EFFECT OF EYESTALK ABLATION ON THE REMATURATION OF FEMALE *MACROBRACHIUM ROSENBERGII* (DE MAN) IN CAPTIVITY

By

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THESIS

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Dedicated To

My Beloved Parents

DECLARATION

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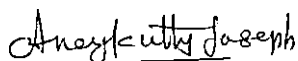
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Introduction

1. INTRODUCTION

The giant freshwater prawn *Macrobrachium rosenbergii* is a commercially important species widely cultured throughout the tropics, subtropics and some parts of temperate regions (New, 2000). The devastating viral infection in marine shrimps has forced many entrepreneurs to grow freshwater prawns as a substitute crop in many coastal districts of India.

But the pace of growth in this vital sector of aquaculture has rather been slow, primarily due to the non-availability of good quality and quantity of seed. For many hatcheries located in tropical regions, availability of larvae is ensured by egg-bearing females obtained from culture ponds or natural sources. However, lack of an assured supply of berried females from the wild, limits the hatchery operations. Controlled production of viable eggs and successful rearing through larval stages are necessary for the reliable supply of seed on which freshwater prawn farming systems depend.

The rapidly growing prawn culture industry, among other areas looks towards crustacean endocrinology, to assist in developing techniques to increase crop yield by stimulated growth and controlled reproduction under captive conditions. Controlled breeding in captive condition provides the advantage of obtaining pure seed as and when required and opens up new avenues of large-scale husbandry. With increasing knowledge of endocrine activity and control of gonadal development in crustaceans, the technique of eyestalk ablation is receiving greater attention as a method of inducing precocious maturation in captivity (Primavera, 1985).

Crustacean eyestalk is known to contain production, storage and release centres of the gonad and moult inhibiting hormones. Published work to date has shown that eyestalk removal is likely to produce either

precocious moulting or gonad development, depending upon the relative interactions of other ambient environmental factors and age of the animal (Adiyodi and Adiyodi, 1970). Eyestalk ablation also leads to predictable peaks in maturation and spawning which facilitates the setting up of production schedules in contrast to scattered spawns for unablated females.

Eyestalk ablation has been successfully used to induce ovarian maturation in various penaeid species (Adiyodi and Adiyodi, 1970; Primavera, 1985). However, very few studies have been carried out in freshwater prawns. No concerted attempt has so far been made in our country on rematuration of spent female of *M. rosenbergii* through eyestalk ablation.

The objectives of the present study are:

1. To find out the role of eyestalk ablation on rematuration of spent female of *M. rosenbergii* in captive conditions.

2. To study the histology of the ovary during rematuration of eyestalk ablated and unablated spent female of *M. rosenbergii*.

Review of Literature

2. REVIEW OF LITERATURE

2.1 DISTRIBUTION OF *MACROBRACHIUM ROSENBERGII*

Species of freshwater prawn of the genus *Macrobrachium* are distributed throughout the tropical and subtropical regions and more than 100 species are known to exist today (New, 2000). In India, the genus is represented by about 40 species out of which 15 are important from fisheries point of view (Jayachandran and Joseph, 1992). The largest and most popular among them, the giant freshwater prawn, *Macrobrachium rosenbergii* is indigenous to Southeast Asia, Northern Oceanica and Western Pacific Island. The species inhabits fresh, brackish and rarely marine waters and is the most commercially important species of the genus *Macrobrachium* (New, 2000). *M. rosenbergii* is distributed from Indus delta of India, China to Asian mainland in fresh and brackishwater areas. In India, it is available in West Bengal, Gujarat and in Kerala state (Tripathi, 1992; Sebastian *et al.*, 1993).

2.2 REPRODUCTIVE BIOLOGY OF *M. ROSENBERGII*

2.2.1 Sexuality

Palaemonid prawns are dioecious, the sexes being distinguished by a number of external characters (Patwardhan, 1937). In general, the females are smaller than the 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.

2.2.2 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 become fully mature, the ovaries occupy the entire carapace cavity and also extend a little into 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 translucent 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 the fifth pereiopods.

Joseph (2002) studied the morphological and histological details of male reproductive system of this species. 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.

2.2.3 Sex ratio

Seasonal variations in sex ratios have been reported in the case of *Macrobrachium spp.* Raman (1967) has reported male domination in the case of *M. rosenbergii* in the catches especially during May-June from the Vembanad lake. Kurup *et al.* (1992) reported a male: female ratio of 1: 1.11 for *M. rosenbergii* from the catches from Vembanad lake. The seasonal variation was also found to be pronounced in this species being of 1:0.17 to 1: 5.87, the females dominating during September to December and males during March to June. Varghese *et al.* (1992) observed the best result of oviposition by maintaining the sex ratio of 1male: 4 female in brood stock rearing of *M. rosenbergii.*

2.2.4 Size at first maturity

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 years respectively. Rao (1967) recorded the mean size of 155 mm as the maturity size in Hooghly estuary. Goorah and Parmeshwaran (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).

2.2.5 Breeding season

The appearance of berried females marks the onset of the breeding season, while the time by which majority of prawns appears to have dehished 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 Hooghly 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).

2.2.6 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, 2001). Small males and blue-clawed males actively take part in mating, insisting little energy on somatic growth; while orange clawed males are characterized by fast growth rate (Sagi, 1984; Ra'anan and Sagi, 1985; Sureshkumar and Kurup, 1998). Harikrishanan and Kurup (1997a) reported seven types of male morphotypes in *M. rosenbergii* viz. small males, weak orange-clawed males, strong orange-clawed males, pre-transforming orange-clawed males, weak blue-clawed males, strong blue-clawed males and old blue-clawed males.

