiment was 215 mm to 260 mm for females and 230 mm to 270 mm for males and for the second experiment it was 155 mm to 230 mm for females and 160 mm to 240 mm for males. The females used in the experiment were berried at least once and their ovaries in most cases were in the stage II of maturity. The males were blue clawed.

3.3 FEED

The animals were fed with fresh clam meat.

3.4 FEEDING

Feed was given *ad libitum*. Feeding was done in the evening. Left over feed was siphoned out in the morning.

3.5 WATER CIRCULATION AND QUALITY MAINTENANCE

Continuous aeration was provided to ensure sufficient dissolved oxygen. About 30% of the water in the tanks was exchanged daily by siphoning and refilling. The exuviae, feed remnants etc., were removed

during the water exchange. In the second experiment water circulation between second and third compartments was maintained at flow rate of about 3 litres/min.

3.6 DETERMINATION OF WATER QUALITY PARAMETERS

The water quality parameters like temperature and pH were checked periodically using Mercury thermometer of 0 to 100° C and Universal indicator (Qualigens) respectively.

3.7 EXPERIMENTAL PROCEDURES

3.7.1 Experimental Observations

3.7.1.1 Experiment I- Effect of isolation from males on ovarian maturation

Experiment was conducted from January to March 2005. Ovarian maturation was monitored in two groups of females which were maintained in fibreglass tanks of capacity 500 litres (Plate 1A). To start with, first group of animals consisted of 12 females only (isolated group) and second group of animals consisted of 12 females cohabiting with 3 blue claw males in the ratio 4:1. The duration of the experiment was 60 days. 5 females died during the experiment.

Ovarian maturation of each animal was monitored daily and the occurrence and incubation of berries was noted. The results were analysed statistically using Fisher's Exact Test and Student's t test.

3.7.1.2 Experiment II- Role of visual and/or chemical stimuli from male on ovarian maturation

Experiment was conducted from August to October 2005. Ovarian growth was monitored in 3 groups of females which were maintained in 3 adjacent glass chambers of 6 glass aquaria (150cm × 34cm × 30cm) with continuous aeration (see Fig 1 and Plate 1B). To start with, there were 12

females in each group and 6 blue claw males. Free interaction between individuals was not allowed to avoid injury and mortality of moulted individuals in the confined conditions. The first water tight compartment contained only females but these had visual contact with male in the next compartment. The second compartment consisted of females cohabiting with male in the ratio 2:1. Females were exposed to visual stimuli and chemical cues from male and also had limited tactile contact with male through a 1 cm gap below the glass partition. The third group in the third compartment consisted of only females with no visual contact with the male but had common water circulation with second compartment and could be thus exposed to any chemical cue (putative male pheromone) from male (see Fig. 1 and Plate 1B). The females of the third compartment were prevented from visual contact with male by dark glass between second and third compartment. The cleft under the dark glass was filled by the heap of gravels. The duration of the experiment was 60 days. 14 females and 2 males died during the experiment.

Ovarian maturation of each animal was monitored daily and the occurence and incubation of berries was noted. The results were analysed statistically using Fisher's Exact Test and Student's t test.

Ovarian maturity stage of each specimen was monitored daily and berried condition and incubation period noted. Regular and ovarian moults were also noted.

3.7.1.3 Calculations and statistical analysis

Fisher's Exact Test was used to compare the number of berried and unberried females between group I and group II in the first experiment and between group I and II, I and III and II and III in the second experiment. Similarly, the number of once berried and twice berried females was also compared. Student's t test was used to compare the average spawning frequency (number of berries per female obtained by dividing the number of

berries in a group by number of females in the group) between the different groups.

3.7.2 Maturity Stages

Ovarian development was classified into five maturity stages based on the colour and the size of ovary observed through the carapace (Chang and Shih, 1995).

3.7.3 Histological Studies

Histological study was carried out on ovaries of *M. rosenbergii* in the different maturity stages during rematuration. Ovaries of females just after a regular moult and a premating moult and an ovary from a mature but unspawned female were also processed for histological study. The ovaries were dissected out and fixed in alcoholic Bouin's fixative. Dehydration was carried out in tertiary butyl alcohol and embedding was done in paraffin wax (congealing point, 58-60° C). Sections were cut at 6-8μm thickness using Rotary Microtome. Conventional procedure of slide preparation was used and staining was done with Harris' haematoxylin and eosin (Weesner, 1960). Mounting was done with DPX mountant.

Composition of alcoholic Bouin's fluid:

Picric acid crystals: 0.5 g

Formaldehyde 40%: 30.0 ml

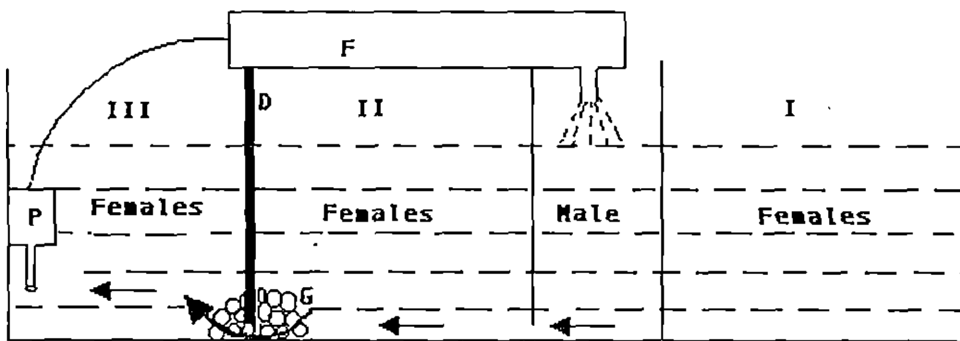
Ethyl alcohol 80%: 75.0 ml

Acetic acid glacial: 7.5 ml

3.7.3.1 Measurement of oocyte diameter in histological sections

Diameter of 30 oocytes in each stage of the rematuring ovaries was measured using a calibrated eyepiece micrometer (Erma, Japan) by standard method (Sumner and Sumner, 1969).

Fig. 1 The arrangement of the glass compartments in the aquarium.



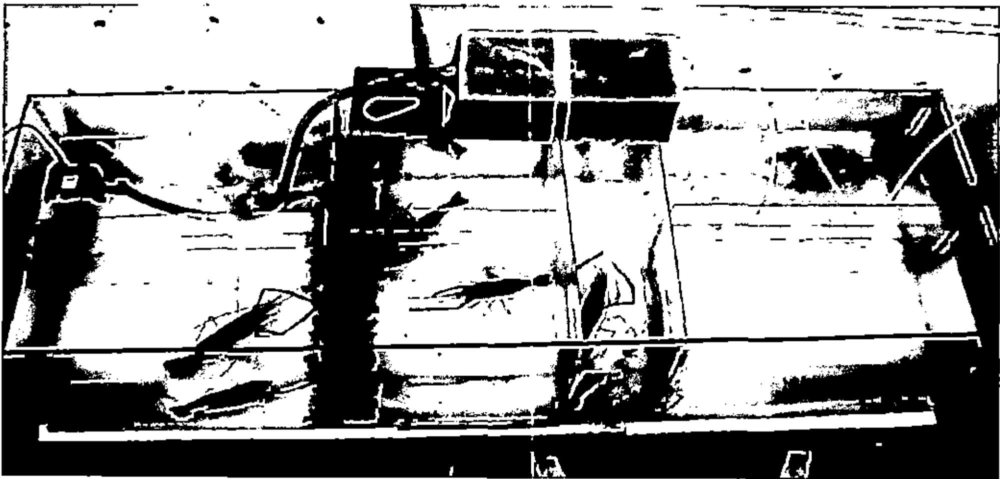
D- Dark Glass

F- Filter

P- Pump

G- Gravels

(A)



(B)

Plate 1. Experimental set up
(A) First experiment - fiberglass tanks
(B) Second experiment - compartments of the glass aquarium

Results

4. RESULTS

4.1 EXPERIMENTAL OBSERVATIONS

4.1.1 Experiment I– Effect of Isolation from Males on Ovarian Maturation

Ovarian maturation and ovulation were monitored in two groups of females; the first group consisted of only females which were kept isolated from males and the second group had females cohabiting with blue claw males.