2.2.7 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 (Jhon, 1957). Mature females may oviposit 3 to 4 times a year under natural conditions. Many investigators (Table 1) have worked out fecundity of *M. rosenbergii*.

Table 1. Fecundity of *M. rosenbergii* worked out by some authors

Region	Size range (cm)	Fecundity	Reference
Kerala waters	18.1-22.9	139,600-503,000	Raman, 1967
Malaysian water	--	60,000-110,000	Ling, 1969
Hooghly estuary	18.0-25.5	12,280-101,700	Rajyalakshmi, 1975
Balgoda lake	5.5-9.0 (carapace length)	82,000-170,000	Jinadasa, 1985
Kolleru lake	15.0-25.5	20,880-162,078	Rao, 1991

2.2.8 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 and 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's thoracic sternum. Soon the spermatophore hydrates, it expands and loses its stickiness except on the surface jointed to the sternum. This expansion often obscures the spermatophore's original morphology and apparently serves to help anchor it.

2.2.9 Moulting and reproduction in females

In freshwater prawns ovarian maturation processes through intermoult and culminates in pre-spawning moult. This pre-spawning moult is of great significance among palaemonids in which new breeding dress is acquired by females in order to carry eggs. Mating takes place a few hours after this moult (Narayanan and Adiyodi, 1992, Sebastian *et al.*, 1993).

2.2.9.1 Mating

Rao (1967) and Ling (1969) have studied the mating behavior of this species. They observed that once the male and female get accustomed to each other, mating behavior 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 to 30 minutes before the female is successfully won over.

2.2.9.2 Spawning and Fertilization

Jayachandran (2001) reviewed the spawning and fertilization 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 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 live 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 the small chelae for fertilization.

The sperm cells are apparently brought into contact with the ova by the mechanical action of the ova passing across the spermatophore on the way to the 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 setae on the first two pleopods and moved into the 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 a gelatinous outer membrane shortly after release from the oviduct.

2.2.9.3 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 pleopods of the mother, to facilitate gaseous and ionic exchange for eggs during incubation. From the 12th day of incubation, the bright orange colour of the eggs gradually lighten and become light grey, deepening to dark to 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).

2.2.9.4 Hatching

During hatching the mother prawn creates 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 decrease and the female postpones the process of hatching.

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2.2.10 Ovarian development in *M. rosenbergii*

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

2.3 ENDOCRINE CONTROL OF REPRODUCTION IN PRAWNS
/SHRIMPS AND OTHER DECAPOD CRUSTACEANS

Reproduction in crustaceans like many other physiological processes is under endocrine control. Moulting and reproduction are major metabolic events involving cyclic mobilization of organic reserves from storage depots to the epidermis and gonad respectively

and though temporally separated, are functions inseparably integrated with one another (Adiyodi and Adiyodi, 1970).

The eyes in decapod crustaceans are generally stalked and movable and are known to contain a variety of hormones or factors apparently responsible for growth, moulting, metabolic rate, water balance, dispersion of retinal pigments and sexual·activity (Lockwood, 1968). The X-organ sinus gland (XO-SG) complex in the eyestalk is believed to produce a hormone controlling both reproduction and moult (Adiyodi and Adiyodi, 1970). Two hormones jointly involved in the control of moult, growth and development have been postulated later to regulate reproduction jointly (Adiyodi, 1980).

Although many observations have been made on the inhibition of reproductive maturation by eyestalk hormone(s), recent research is focused mostly on brain, thoracic ganglion (TG), ovary and mandibular organ (MO) 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 penaeid shrimp gonad inhibiting hormone (GIH) is secreted from the XO-SG complex and inhibits vitellogenesis and gonad stimulating hormone (GSH) is secreted from the thoracic ganglion and brain and stimulates vitellogenesis (Yano, 1992a).

2.3.1 Gonadal maturation

There is still much speculation and difference of opinion about the process of gonad maturation and the model of Adiyodi and Adiyodi (1970) is still currently accepted (Adiyodi, 1985). This scheme proposes that the actions of moult inhibiting hormone (MIH) and GIH are antagonistic and also that there is a GSH, produced by the brain and the thoracic ganglion. Moulting occurs when the titres of MIH and

GSH are low and those of GIH and moulting hormone(s) 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 (AG) by inhibiting its secretion; in its absence maturation takes place (Payen *et al.*, 1967). Moulting and reproduction are generally held to be 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). In species where moulting and ovarian development alternate, MIH and GIH must act antagonistically (Dull *et al.*, 1990).

2.3.2 Inhibitory factors of gonad maturation

2.3.2.1 Gonad inhibiting hormone (GIH)

In decapods, it is well known that the removal of eyestalk induces ovarian activity (Adiyodi and Adiyodi, 1970). The existence of a gonad inhibiting principle in the eyestalk of decapod crustacean was first demonstrated in *Palaemon serratus* by Panouse (1943). The neuroendocrine complex produces an inhibitory hormone; when this is removed precocious gonadal development ensues. This was later confirmed in many decapod crustaceans, in *Cambarus* (Stephens, 1952); in *Uca* (Brown and Jones, 1949); in *Lysmata* (Carlisle, 1953) and *Carcinus* (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 size which precedes the breeding season may be inhibited by injections of extracts of whole eyestalk or sinus gland or medulla-terminalis ganglionic x-organ (MTGX) (Carlisle, 1953). Knowles and Carlisle (1956) took this result as evidence for existence of an ovary-inhibiting hormone (OIH).

Eyestalk ablation 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, injections of eyestalk extract supplies the inhibitor, which keeps the ovary in check.