4.1.1.1 Ovarian maturation in isolated females (group I)

Observations were made for 60 days in the isolated females. Out of 9 females, 4 females became berried (44.44%) and 5 females remained unberried (55.55%). None of the berried females became berried subsequently during the experimental period. So total number of berries was 4 (Table 1).

Four berried females were carrying the unfertilized eggs (incubation period) in their pleopods for 4-6 days only.

4.1.1.2 Ovarian maturation in females cohabiting with males (group II)

Observations were taken for 60 days in females cohabiting with blue claw males. Out of 11 females, 6 became berried (54.55%) and 5 remained unberried (45.45%). One of the females became berried twice during the period of observation. Total number of berries was 7. Observations are presented in Table 1.

Berried females were carrying the fertilized eggs (incubation period) for 15-21 days.

Table 1: Details of ovarian maturation in the first experiment.

Group	Total	Berried			Unberried		B.	O.B.	T.B.	A.S.F.
		No.	%	I.P.	No.	%				
I	9	4	44.44	4-6	5	55.55	4	4	0	0.44
II	11	6	54.55	15-21	5	45.45	7	6	1	0.64

I.P.- Incubation Period (Days)

B- Number of Berries

O.B.- Once Berried

T.B.- Twice Berried

A.S.F.- Average Spawning Frequency

Table 2: The comparison of berried and unberried females between group I (without male) and group II (with male).

	Berried	Unberried	Total
Group I	4	5	9
Group II	6	5	11
Total	10	10	20

Probability (P) = 0.5, which is more than 0.05. There is no significant difference between these two at 5% level.

Table 3: The comparison of spawning frequencies in females between group I (without male) and group II (with male).

Group	Total	No. of Berries	Average Spawning Frequency
I	9	4	0.44
II	11	7	0.64

Calculated $t = 0.1401 < 2.101$ (table value) for $\alpha = 0.05$. Hence, there is no significant difference between these two groups.

4.1.1.3 Comparison of ovarian development in group I and II

Comparison of berried and unberried animals was made between isolated females (Group I) and females cohabiting with males (Group II) (Table 2).

The comparison of results was done using Fisher's Exact Test (Table 2). The calculated probability was more than 0.05, which implies that there is no significant difference between these two groups as regards the number of berried females. So isolation from males has no effect on the ovarian maturation as indicated by the number of berried females.

4.1.1.4 Comparison of average spawning frequencies between group I and II

Average spawning frequencies were calculated for group I and group II and were compared using Student's t test (Table 3) which showed no significant difference between the two groups, again showing that ovarian maturation is unaffected by isolation from males.

4.1.2 Experiment II – Role of Visual and/or Chemical Stimuli from Males on Ovarian Maturation

For studying regulatory influence of visual and/or chemical stimuli on ovarian maturation, observations were made on three groups of females (Fig. 1 and Plate 1B).

4.1.2.1 Ovarian maturation in group I (isolated females receiving only visual stimuli from male)

In this group isolated females were receiving only visual stimuli and no chemical cues from male kept in the adjacent glass chamber. All the 8 females were berried and 4 among them were berried twice. So total number of berries was 12. The average spawning frequency was 1.5.

Berried females were carrying the unfertilized eggs in their pleopods for 1-4 days. Results are shown in Table 4.

Table 4. Details of ovarian maturation in the second experiment.

Group	Total	Berried			Unberried		No. of Berries	O.B.	T.B.	A.S.F.
		No.	%	I.P.	No.	%				
I	8	8	100	1-4	0	0	12	4	4	1.5
II	6	6	100	2-3	0	0	7	5	1	1.17
III	8	8	100	1-6	0	0	9	7	1	1.13

I.P.- Incubation Period (Days)

O.B- Once Berried

T.B.- Twice Berried

A.S.F.- Average Spawning Frequency

4.1.2.2 Ovarian maturation in group II females (cohabiting with males receiving visual as well as chemical stimuli from male)

In this group of females receiving visual as well as chemical stimuli and limited physical contact from males, all the 6 females were berried and one among them was berried twice and another had mature ovary a second time, but failed to spawn. Thus total number of berries was 7. The average spawning frequency was 1.17.

Berried females were carrying the unfertilized ova in their pleopods for 2-3 days. Results are shown in Table 4.

4.1.2.3 Ovarian maturation in group III (isolated females receiving only chemical stimuli from male)

In this group of females receiving only chemical stimuli from male (through common water circulation), all the eight females were berried. One among them became berried twice. So the total number of berries was 9. The average spawning frequency was 1.13.

Berried females were carrying the unfertilized ova in their pleopods for 1-6 days. Results are shown in Table 4.

4.1.2.4 Comparison of total number of berried females and total number of unberried females between the three groups of females

The comparison of observations from three groups was done using Fisher's Exact Test.

When the comparison was made between I and II groups (Table 5), the calculated probability was more than 0.05, which implies that there is no significant difference between these two groups. So isolated females which had no chemical cues but only visual stimuli from male had ovarian maturation and ovulation in the same way as that of females in the group II which had chemical and visual stimuli from male. There is no evidence of any block in maturation due to lack of chemical cues from male.

Table 5. The comparison of berried and unberried females between group I and II.

	Berried	Unberried	Total
Group I	8	0	8
Group II	6	0	6
Total	14	0	14

Probability (P) = 1, which is more than 0.05. Hence, there is no significant difference between these two at 5% level.

Table 6: The comparison of berried and unberried females between group I and III.

	Berried	Unberried	Total
Group I	8	0	8
Group III	8	0	8
Total	16	0	16

Probability (P) = 1, which is more than 0.05. Hence, there is no significant difference between these two groups at 5% level.

Table 7: The comparison of berried and unberried females between group II and III.

	Berried	Unberried	Total
Group II	6	0	6
Group III	8	0	8
Total	14	0	14

Probability (P) = 1, which is more than 0.05. Hence, there is no significant difference between these two groups at 5% level.

Similarly, when comparison was made between I and III group (Table 6), the calculated probability was found to be more than 0.05, showing that there is no significant difference between these two groups. This indicates that in isolated females receiving either visual stimuli alone or chemical stimuli alone from male, ovarian maturation and ovulation proceeded in the same rate revealing no particular effect of chemical cues from male.

When comparison was made between II and III group (Table 7), the calculated probability was more than 0.05, which implies that there is no significant difference between these two groups. This shows that chemical stimuli alone or visual and chemical stimuli in combination had the same effect on ovarian maturation and ovulation.

4.1.2.5 Comparison of once berried and twice berried females between group I, II and III.

The comparison of once berried and twice berried females was done using Fisher's Exact Test between group I and group II (Table 8), between group I and group III (Table 9) and between group II and group III (Table 10). In all the comparisons the calculated probability was more than 0.05, which implies that there is no significant difference between these groups at 5% level. If there was suppression of second maturation in group I due to lack of chemical cues from male, it would have become evident in this analysis. The results show the absence of such an effect.

4.1.2.6 Comparison of average spawning frequencies between group I, II and III

Average spawning frequencies were calculated for group I, II and III and were compared using Student's t test (Table 11) which showed no significant difference between the three groups. As the lack of chemical cues or visual stimuli did not make any difference in average spawning frequency, it can be inferred that none of these has a singular influence on ovarian maturation.

4.1.3 Observations of Water Quality Parameters

The water quality parameters such as temperature, pH were checked periodically and they were in the following range:

Temperature: 23° C – 27° C

pH : 7.0 – 8.0

4.2 BIOLOGICAL OBSERVATIONS

4.2.1 Maturity Stages

Ovarian development is classified into five stages: stage I, stage II, stage III, stage IV and stage V (Chang and Shih, 1995). The classification is based on the colour and the size of the ovary observed through the external carapace.

Details regarding ovarian maturation and classification of different stages are given in Table 12. The ovary of stage I is translucent. It occupies the posterior region of carapace cavity. The ovary is without pigments or yolk. Ovary has no apparent ovarian tissue.

The ovary with small spots of chromatophores and yellow colour found near the posterior part of the carapace is defined as stage II.