Though Carlisle (1954) proposed specificity of GIH, Adiyodi and Adiyodi (1970), quoting Carlisle (1953), Otsu (1963), Payen *et al.* (1967) and Juchault and Legrand (1967), argue that ovary-inhibiting hormone (OIH) and testis inhibiting hormone (TIH) are 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 adult but also in immature stage 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 gonad 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, 1992a). In adult female of several species of decapods eyestalk ablation results not only in moulting, as in juveniles and some adults, but also in premature yolk deposition in the ovary, both during the non-breeding season and 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 i.e. growth restraining hormone (GRH) that exercises its influence on the target processes namely, growth and

reproduction. Crustacean eyestalk contains hormone that inhibits moult and reproduction, but the course of events initiated by eyestalk ablation varies with species, age of individuals, season and other factors (Adiyodi, 1985; Quackenbush, 1986; Fingerman, 1987). The production of GIH has been shown to be seasonal (Adiyodi and Adiyodi, 1970; Bomirski and Kelk, 1974; Kelk-Kawinska and Bomirski, 1975).

Subramoniam and Keller (1993) demonstrated the inhibition of oocyte growth in shrimp, *Atylephra desmaresti,* by administration of sinus gland extract from the lobster, *Homarus americanus.* Quackenbush and Kelley (1987) showed that partially purified eyestalk extract from the shrimp *Penaeus vannamei* could inhibit ovarian synthesis of vitellogenin in the crab, *Uca pugilator in vitro,* while Eastman-Reks and Fingerman (1984) found inhibition of vitellogenin production in ovaries of cultured crab. Interestingly almost all the GIH bioassay thus for found have been heterologous, implying lack of species specificity of this peptide (Subramoniam 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 ovaries in cultured species (Paulus, 1984; Paulus and Laufer, 1987; Quackenbush, 1989; Yano, 1992a). The fact that cyclic AMP (Adenosine monophosphate) can mimic this inhibitor 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).

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* (Chang *et al.*, 1990; Soyez *et al.*, 1991) and in the shore crab, *Carcinus maenas* (Webster and Keller, 1986). A similar immunological study in the lobster indicates that GIH and CHH share common antigens (Subramoniam and Keller, 1993).

2.3.2.2 Other inhibitory factors

The androgenic gland, which is responsible for the masculinisation of the animals, seems to produce a number of compounds including farnesyl acetone, a molecule similar in structure to methyl farnosoate (Ferzon *et al.*, 1978), which 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 (octamine and serotin) inhibited by methyl farnosoate synthesis in *Libinia emarginata* (Homola *et al.*, 1989). Serotin 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 inhibit mandibular organ synthesis of methyl farnosoate 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.*, 1987b).

2.3.3 Stimulatory factors for control of reproduction
2.3.3.1 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 *Penaeus vannamei* suggested its existence because eyestalk ablation caused precocious ovarian growth in adult, but not in juveniles. This led them to reason that not only was the absence of GIH required for ovarian growth in adult, but the presence of a stimulatory hormone was also necessary. Otsu (1963) also observed that implantation of adult TG was effective in triggering maturation of ovary in eyestalk ablated juveniles. The experiments of Hinsch and Bennett (1979) using *L. emarginata*, Gomez (1965) using *P. hydrodromous* with both brain and TG and Takayanagi *et al.* (1986b) using the shrimp, *Paratya compressa* also proved that GSH from TG has a role to play in ovarian maturation. Extracts of TG of reproductive *Uca pugilator* stimulates ovarian growth in adult crabs (both intact and ablated) while TGE 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 TG in stimulating ovarian growth in *Macrobrachium kistensis*. Yano and Wyban (1992) propose a GSH-releasing hormone (GSH-RH) from brain of the *P. vannamei*. From all these findings, Yano (1992b) speculates that in immature females the ovarian stimulating principle is absent or yet not functioning. Yano (1992b) found that TGE prepared from maturing females is effective in increasing serum vitellogenin (Vg) in *P. japonicus* and suggested that GSH also stimulates Vg synthesis and/or secretion into the blood in penaeid shrimp. He further noted that brain extract prepared from maturing females induced Vg secretion in *P. japonicus* suggesting a brain hormone stimulate release of GSH in penaeid shrimps.

Implantation of brain and TG into the male *Peratelphusa hydrodromous* result in precocious maturation of testis and even in hypertrophy of the vas deferens (Gomez, 1965). This observation together with the finding of Otsu (1963) that the TG effectively accelerated ovarian development in young female of *Potaman dehaani* and the experiment of Yano *et al.* (1988) where the TG implantation of mature female *H. americanus* into *P. vannamei* induced ovarian growth, suggest that GSH is effective in both sexes in the different genera and is present in TG as well as in the brain of crabs and shrimps.

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

2.3.3.2 *Juvenile hormone (JH)*

The role of terpenoid hormones unique to arthropods and collectively known as Juvenile hormone (JH) or juvenoid has been established in insect reproduction (Raabe, 1982; Laufer *et al.*, 1992).

Downer and Laufer (1983) proposed that 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 factors in decapod crustaceans (Subramoniam and Keller, 1993). Since both the arthropod subphyla, 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 may also have a functional JH for development and reproduction (Chang *et al.*, 1992; Laufer *et al.*, 1992). This view is supported by considerable literature. There are reports of insect JH or related compounds having biological activity in crustacea and of crustacean tissue having JH activity in insects. Schniederman and Gilbert (1958) detected some JH activity in the eyestalk of the crustacea. Laufer *et al.* (1987a) identified a sequiterpenoid compound methyl farnosoate in the mandibular organ as well as in the haemolymph of the spider crab. The methyl farnosoate, 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 crustacean is structurally similar to the corpora allata of insects (Chandennert, 1956; Le Roux, 1968; Byard *et al.*, 1975). After critically reviewing the literature in this field Subramoniam and Keller (1993) proposed methyl farnosoate as the crustacean juvenoid probably involved in the stimulation of vitellogenesis and farnesoic acid as a pre-hormone which could undergo conversion to methyl farnosoate 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 farnosoate by the mandibular organ of the crayfish,

Procambarus clarkii. Experiments of Laufer and Landau (1991) also indicated that RPCH has mandibular organ stimulating activity in *P. clarkii* and *Libinia emarginata.*

2.3.3.3 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 (Couch and Hagino, 1983; Adiyodi, 1985). 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).

2.3.3.3.1 Ecdysteroids (ECDs)

The moulting hormone (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 growing evidence to suggest that in insects and crustaceans, ECDs which are primarily MH (Adiyodi and Adiyodi, 1970) are also present 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 ECDs, especially ecdysone in ovaries of crab *C. maenas*, whose titre in ovary registered a gradual increase with the process of vitellogenesis, with peak levels detected on termination of the process.

Young *et al.* (1993) suggested that ECDs may be synthesized or get accumulated in the gonads. Chang (1992) has observed that y-organ removal can result in either stimulatory or inhibitory effect on vitellogenesis depending on species, age and stage in the moult stage and reproductive cycle. Young *et al.* (1993) have opined that though ECDs are primarily moulting hormones, they may also have secondary effect on reproduction.

2.3.3.3.2 Other steroids

Evidence from scattered works suggest that the crustacean ovary might play a role in the biosynthesis of steroid hormones. 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 trituberculatus* possess the enzyme involved in the conversion of progesterone to hydroxyprogesterone, testosterone and deoxycorticosterone. Teshima and Kanazawa (1971) detected progesterone and testosterone in the ovaries of *Panulirus japonicus*.

A number of steroids including testosterone, progesterone and pregnenolone have been identified in the gonads and serum of the cray fish *Astacus leptodactylus* and lobster, *H. americanus* (Burns *et al.*, 1984; Ollivier *et al.*, 1986) and the shrimp, *P. monodon* (Young *et al.*, 1992).

2.3.3.4 Other factors

Tenson *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 hyperglycaemia hormone (CHH). The possible existence of such an ovary stimulating hormone in the sinus gland and working of this peptide antagonostically to GIH or

synergistically with the putative GSH is yet to be evaluated (Subramoniam and Keller, 1993).

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

Beltz (1988) reported that octamine and serotin not only affect the mating behavior of lobster, *H. americanus* 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).

2.4 METHODS OF INDUCING MATURATION IN PRAWNS

Securing of ripe spawners from wild is costly and uncertain. This has generated interest in the induced maturation of prawns under controlled conditions. Development and management of shrimp broodstock is now an integral part of hatchery (Muthu and Laxminarayana, 1982; Muthu, 1983). Male shrimps generally mature in captivity so that induced maturation mainly concerns females and so studies on reproduction have predominantly focused on female maturation (Primavera, 1985,1988). To avoid dependence entirely on natural brood stock, and to have a year round hatchery operation of freshwater prawn induced maturation in captivity is essential. Although a number of published works on induced maturation in marine shrimps are available, very few works have been done on this aspect in freshwater prawns (Murugadass *et al.*, 1988). In general environmental,

nutritional and endocrine manipulations are the three basic approaches employed singly or in combination to induce ovarian maturation in crustaceans (Primavera, 1985).

2.4.1 Environmental

All animals are known to select the most appropriate time for breeding. As such the fluctuations of environmental factors have a definite influence in stimulating maturation and subsequent reproductive processes. The important among the environmental factors are light, salinity, temperature and pH. In maturation systems these factors are set at optimum levels.

2.4.1.1 Light

The presence of an eyestalk mediated control mechanism for reproduction in shrimps emphasizes the importance of light on maturation. Increase in photoperiod resulted in gonadal maturation of *P. japonicus* (Laubier and Laubier, 1979) and *P. kerathurus* (Lumare, 1979) in the subtropical regions where there is a profound variation in photoperiod with season.

Among various environmental parameters, light may play a significant role in maturation. Various workers have tested the effect of light quality (Kelemec and Smith, 1980; Pudadera and Primavera, 1981), photoperiod (Laubier and Laubier, 1979), and light intensity (Chamberlain and Lawrence, 1981a) on different penaeid species.

Although moulting in captivity has not been observed to be synchronized, a greater number of females moulting in the light phase of moon is in agreement with that observed for penaeid prawns in wild (Kirkegard and Walker, 1970). Black colour of the tank is comparatively more suitable than other colours because it absorbs more light enabling the female to mature more properly (Emmerson, 1980).

2.4.1.2 Temperature

Temperature of water in the maturation facility is found to influence the rate of all physiological functions including the rate of maturation. Controlling temperature has been instrumental in inducing maturation in temperate prawns such as *P. japonicus* (Laubier, 1978) and *P. esculentus* (Crocos and Kerr, 1986). However in tropics this technique is less effective.

2.4.1.3 Salinity

Salinity as a factor has a profound influence on gonadal development. For normal development of gonad optimum levels of salinity is to be maintained. Studies on the influence of salinity on reproduction of palaemonids are very limited. .