In stage III the ovary is orange in colour and occupies the cephalothoracic cavity from the posterior part of the carapace to the area just in front of epigastric tooth.

Stage IV begins with the growth and extension of the ovary to the area of epigastric tooth. Ovary is orange coloured.

In stage V the ovary has grown to the anterior part of the carapace cavity. Colour of the ovary is dark orange when seen through carapace.

Table 8: The comparison of once berried and twice berried females between group I and II.

	Once Berried	Twice Berried	Total
Group I	4	4	8
Group II	5	1	6
Total	8	6	14

Probability (P) = 0.238, which is more than 0.05. Hence, there is no significant difference between these two groups at 5% level.

Table 9: The comparison of once berried and twice berried females between group I and III.

	Once Berried	Twice Berried	Total
Group I	4	4	8
Group III	7	1	8
Total	11	5	16

Probability (P) = 0.141, which is more than 0.05. Hence, there is no significant difference between these two groups at 5% level.

Table 10: The comparison of once berried and twice berried females between group II and III.

	Once Berried	Twice Berried	Total
Group II	5	1	6
Group III	7	1	8
Total	11	3	14

Probability (P) = 0.692, which is more than 0.05. Hence, there is no significant difference between these two groups at 5% level.

Table 11. Comparison of average spawning frequencies between females of group I, II and III.

Group	Total Females	Total Spawnings	Average Spawning Frequency
I	8	12	1.5
II	6	7	1.167
III	8	9	1.125

Between I and II group,

Calculated $t = 0.638 < 2.179$ (table value) for $\alpha = 0.05$. Hence, there is no significant difference between these two groups.

Between I and III group,

Calculated $t = 1.769 < 2.145$ (table value) for $\alpha = 0.05$. Hence, there is no significant difference between these two groups.

Between II and III group,

Calculated $t = 0.925 < 2.179$ (table value) for $\alpha = 0.05$. Hence, there is no significant difference between these two groups.

4.2.2 Histological Examination of Ovary

Histological studies of the ovaries of *M. rosenbergii* revealed the presence of oocytes in various stages of development in the rematuring ovary.

4.2.2.1 Germogen

Each lobe of the ovary contains a thin finger shaped invagination of the mesial wall tissue, the germogen or germinal zone from where new oocytes are budded off. There are many such invaginations and hence, it is presumed that the germogen is not concentrated at one point.

4.2.2.2 Growth of ova

The growth of ova is a dynamic process comprising a generative phase or proliferative phase and a vegetative phase or growth phase.

According to size, yolk deposition and contact with the follicle cells, the oocytes are divided into the following phases.

I. Oogonia

Small, rounded cells with large central nucleus and a thin rim of cytoplasm in the central zone (CZ) of the ovary. No follicle cells observed. This stage represents the newly budded off cells of the germogen and lie in close vicinity of germogen (Plate 2 A).

Table 12. Colouration and the relative size of the ovaries in different developmental stages.

Stage	Colouration	Relative Size
I	White/Creamy	Small, cannot be seen through carapace
II	Yellow	Small, fills ¼th part of cephalothorax
III	Orange	Large, fills ½ of the cephalothorax
IV	Orange	Large, fills ¾th of the cephalothorax
V	Dark Orange	Very large, fills full cephalothorax i.e. extends anteriorly to the base of the second or third last spine of the rostrum

II. Previtellogenic oocytes

The previtellogenic oocytes (PVO) are of varied shapes possibly because of pressure exerted by the surrounding cells. These primary oocytes are seen crowded around the germinal zone immediately outer to the secondary oogonia (Plate 2 A). The characteristic feature of the oocyte at this stage is the possession of the large dense active nucleus (N) (Plate 2B). At a later stage the nucleus of primary oocyte becomes enlarged and less dense, called the germinal vesicle. The germinal vesicle is round with a nucleolus (NL) and occupies major part of the oocyte with only a thin film of cytoplasm around it. Chromatin material is in the form of deeply staining granules. A large amount of basophilic cytoplasm (CYT) is acquired by previtellogenic oocyte. Yolk formation has not yet begun. The previtellogenic oocytes in the later stage become surrounded partly by follicle cells. The oocytes measure about 52.8-74.8 μm at their broadest points.

III. Early vitellogenic oocytes (Vitellogenesis I- Endogenous vitellogenesis)

This stage is characterized by the appearance of the yolk vesicles (YV) in the ooplasm (Plate 3 B). The oocytes increase enormously in size ranging from 110-132 μm in diameter at their broadest. The germinal vesicle is prominent and with a nucleolus in the centre. Characteristically the oocytes of this stage have an increased volume of the cytoplasm. In the cytoplasm appear many small oval lipid vesicles; to start with these are peripheral, arranged in one or two rows but subsequently as their number increases this arrangement is lost and the entire cytoplasm gets filled with vesicles.

From this stage onwards two groups of oocytes could be noticed – the oocytes concentrated around the germogen without lipid vesicles and the outer zone of oocytes with lipid vesicles (Plate 3 A). The oocytes seen around the germogen are small and are in the previtellogenic phase. The

oocytes of the outer zone are much developed and continue growth to enter into the next stage (late vitellogenesis) and finally attain maturity and are ovulated and extended into the brood chamber. During this time the immature oocytes remain dormant as immature stock. After ovulation of the first batch a second batch of oocytes from the immature stock enter into early vitellogenic phase. In the early vitellogenic stage small round follicular cells (FC) appeared completely around the oocytes.

IV. Late vitellogenic oocytes (Vitellogenesis II- Exogenous vitellogenesis)

This stage is characterized by the appearance of yolk platelets (YP) in between the lipid vesicles. The nucleus is still visible (Plate 4 A). The nucleolus may or may not be visible. Fully formed platelets are seen at the periphery (Plate 4 B). The oocytes at this stage attain a diameter of 184.8-242 μm.

V. Mature oocytes

The entire ooplasm becomes acidophilic. Concurrence with maturity the germinal vesicle becomes smaller and inconspicuous and finally the nuclear membrane disappears and the original location of the germinal vesicle is indicated by clumps of chromatin. This early disappearance of the nuclear membrane does not however, mark the end of the growth period of the oocyte. The oocytes attain their utmost size in this phase (Plate 5 A). No follicle cells are visible around them and they are surrounded by a vitelline membrane (VM). These oocytes have a diameter ranging from 390-480 μm.

4.2.2.3. Spent ovary

Sections of the ovary 2 hours after spawning showed two specific regions; a central region with the developing oocytes up to the previtellogenic stage as described earlier and a peripheral region of empty follicles (EF) and trabeculae. The nuclei of the follicle cells are prominent. An unovulated ovum is also seen (UO) (Plate 5 B).

4.2.2.4. Spent recovering ovary after a regular moult

In this section central zone is prominent with oocytes in different stages up to previtellogenic stage. During the whole regular moulting cycle no vitellogenesis has been observed. In the peripheral area there are empty trabeculae and follicles and cells with prominent nuclei (Plate 6 A).

4.2.2.5 Unovulated atretic ovary undergoing resorption

Section of an ovary of a female which failed to spawn even after 5 days of moulting shows signs of resorption. Peripheral ova are disintegrating with yolk (Y) undergoing absorption. An unovulated atretic ovum (UAO) can be seen. The trabeculae (TR) from the ovarian wall (OW) and scattered cells are prominent (Plate 6 B).

4.2.3 Oocyte Diameter Measurements of the Histological Sections

Diameters of 30 oocytes of each stage were measured. The results of oocyte diameter measurements are presented in Table 13.

In the previtellogenic oocytes (stage II) the diameter ranges from 52.8-74.8 µm.

In the early vitellogenic oocytes (stage III) the diameter ranges from 110-132 µm.