Salinity is known as a major factor limiting the reproductive capability of those *Macrobrachium*, which undertake breeding migration. Ignatius (1989) observed that a salinity level of 6-12 ppt is congenial for ovarian development as compared to a lower level of 0 ppt and higher level of 18 ppt in *M. idella*. In many species as in *M. rosenbergii*, the early growth phase is spent in the upper stretches of the rivers and the adults migrate to lower reaches for breeding. The number and size of egg in *M. nipponense* in freshwater is found to be different from those living in brackishwater region (Moshika, 1983,1984).

2.4.1.4 pH

pH as a factor is known to influence the gonadal development. A higher pH of 8.2 induces maturation in *P. indicus* (Muthu *et al.*, 1984). Similar higher values of pH such as 7.1-8.6 (Emmerson, 1980), 7.8-8.1 (Primavera *et al.*, 1982), 8.2 (Aquacop, 1983), 7.1-8.6 (Emmerson *et al.*, 1983) for *P. indicus* and 8.2 (Aquacop, 1977,1979,1980,1983), 7.8-

8.1 (Primavera, 1978), 7.9-8.1 (Pudadera and Primavera, 1981), 7.8-8.0 (Ruangpaint *et al.*, 1981) for *P. monodon* have been reported. The optimum pH reported for *M. rosenbergii* is 7.0-8.5 (New and Singholka, 1982).

2.4.2 Nutritional method

Nutrition is profoundly important for reproduction and success of reproduction closely relates to nutrient ingestion accompanying ovarian development (Bray and Lawrence, 1992). Live or fresh frozen components used are expensive, may deteriorate water quality and vary in nutritional quality with species, age, maturation state, season and location (Bray *et al.*, 1990). Broodstock are generally fed *ad libitum* with fresh and frozen clam (Muthu and Laxminarayana, 1979), squid meal (Aquacop, 1975,1977,1979), marine worms (Ponnuchamy *et al.*, 1981), shrimp (Beard *et al.*, 1977), fish (Halder, 1978,1980), supplemented with dried pellets (Moore *et al.*, 1974; Aquacop, 1977,1979).

The effect of ascorbic acid on the sperm and spermatophore quality of *P. vannamei* has been recently documented (Leung-Trujello and Addison, 1990). Generally diets containing artificial and natural food give the best results (Galgani *et al.*, 1989).

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2.4.3 Endocrine methods

2.4.3.1 Use of exogenous hormones

Crustacean gonads secrete steroids more usually identified with vertebrate and also possess the enzymatic capacity to synthesize vertebrate sex steroids (Burns *et al.*, 1984). Donahue (1940, 1948) found that estrogen in the ovary of *Panilurus argus* and showed that testis of *Homarus americanus* contains testosterones. Human chorionic gonadotropin stimulated oogenesis of sand shrimp *Crangon crangon*

(Bomirski and Kelk, 1974) and vitellogenin synthesis in *Idothea balthica* (Souty and Picaud, 1984). Similarly, Kulkarni and Nagabhusanam (1979) in *Parapenaeopsis hardwickii* and Yano (1985) in *Metapenaeus ensis* reported that progesterone produced ovarian maturation. In *Crangon crangon*, bouine gonadotropin follicle stimulating hormone and leutinizing hormone produced ovarian maturation (Zukowska-Arenda rezyk, 1981). Kulkarni *et al.* (1979) reported that there is significant difference in oocyte diameters of progesterone injected and control prawns of *Parapenaeopsis hardwickii*.

Jhonson (1995) conducted an experiment on induced maturation of *Penaeus indicus* using exogenous hormone. He revealed that complete development of ovary and spawning of shrimp could not be achieved by the mere application of progesterone or tocopherol, in the presence of endogenous GIH from the eyestalk.

2.4.3.2 Eyestalk ablation

Removal of eyestalk results in precocious maturation of ovaries (Panouse, 1943) and testis (Demeusy, 1953). Subramoniam (1988) reported that interbreeding and inter moulting period can be shortened by eyestalk ablation. The knowledge of the x-organ sinus gland complex secreting hormones having inhibitory roles on the reproduction and moulting process has led to the extensive use of eyestalk ablation technique to induce maturation in prawns. A number of workers tried to induce maturation in the shrimps and prawns by resorting to eyestalk ablation. (Table. 2)

With the increasing knowledge of endocrine activity and its control on gonadal development in crustaceans, the technique of eyestalk ablation is receiving increased acceptance as a method of inducing precocious maturation of ovary and subsequent spawning.

Destalking has been accompanied through different methods. The simplest method is to cut the eyestalk near its base with a pair of sharp scissors (Arnstein and Beard, 1975; Lumare, 1979). However this leads to profuse bleeding in delicate species and results in high mortality. Caillouet (1973) performed ablation by cutting the eyestalk near the base with the help of sharp scissors and the wound cauterized immediately with a pencil type soldering iron to avoid loss of haemolymph. Primavera (1978) incised the eyeball with a sharp blade, allowed the fluid to ooze out and then squeezed out the content of the eyeball outwards two to three times to destroy the tissue. Rodriquez (1979) crushed out the eyestalk by pressing between fingers. The last two methods are suited only for hard-shelled species like *P. monodon*.

Muthu and Laxminarayana (1979) used medial electrocautery apparatus to accomplish destalking, such a method resulting in simultaneous cutting and sealing of the wound thus ensuring 100 percent survival of the destalked animals.

Makinouchi and Primavera (1987) comparing different technique of ablation in *P. indicus* reported 90-100 percent survival in eyestalk cauterized females, which was comparable to the unablated treatment. Significantly low survival rate was recorded in females, which were ablated by pinching resulting from stress.