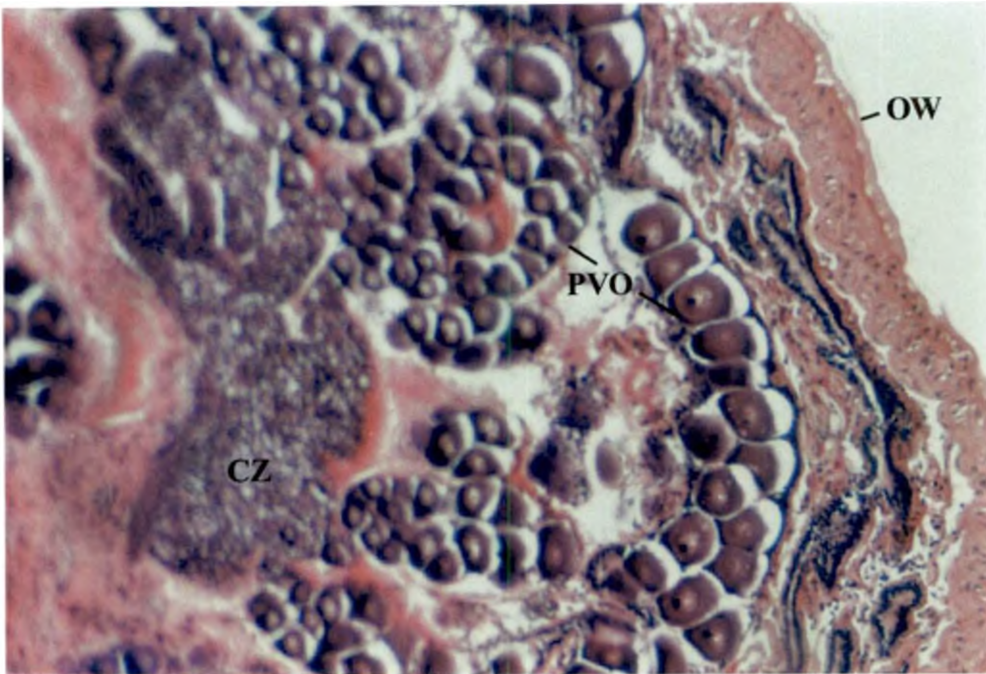
In the late vitellogenic oocytes (stage IV) the diameter ranges from 184.8-242 µm.

In the mature oocytes (stage V) diameter ranges from 390-480 µm.

The ova diameter measurements taken for different histological stages indicate that diameter was highest for mature oocytes (stage V) and lowest for previtellogenic oocytes (stage II).

Table 13: Details of oocyte diameter measurements for different maturity stages during rematuration of ovary.

Ovarian Maturity stage	Range of Oocyte diameter (μm)	Average Oocyte diameter (μm)
II	52.8 - 74.8	68.38
III	110 - 132	121.79
IV	184.8 - 242	216.33
V	390 - 480	443.5



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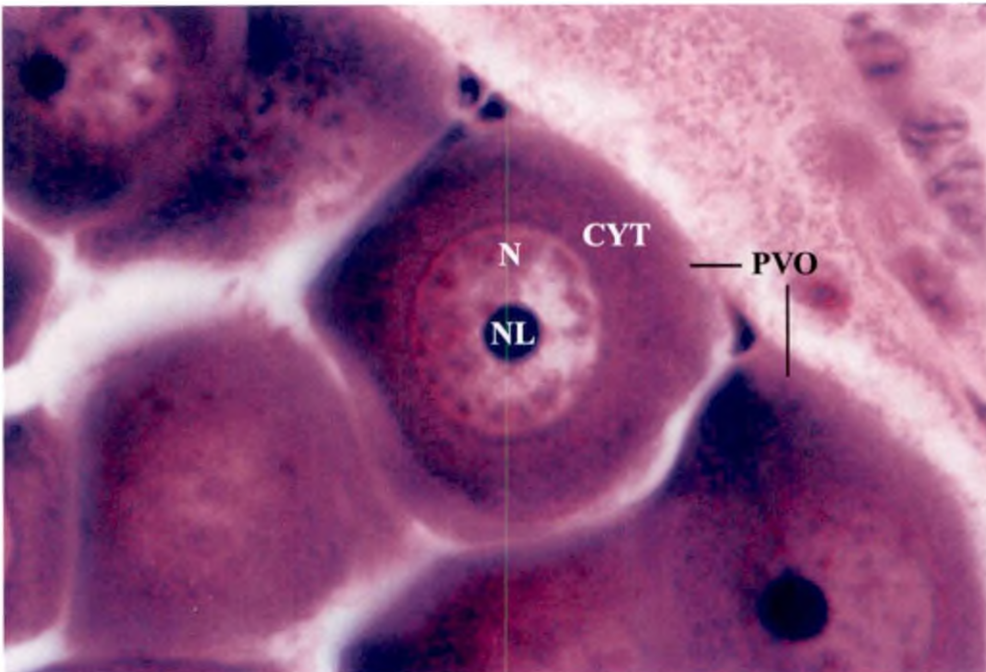
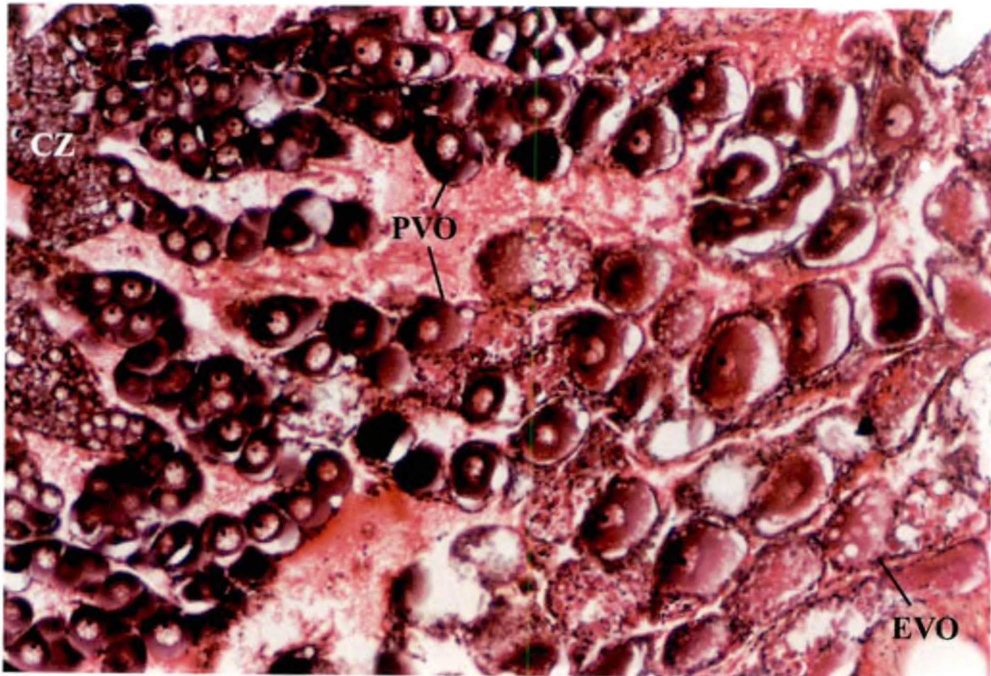


(B)

Plate 2. Photomicrographs of ovary of *M. rosenbergii* in maturity stage II,
(A) showing ovarian wall (OW), previtellogenic oocytes (PVO) and central zone (CZ) x 240
(B) showing previtellogenic oocytes (PVO), cytoplasm (CYT), nucleus (N) and nucleolus (NL) x 2400



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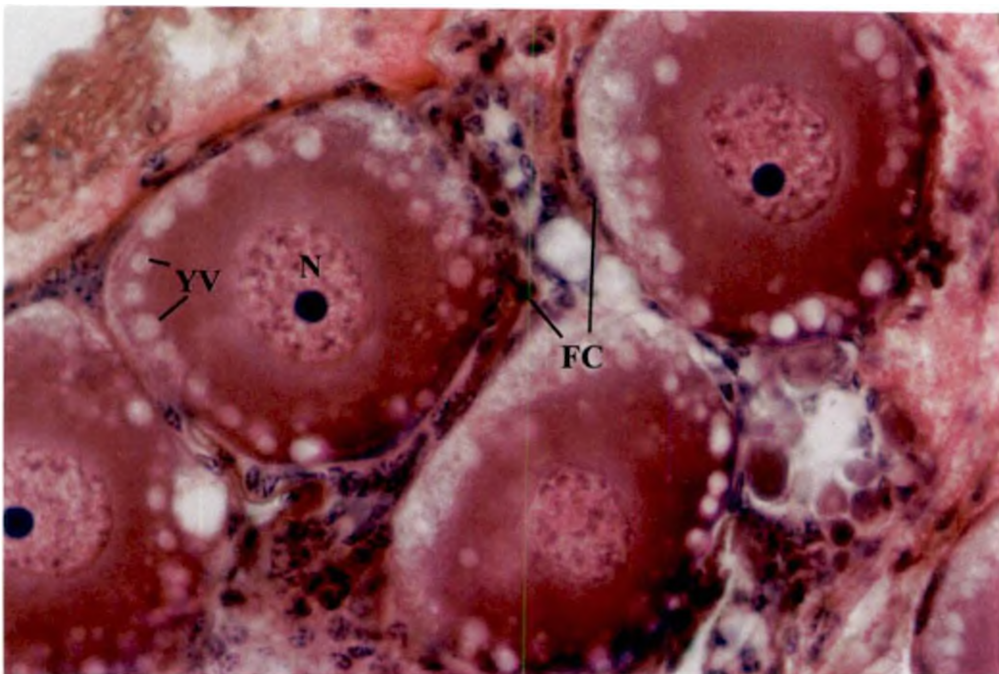
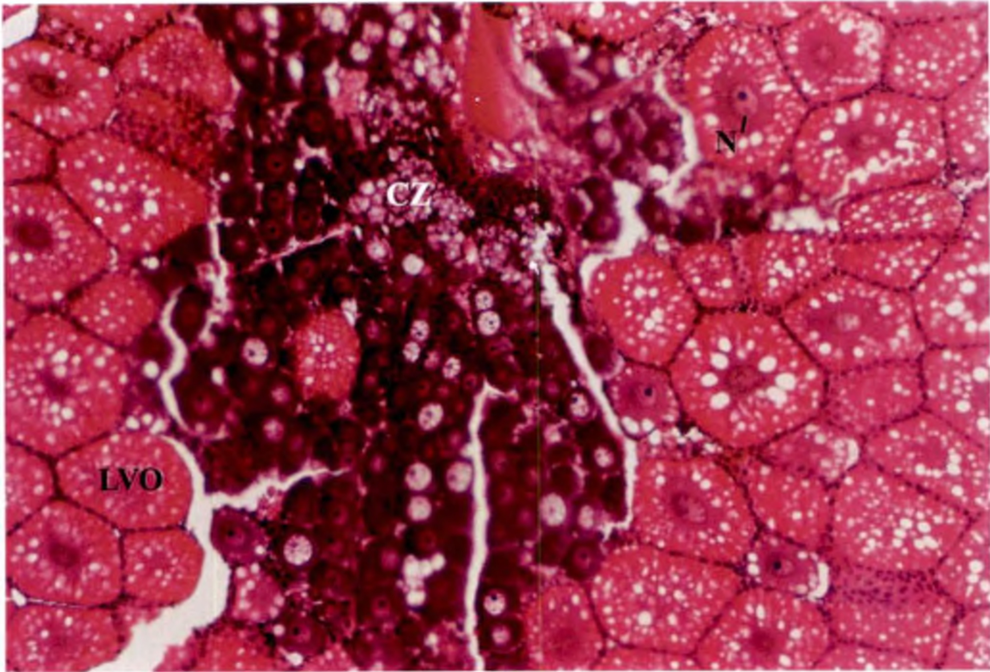


(B)

Plate 3. Photomicrographs of ovary of *M. rosenbergii* in maturity stage III,
(A) showing previtellogenic oocytes (PVO), early vitellogenic oocytes (EVO) and central zone (CZ) x 240
(B) showing yolk vesicles (YV), follicle cells (FC) and nucleus (N) x 960



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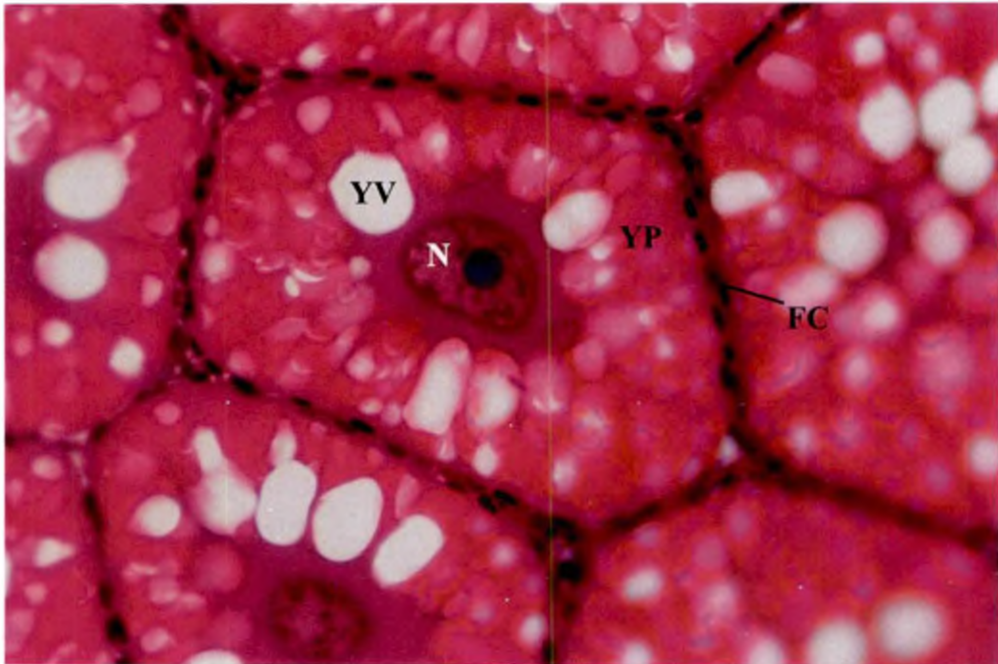

(B)

Plate 4. Photomicrographs of ovary of *M. rosenbergii* in maturity stage IV,
(A) showing late vitellogenic oocytes (LVO), nucleus (N), central zone (CZ) x 240
(B) showing yolk vesicles (YV), yolk platelets (YP), nucleus (N) and follicle cells (FC) x 960

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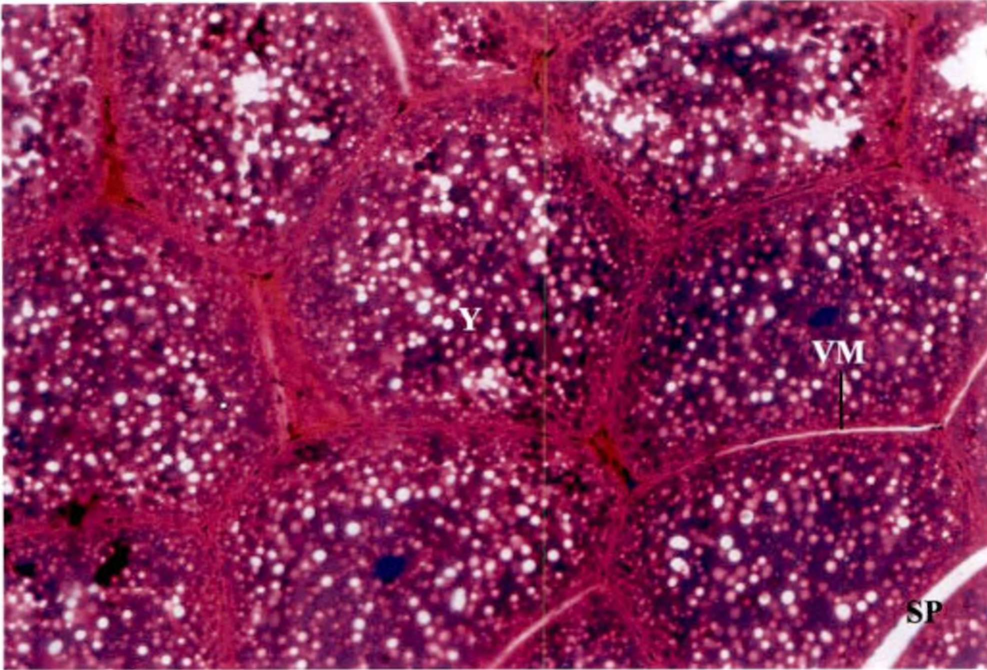