Ablation has been done both unilaterally and bilaterally and the later resulted in high initial mortality. In this case though full development of gonads was observed, spawning did not take place, the ovaries regressed gradually and the prawns died within a month. Loss of balance and spiral movement were also observed (Caillouet, 1973; Aquacop, 1975). However, Alikunhi *et al.* (1975) and Rajyalakshmi *et al.* (1988) reported spawning in bilaterally ablated prawns.

Table 2. Eyestalk ablation experiments on reproduction in Shrimps and Prawns

Species	Source
Artemesia longinaris	Petriella and Diaz (1987)
Penaeus canaliculatus	Choy (1987)
P. duorarum	Idyll (1971); Caillouet (1973)
P. indicus	Emmerson (1980); Muthu and Laxminarayana (1979); Primavera *et al.* (1982); Makinouchi and Primavera (1987)
P. japonicus	Oltra and San Feliu (1990)
P. kerathurus	Lumare (1979); Oltra and San Feliu (1990)
P. merguiensis	Alikunhi *et al.* (1975); Beard *et al.* (1977)
P. monodon	Alikunhi *et al.* (1975); Chen (1977); Aquacop (1977,1979,1980); Santiago (1977); Halder (1978); Primavera (1979); Rodriquez (1979); Primavera and Boroglan (1977); Rajyalakshmi *et al.* (1988); Beard and Wickins (1980); Emmerson (1983); Pernomo and Hamami (1983); Sudarsanam *et al.* (1990); Lumare *et al.* (1996)
P. orientalis	Arnstein and Beard (1975)
P. semisulcatus	Browdy and Samocha (1985a)
P. setiferus	Brown *et al.* (1979)
P. stylirostris	Aquacop (1979); Chamberlain and Lawrence (1981b); Bray and Lawrence (1990)
P. vannamei	Aquacop (1979); Chamberlain and Lawrence (1981b); Yano and Wyban (1993)
Caridina rajadhari	Persis and Sarojini (1985)

Table 2. Continued

Species	Source
Macrobrachium equidens	Bijulal (1994)
M. idella idella	Jayachandran and Jose (1993); Sherine (1998)
M. malcolmsonii	Murugadass *et al.* (1988)
M. nobilii	Kumari and Pandian (1987)
M. rosenbergii	Huang *et al.* (1981); Chakravarty (1992); Koshio *et al.* (1992); Wilder *et al.* (1994); Karplus and Hulata (1995); Perez Cruz *et al.* (1995); Sanjeeviraj *et al.* (1997); Okumura and Aida (2001)

The high mortality and inability to spawn after bilateral removal of eyestalk prompted researchers to abandon this method (Muthu and Laxminarayana, 1982).

Temporal synchronization of eyestalk with moult cycle is an important factor for the production and synchronization of egg production and relating reproduction (Emmerson, 1980). Ideally, ablation should be undertaken during intermoult for maturation to follow; during premoult, leads to moulting subsequently longer latency period (Aquacop, 1979; Primavera, 1979) and during post moult to mortality because of added stress of the female and excessive loss of haemolymph (Aquacop, 1979). Animals ablated early in the moult cycle get enough time between ablation and spawning (Browdy and Samocha, 1985a).

The latency period from the ablation to the onset of maturation depends on age, source of broodstock and the stage of moult cycle (Muthu and Laxminarayana, 1982). The number of moults before which maturation is induced is reduced by eyestalk ablation (Aquacop, 1982; Crocos and Kerr, 1986).

Browdy and Samocha (1985a) reported a significant decline in mating success in ablated *P. semisulcatus* females. Muthu and Laxminarayana (1984) conducted artificial impregnation in unilaterally ablated specimens of *P. indicus* and reported that such a technique can yield viable egg which could be reared to post larval and subsequent stages. Eyestalk ablated specimens of *P. canaliculatus* mated at much smaller sizes (Choy, 1987). Petriella and Diaz (1987) reported that unilateral eyestalk ablation accelerates gonadal maturation and more than doubles the individual moulting rate in *Artemesia longinaris*. Sudarsanam *et al.* (1990) reported higher moulting in ablated *P. monodon* compared to non-ablated.

The number of eggs spawned may vary according to species. The effect of unilateral ablation on spawn size is poorly defined (Browdy and Samocha, 1985a; Primavera *et al.*, 1982) and Santiago (1977) reported no significant effect of ablation on fecundity. Microscopic examination suggested the progeny of unilateral ablation to the normal and viable (Santiago, 1977). Increased in fecundity has been demonstrated in ablated females in a number of species (Primavera, 1985). Murugadass *et al.* (1988) reported that ablated *M. malcolmsonii* carried more clutches of eggs and that there was an increase in the number of eggs per clutch. Egg production in ablated species was double in comparison to unablated control. Choy (1987) reported lower fecundity in ablated *P. canaliculatus*.

The number of larvae produced depends on the factors like female size, fertility of males and occular ablation (Holtschmit and Romero, 1991). While eyestalk ablation leads to predictable maturation and spawning it has been reported that ablation of captivity reared females result in reduced hatch rates (Lumare, 1979; Emmerson, 1980; Primavera and Posodas, 1981). Employing different types of ablation Makinouchi and Primavera (1987) noticed that the hatching rates of

unablated *P. indicus* were significantly higher than females ablated by pinching the eyestalk, but not from those ablated by tying and cautery. The inconsistent quality of eggs particularly with successive spawning has been attributed to the abnormal rapidity of maturation and over stimulatory following ablation. This decline in egg fertility limits the reproductive life of ablated specimens necessitating the replacement of broodstock few weeks after ablation (Aquacop, 1975; Beard and Wickins, 1980; Simon, 1982; Primavera, 1983).

Browdy and Samocha (1985a) did not find any reduction in hatch rates insubsequent spawning within an intermoult period and concluded that a single mating is sufficient to fertilize four spawnings in *P. semisulcatus*. The number of larvae produced is known to have increased by the synchronization of moult cycle and reproductive cycle. Hatching success between ablated and unablated treatments did not vary in *P. vannamei* (Chamberlain and Lawrence, 1981a, b), *P. stylirostris* (Chamberlain and Lawrence, 1981b) and *P. semisulcatus* (Browdy and Samocha, 1985a). Decrease in hatching success for successive spawning has been observed for ablated *P. monodon* (Primavera and Boroglan, 1979; Emmerson, 1983) and *P. indicus* (Emmerson, 1980). While no such change was evident in unablated females.

According to Browdy and Samocha (1985a) deterioration in sperm quality described by many authors is a direct result of worsening conditions of animals over time and is not an effect of ablation. The extent to which females can remature is important in terms of recycling spawners. Unilaterally ablated female prawns repeatedly mature and spawn viable eggs (Aquacop, 1979,1982; Lumare, 1979; Brown *et al.*, 1980; Beard and Wickins, 1980; Lawrence *et al.*, 1980). Yano and Wyban (1993) showed that eyestalk ablation increased spawning frequency in female *Penaeus vannamei* under tank culture conditions.

Experiment conducted by Racotta *et al.* (2000) revealed that the proportion of spawning females and spawning frequency was greater for ablated females than unablated females of *P. vannamei.*

Eyestalk ablation in males has received little attention despite the demonstration of male ablation yielding increased gonad size and precocious development (Adiyodi[9] and Adiyodi, 1970; Young, 1971; Lawrence *et al.*, 1979).

Several experiments were also carried out on freshwater prawns. Huang *et al.* (1981) studied the effect of eyestalk ablation on growth and moult of *M. rosenbergii* and found shortening of moult interval in ablated individuals. Kumari and Pandian (1987) reported that unilateral eyestalk ablation in juvenile *Macrobrachium nobilii* advanced the onset of sexual maturity. Chakravarty (1992) noted that ablation of eyestalks induces precocious moulting and accelerate growth in male prawn of *M. rosenbergii.* Wilder *et al.* (1994) observed that methyl farnosoate administration induced vitellogenin production in ablated *M. rosenbergii.* 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.* Sanjeeviraj *et al.* (1997) revealed that smaller size groups of *M. rosenbergii* responded quickly than the bigger size groups in a attempt to increase the frequency of moulting through eyestalk ablation. 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*.

3. MATERIALS AND METHODS

The experiment was conducted for 60 days from September to November, 2002.

3.1 EXPERIMENTAL LABORATORY:

The experiment was conducted in the wet laboratory of the Department of Aquaculture, College of Fisheries.

3.2 EXPERIMENTAL TANKS:

The experiment was conducted in round cement cisterns and oval fiberglass tanks. Round cement cistern having a capacity of 100 litres served as hatching tank whereas the oval fiberglass tank having a capacity of 800 litres used as rematuration tank.

3.3 EXPERIMENTAL ANIMALS:

Farm reared healthy berried females of *M. rosenbergii* from a single aged population were brought to the *Macrobrachium* hatchery of the College and maintained in oval fiberglass tanks. The length and weight was measured for each berried females and the length and weight varied from 145-170 mm and 35-45 g respectively. Sufficient numbers of strong blue clawed (SBC) males of *M. rosenbergii* were procured from the wild for mating purpose to be introduced along with the spent females. The length and weight of the SBC males varied from 210-250 mm and 140-160 g respectively.

3.4 FEED:

The animals were fed with fresh clam meat.
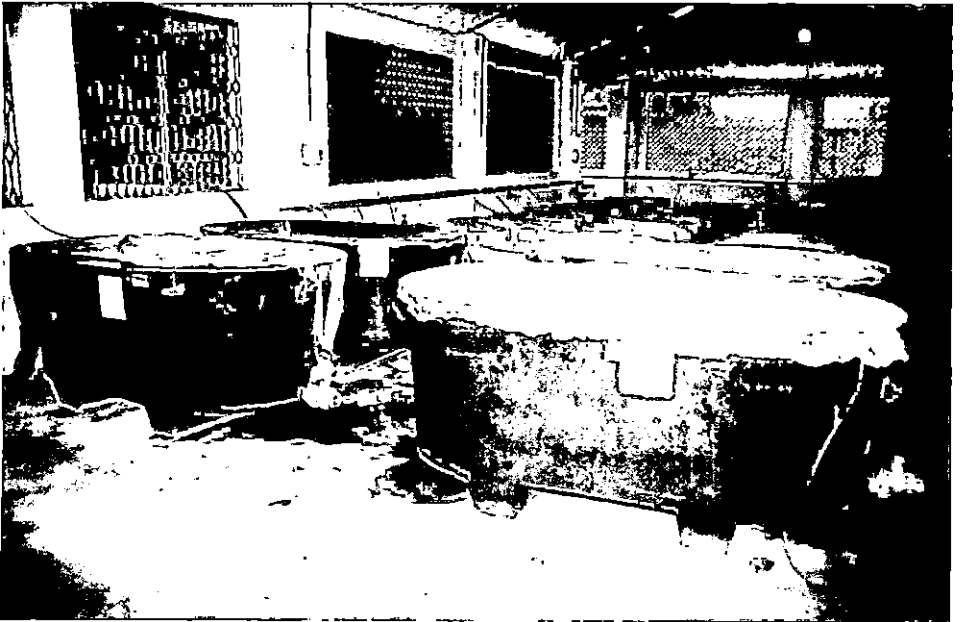
Plate 1. Experimental setup

3.5 EYESTALK ABLATION:

Eyestalk ablation was effected in the early hours of the day by severing the left eyestalk from the body at narrow proximal end in the region of articulating membrane using a pair of fine sterilized scissors (Caillouet, 1973). The ablation was done after dipping the spent female prawns in antibiotic (oxytetracycline - 5 ppm) treated water.