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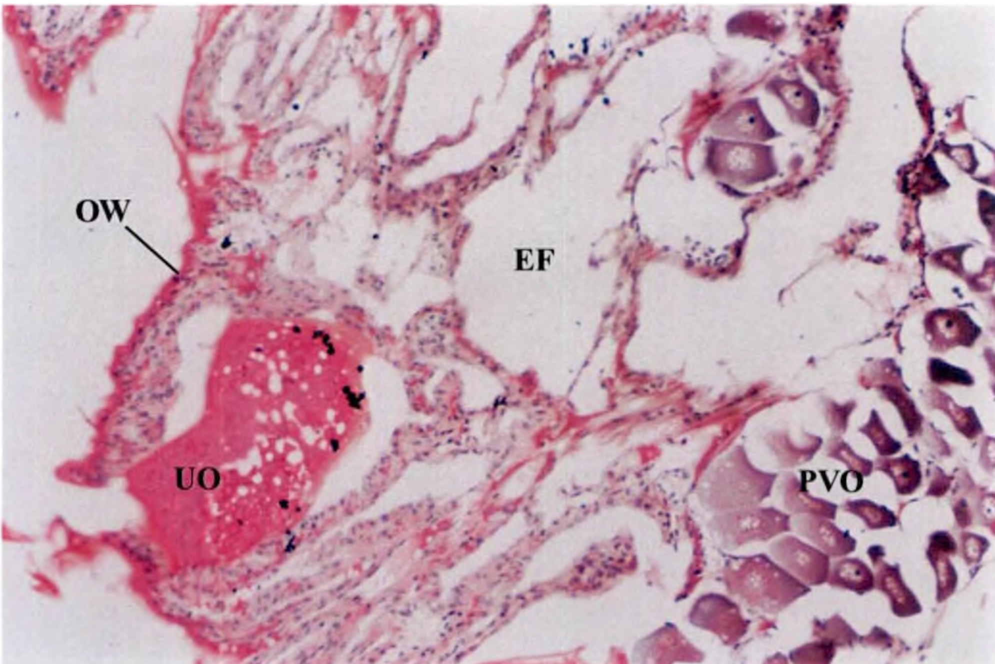

(B)

Plate 5. Photomicrographs of ovary of *M. rosenbergii,*

(A) in maturity stage V showing yolk (Y), vitelline membrane (VM) and spaces between follicles (SP) x 240

(B) spent showing empty follicles (EF), previtellogenic oocytes (PVO), unovulated oocyte (UO) and ovarian wall (OW) x 240

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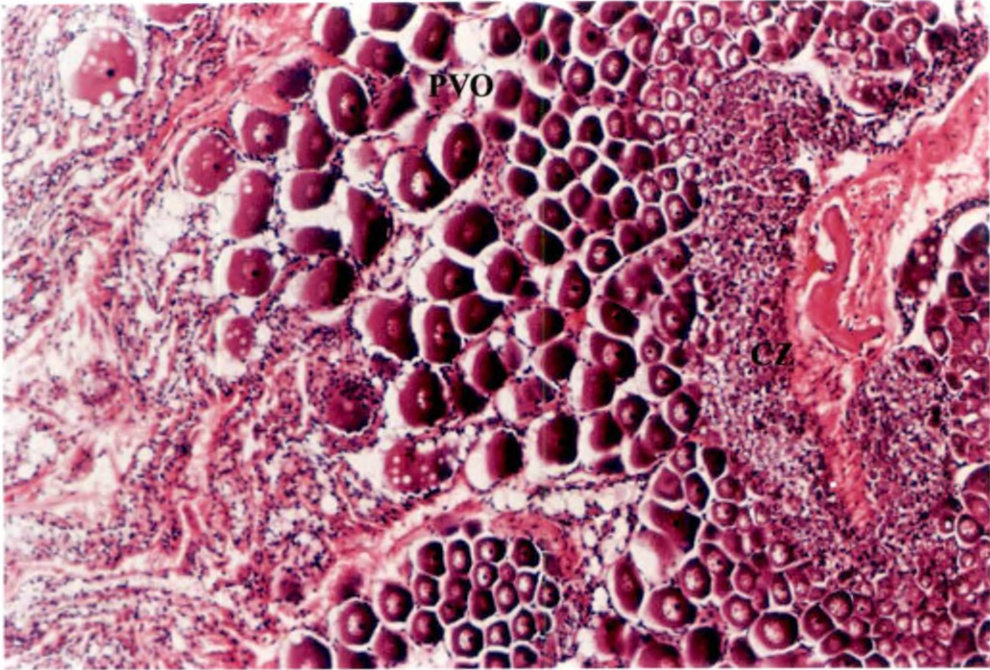

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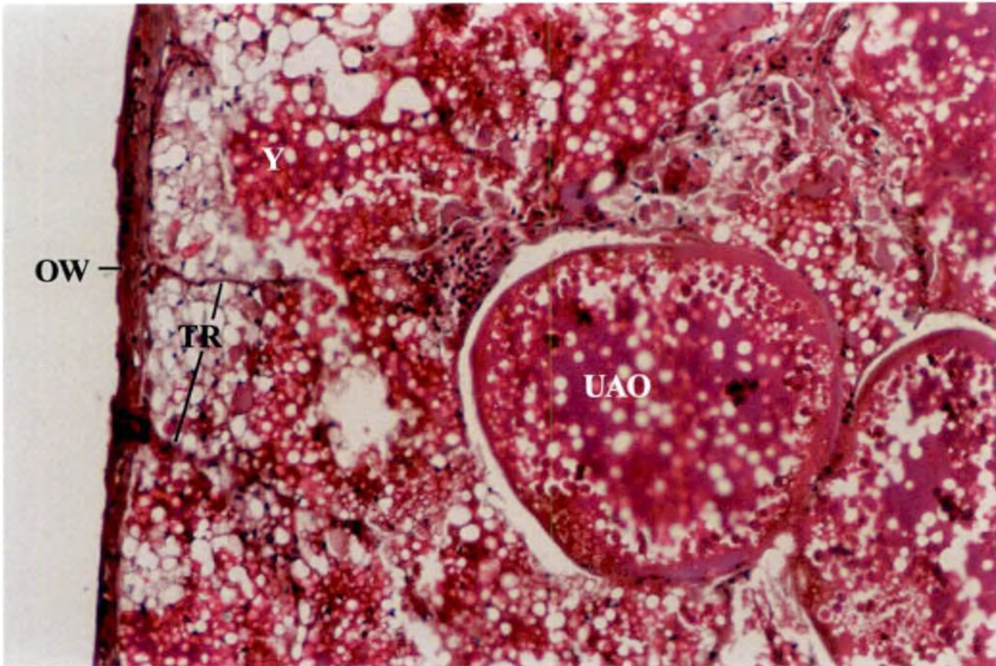

(B)

Plate 6. Photomicrographs of ovary of *M. rosenbergii*,

(A) spen t recovering ovary after a regular moult showing previtellogenic oocytes (PVO) and central zone (CZ) x 240

(B) unovulated ovary undergoing resorption showing unovulated atretic ovum (UAO), trabeculae (TR), ovarian wall (OW) and yolk (Y) x 240

Discussion

5. DISCUSSION

5.1 EXPERIMENTAL OBSERVATIONS

5.1.1 Effect of isolation from males on ovarian maturation (Experiment- I)

Observations on ovarian maturation in isolated females have been reported only in a few species of freshwater prawns/shrimps. In the small sized atyid shrimp, *P. compressa,* ovaries failed to develop in the absence of males. There was no vitellogenesis in these isolated females (Takayanagi *et al*., 1986a). In the small sized palaemonid prawn, *M. kistnensis*, the ovarian vitellogenic growth was significantly low in isolated females (Nagabhushanam *et al.,* 1989). In the medium sized palaemonid prawn, *M. idella,* in females reared in isolation from males, vitellogenesis was blocked in stage IV i.e., the stage of the begining of exogenous vitellogenesis (Jayachandran and Jose, 1993). This suppression of ovarian maturation was more pronounced in the second ovarian cycle after isolation. These animals also showed severe hypophagia. After a period of 48 days' isolation, when males were introduced back, all those symptoms were ameliorated, vitellogenesis was resumed and all the animals spawned (Jayachandran and Jose, 1993). In *M. idella*, the experiment was conducted in the post breeding season i.e., January to March and the control females cohabiting with males were showing ovarian maturation normally. The present study was conducted during post-breeding season (January to March) on specimens collected from the wild during December as was done for *M. idella.*

In *M. rosenbergii,* the large sized palaemonid prawn, in the male deprived group I females, the percentage of berried females (45%) as well as average spawning frequency (0.44) were less than in group II females cohabiting with males (55% and 0.64 respectively), but the difference was not statistically significant (Table 1 and 3). So the conclusion can only be that in this species there is no indication of a male influence on ovarian

maturation. It has to be noted here that the average spawning frequency was low even in the females cohabiting with males (0.64). This is probably due to the fact that it was the post breeding season and though *M. rosenbergii* is known to breed freely in captivity, the percentage of spawning females varies in different months of the year (Damrongphol *et al.*, 1991). The females cohabiting with males had fertilized ova in the berry which they incubated for 15-21 days and the male deprived females had unfertilized ova which they incubated only for 4-6 days which is in general agreement with the already published data (Jayachandran, 2001). Damrongphol *et al.* (1991) have reported that the intermoult period between consecutive premating moults in berried females was about ten days longer than that in unberried females in *M. rosenbergii* indicating delay in maturation in berried females.

5.1.2 Effect of visual and/or chemical cues from male on ovarian maturation and ovulation (Experiment II)

The experiment was conducted during the peak breeding season, August to October. The females cohabiting with males in the II compartment had only limited physical contact and could not copulate and fertilize the ova and hence period of incubation of berries in cohabiting group and the isolated groups would be identical.

In the present experiment, the design of aquarium and the arrangement of the three compartments (Fig 1. and Plate 1B) were similar to that used for *M. idella* by Patil (2001). Compartment I which housed only females was also water tight, not allowing any chemical cues from male to reach, but the females could see the male. The II compartment had females and males cohabiting but with limited physical contact and in III compartment, females received only chemical cues from male and no visual signals. Results are given in Table 4. There was 100% spawning in all the three groups. This is in contrast to the result reported for *M. idella* (Patil, 2001) where there was 0% spawning in the I compartment of isolated females

which received no chemical cues from the male, though they had visual signals from male.

The statistical analysis of the results comparing total number of berried and unberried females between the three groups using Fisher's Exact Test are given in the Table 5, 6 and 7. It is obvious that during the period of 60 days all the females in the three groups had spawned at least once and there was no difference between them in this parameter. In *M. idella* (Patil, 2001) considered for comparison only the animals which became berried in the second breeding cycle. The present data on the number of females which became berried twice in the three groups are given in Table 4. In group I out of 8 animals 4 became twice berried (50%), in group II out of 6 animals 1 became twice berried (16.66%) and in group III out of 8 animals 1 became twice berried (12.5%). Compared to the result in *M. idella* (spawning was 0% in the isolated females) in the present study, 50% spawning was observed which is more than the other groups of females cohabiting with males and females and those receiving only chemical cues. The statistical analysis (Tables 8, 9 and 10) however showed no significant difference between three groups leading to the conclusion that in isolated females ovarian maturation and ovulation takes place in the same way as in those cohabiting with male or those receiving only chemical cues from males. Average spawning frequency was also calculated for each group (Table 4) and analysed by Student's 't' test (Table 11). Though average spawning frequency was highest in group I of isolated females (1.5), statistical analysis showed no significant difference between the three groups. The average spawning frequencies obtained in the present study in the three groups were comparable to the value of 1.28 reported by Vadher (2003) based on a study during breeding season at the same location. This again shows that normal ovarian maturation and ovulation occurred in all the three groups.

Thus the second experiment also does not give any evidence for a chemical factor released by males playing a role in ovarian maturation and ovulation in *M. rosenbergii.* This is in contrast to the condition in the related species *M. idella* and *M. kistnensis.* In the three species *P. compressa, M. kistnensis and M. idella* where an ovary stimulating pheromone has been reported in the males, it is present in the testes and vas deferens extracts. In the former two species, the pheromone was shown to act through the release of GSH from brain/thoracic gland. In these species pheromone may help to avoid waste of energy invested in ovarian maturation when males are not around for mating.

Though Das *et al.* (1999) reported delayed ovarian maturation in isolated *M. rosenbergii* and *M. malcolmsonii,* no critical comparison of results are possible as their experimental data are not published.

The large sized freshwater prawn, *M. rosenbergii* exhibits wide range of intraspecific racial and regional variations in wild populations. The presence of male morphotypes exhibiting differential growth and sexual behaviour patterns make this species unique. Chemical cues from freely interacting animals are known to cause differential growth in the juveniles while chemical cues from individually housed animals do not have this effect (Ismael and New, 2000). Similarly regarding sexual attraction also complexity exists and the stage of maturity of ovary plays a major role (Ismael and New, 2000). Only females with ripe ovaries are attracted to blue claw males, but blue claw males are not attracted to chemical cues from ripe females. Some blue claw males fight only in the presence of ripe females while others fight all the time. Females approach the blue claw males only in the late mature, premoult stage and only then they are entertained. At the same time small males are attracted to chemical cues from females with ripe ovary. Confining the females close to blue claw males throughout the experimental period as done in the present study, may

not happen in a natural condition. Moreover the role of small males vis-à-vis female sexual maturation is also not investigated.

From the present study it can be concluded that unlike in the related species of *M. idella* and *M. kistnensis*, the ovarian maturation and ovulation in *M. rosenbergii* is independent of any pheromonal influence from blue claw males at least in the two maturation cycles following isolation.

5.2. BIOLOGICAL OBSERVATIONS

5.2.1 Maturity Stages and Histology

The ovarian maturation is classified into 5 stages: stage I, stage II, stage III, stage IV and stage V. Here, the classification was done based on the colour of the ovary and size of the ovary in relation to carapace cavity. This classification is adopted from the work already carried out by Chang and Shih (1995). For easy reference the details are given in Table 12.

The present histological studies of the ovary of *M. rosenbergii* agree well with work already done for the species by O'Donovan *et al.* (1984), Damrongphol *et al.* (1991) and Chang and Shih (1995). It was found that the germogen is not at isolated regions within the ovary which is in contrast to the germogen described for *M. idella, M. equidens equidens* and *M. equidens pillai* (Jayachandran and Joseph, 1988; Sebastian, 1993). Adiyodi and Subramoniam (1983), while reviewing germarium in crustacea, had grouped it into 5 broad categories.

1. Peripheral, 2. Peripheral but confined to lateral or ventral regions as a thin band, 3. Central as a germinal cord, 4. Germ nests and 5. Peripheral with germinal nest. The germarium of the prawn studied here in all probability belongs to fifth category. This is quite in agreement with the germarium of *M. lamarrei* (Charles and Subramoniam, 1982).

Sequential changes of oocyte growth within the ovary in palaemonids are meagre (Nadarajalingam and Subramoniam, 1982; Jayachandran and Joseph, 1988; Sebastian, 1993). The study confirms the

general pattern of oocyte development observed in *M. idella, M. lamarrei, M. equidens equidens* and *M. malcolmsonii* but differs very much from *M. equidens pillai* in the nature of yolk deposition (Sebastian, 1993).

5.2.1.1 Spent ovary

Microscopic structure of ovaries of *M. rosenbergii* shortly after ovulation and oviposition has been described by O'Donovan *et al.* (1984). The present study is in agreement with their observation. Periphery of the ovary contained empty trabeculae from which ripe ova were ovulated (Plate 5 B). The network of follicular cells left over in the ovaries later form the new follicular envelope around the oocytes which begin their secondary vitellogenesis. In the centre of ovary, previtellogenic oocytes which are the candidates for the next vitellogenic maturation can be seen. An unovulated ovum is also seen in the section.

5.2.1.2 Spent recovering ovary after a regular moult

According to Damrongphol *et al.* (1991) after spawning, the ovaries of reproductively active females returned to stage I (ovary with mainly oocytes in primary vitellogenesis in the central area and oocytes in the secondary vitellogenesis at the periphery) and premating moult followed, resulting in spawning. Some females whose ovary was in stage 0 (previtellogenic oocytes) passed on to a regular moult. But in the present study, all the spent ovaries were in previtellogenic stage (equivalent to stage 0 of Damrongphol *et al.*, 1991). The section of an ovary of a female just after a regular moult shows predominantly previtellogenic oocytes in the centre and empty cordons in the periphery (Plate 6A). No sign of vitellogenesis was found in the ovary during a regular moulting cycle.

5.2.1.3 Unovulated atretic ovary undergoing resorption

A female of group II of II experiment with ripe ovary failed to spawn and underwent resorption. The ovary became yellowish-orange and the whole body also became yellowish-orange. By the end of a week the prawn regained the natural colour, underwent subsequent ovarian maturation and spawned. In order to study the structure of an ovary of a female under similar condition preovulatory atretic ovary 5 days after moulting was processed histologically. O'Donovan *et al.* (1984) reported that in *M. rosenbergii* not all the ripe eggs were ovulated and some were resorbed in the ovary and unemptied ovaries at ovulation time were mentioned in many crustaceans. Atretic oocytes with yolk material undergoing resorption can be seen in the section (Plate 6 B). Connective tissue trabeculae with blood spaces and continuous with the ovarian wall can be seen with cells probably playing a role in resorption. In a condition of absence of male and thus mating, spawning is a waste of energy and follicular atresia has been reported in many fishes in conditions of insufficient hormonal support or stress (Nagahama, 1993). By resorting to blocked ovulation and resorption of atretic oocytes, the animal saves energy. It may also be noted here that in *M. idella*, Patil (2001) reported signs of atresia in the male deprived female ovary in which maturation was blocked at stage IV. But in this species ovaries resumed secondary vitellogenesis and successfully spawned, when male was introduced.

Patil (2001) reported that in male deprived females (isolated females) the ovarian maturation is arrested at stage IV (ovary fills ½ carapace cavity, beginning of massive exogenous vitellogenesis). However, the colour is dull yellow or white instead of green. The histological picture of these ovaries shows the absence of yolk platelets and other features like presence of spaces in the ooplasm and clumped chromatin, similar to that reported in *P. clarkii* in the oocytes of resorptive ovary, which has lost the hormonal support (Kulkarni *et al.*, 1991).

In *P. compressa*, Takayanagi *et al.* (1986 b) found that in isolated females ovary remained white in maturity stage II (where only 4 maturity stages were identified). Progress of maturation to stage III where the ovary is brown in colour and the ooplasm is with large number of oil droplets is dependant on the presence of male (or male pheromone). The conclusion emerging from their studies is that in the absence of male, ovarian maturation is blocked at the stage of massive vitellogenesis. A similar observation has also been made in *M. kistnenesis* (Nagabhushanam *et al.*, 1989), and they arrived at similar conclusion based on the change of colour of ovary and mean oocyte diameter. However, in the present study there was no any kind of block to vitellogenesis at any stage of ovarian maturation in any experimental group. Thus, the histological studies corroborated the biological observations.

Summary

6. SUMMARY

Macrobrachium rosenbergii (de Man, 1879) is a commercially important freshwater prawn which exhibits wide range of intraspecific, racial and regional variations manifested as male morphotypes, differential growth and intricate sexual behaviour patterns. The presence of a male derived ovary stimulating pheromone has been reported in two freshwater prawns viz., *Macrobrachium kistnensis* and *Macrobrachium idella* and the shrimp *Paratya compressa*. The object of the present study was to investigate the presence if any of a similar male pheromone in *M. rosenbergii.* Biological and histological observations were made. The methodology, important results and conclusions of the study are as follows.

1. Mature females and blue claw males of *M. rosenbergii* were collected from Cochin backwaters. The size range for experimental animals was 155 mm to 260 mm for females and 160 mm to 270 mm for males. They were kept in aerated freshwater under natural photoperiod and fed with fresh clam meat.

2. Based on morphology and histology, five maturity stages can be identified in *M. rosenbergii* as described earlier. In the rematuring ovaries, stages II-V were identified viz., stage II (Previtellogenic oocytes), stage III (Early Vitellogenic- endogenous vitellogenesis), stage IV (Late Vitellogenic- exogenous vitellogenesis) and stage V (Mature oocytes). Oocyte diameter was also measured which conformed to the reported data. A rare case of atretic ovary in a ripe unspawned female was also described.

3. The first experiment was conducted to investigate whether there is a male factor influencing ovarian maturation in females. In this, group I females were kept isolated from males and group II females cohabited with males. The observation period was 60 days. Ovarian maturation was monitored daily and the occurrence and incubation of berries noted. Comparing the two parameters viz., number of berried females and average spawning frequency between the two groups, no particular effect of male deprivation

on female maturation was found. The number of berried females was 44.4% in group I and 54.55% in group II and the average spawning frequency was 0.44 in group I and 0.64 in group II. In this experiment conducted during the beginning of the reported post breeding season of wild population (January- March), average spawning frequency was low in both groups and no effect of male factor influencing ovarian maturation was found.

4. The second experiment was conducted during the peak breeding season (August- October) for a period of 60 days to investigate the role of visual and/or chemical cues on ovarian maturation and spawning in specially designed aquaria with three compartments. In first water tight compartment only females (group I) were kept which had no chemical cues but received visual signals from male in the adjacent (middle) compartment which had both females and males (group II). The third compartment also had females only (group III) but they received chemical cues from the male in the middle compartment through common water circulation. These females however could not see the male due to the dark partition separating them. All the parameters mentioned for the previous experiment were monitored in this case also. Comparison of number of berried females, number of twice berried females and the average spawning frequency between the three groups, revealed no significant difference between them. All the animals became berried in the three groups. The percentage of twice berried females were 50%, 16.66% and 12.5% and average spawning frequencies were 1.5, 1.167 and 1.125 in the groups I, II and III respectively. The statistical analysis showed no significant difference between them. The females receiving no chemical cues from male showed the same rate of ovarian maturation and spawning.

5. The study concludes that unlike in the related small and medium sized species *P. compressa*, *M. kistnensis* and *M. idella*, in the giant freshwater prawn, *M. rosenbergii*, ovarian maturation and ovulation are independent of

any pheromonal influence from blue claw males during the test period of 60 days.

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172518

INFLUENCE OF A PUTATIVE MALE PHEROMONE ON OVARIAN MATURATION AND OVULATION IN THE FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII* (DE MAN, 1879).

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ABSTRACT OF THE THESIS

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ABSTRACT

Discovery of a male primer sex pheromone with a specific stimulatory effect on ovarian maturation and ovulation in the small sized freshwater shrimp *Paratya compressa* and the prawns *Macrobrachium kistnensis* and *M. idella* is considered very significant. In this context, it was the objective of the present study to investigate this aspect in the commercially important giant freshwater prawn *Macrobrachium rosenbergii* (de Man 1879).

Two sets of experiments were carried out using sexually mature females and blue claw males collected from wild. The first experiment (to study the effect of male deprivation on ovarian maturation) had two groups of females. Group I had isolated females (without males) and group II had females cohabiting with males. Ovarian maturation was monitored daily for 60 days. Maturity stages and the number of berried females were noted and average spawning frequency calculated. There was no difference in the percentage of berried females and average spawning frequency between the two groups and hence no evidence for blocked ovarian maturation in isolated females due to the lack of a male factor.

The second experiment was done to delineate the role of visual and/or chemical cues on ovarian maturation in *M. rosenbergii*. Specially designed glass aquaria were used which had three compartments for three groups of females. Group I females were isolated and received only visual signals and no chemical cues from the males. Group II females were cohabiting with males. Group III females were also isolated from males but received only chemical cues from males through a common water circulation. The same parameters as in the first experiment were monitored for 60 days and no significant difference found between the three groups. Histological study also showed normal maturation. The conclusion is that

there is no evidence for an ovary stimulating pheromone in *M rosenbergii* thus adding one more feature to the distinctiveness of this species.