3.6 EXPERIMENTAL PROCEDURE:

3.6.1 Experiment to study the effect of eyestalk ablation on rematuration of spent female of *M. rosenbergii*

Forty healthy berried females were selected and individually stocked in 40 nos. of cement cisterns of 100 litres capacity. Estimation of larval hatch fecundity of the experimental animal was individually carried out during first time hatching.

Eyestalk ablation was performed in 20 nos of spent females and remaining 20 nos kept unablated. Ablated and unablated females were introduced separately in oval fiberglass tanks of 800 litres capacity immediately after hatching along with male at the ratio of 1 male: 4 females for rematuration.

Observations on moulting frequency, spawning frequency, larval hatch fecundity and incubation period of ablated and unablated spent females of *M. rosenbergii* were individually carried out for the period of 60 days.

3.6.2 To study the histology of the ovarian development during rematuration of eyestalk ablated spent female and normal spent female at weekly intervals

Histological study of the ovary during rematuration of the eyestalk ablated and unablated female prawns were carried out from spent ovary to the ripe ovary at each week of development. For

histological study, female prawn from the above experiment was taken out from both ablated and unablated individuals at each week interval.

The ovaries were dissected out and fixed in aqueous Bouin's fixative for 24 hours.

Composition of aqueous Bouin's fixative:

Picric acid (saturated aqueous solution)	-	75 ml
Formaldehyde (40 percent)	-	25 ml
Glacial acetic acid	-	5 ml

After fixation, the tissues were processed following the technique of Pantin (1948).

1. Transferred to 70% alcohol.

2. Transferred to 90% alcohol for 2 hrs.

3. Transferred to 95% alcohol for 1 hr.

4. Transferred to two changes of absolute alcohol for 1 hr each.

5. Placed the tissue in 1:1 mixture of absolute alcohol and methyl benzoate for 30 minutes.

6. Cleared in methyl benzoate until the tissues became transparent.

7. The tissues were transferred to xylene saturated with paraffin wax at melting point 58-60 °C for 6 hrs.

8. Infiltered the tissue in 2-3 changes of molten paraffin wax of melting point 58-60 °C for 1 hr each.

9. Embedded the tissue in paraffin wax of melting point 60-62 °C.

The blocks were sectioned at 6-8 μ thickness and mounted on glass slides. Mayer's Haemalum stain was used (Humason, 1982).

Staining technique with Mayer's Haemalum (Humason, 1982):

Mayer's Haemalum:

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

Scott's solution:

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

Eosin Y:

Eosin Y	-	2 g
70% Ethyl alcohol	-	1000 ml
Glacial acetic acid	-	5 ml

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

Procedure:

1. Deparaffinised and hydrated slides to water.
2. Stained in Mayer's Haemalum – 3 minutes.
3. Washed in running water – 3 minutes.
4. Blued in Scott's solution – 3 minutes.
5. Washed in running water – 3-5 minutes.
6. Counted stained in Eosin Y- 1-2 minutes.
7. Dehydrated quickly through 70% and 90% alcohols.
8. Dehydration cleared in xylene and mounted with DPX.

The slides were examined by light microscopy and photographed using a binocular microscope and Nippon camera combination at high power.

3.6.3 To study the ovarian index of eyestalk ablated and unablated spent females of *M. rosenbergii* at each week interval

For ovarian index study 24 nos. of spent females of *M. rosenbergii* were used. After first time hatching 12 nos of prawn were ablated and another 12 nos were kept unablated. The ablated and unablated spent female prawns were kept along with male in the ratio of 1male : 4 females for mating separately. The ovarian index at each week interval was estimated for eyestalk ablated and unablated spent female.

3.6.4 Experimental tank management

3.6.4.1 Stocking

The round cement cisterns which served as hatching tanks were stocked with one berried female at a time. While the oval fiberglass tanks which served as rematuration tank were stocked with 1 male and 4 spent females. All the spent females of both the groups were identifiably marked by making small cut on the posterior end of uropod.

The water levels in the hatching tank and rematuration tank were maintained at 50 cm and 70 cm respectively. The rematuration tanks were covered with plastic mesh nets to prevent escape of prawns by jumping. Hideouts using bricks and tiles were provided at the bottom of the tanks as a shelter for the moulting prawns to reduce cannibalism.

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3.6.4.2 Feeding

Fresh clam meat was given *ad libitum*. Feeding was done twice a day during morning and evening, after removing the left over feed of the previous feeding.

3.6.4.3 Water quality maintenance

Continuous mild aeration was provided to ensure sufficient dissolved oxygen. About 20% of the water in the tanks was exchanged daily by siphoning and refilling. The exuviae, feed remnants etc., were removed during the water exchange. The animals were closely examined for health and to assess their number during morning, noon, evening and night.

3.7 ANALYSIS OF WATER QUALITY PARAMETERS:

The following instruments / methods were used for the analysis of water quality parameters.

Dissolved-oxygen	:	Winkler's method (Strickland and Parsons, 1972)
pH	:	By using Universal indicator (Qualigens)
Temperature	:	By using Mercury thermometer of 0 to100 ° C

3.8 EVALUATION METHODS:

3.8.1 Moulting frequency: (Guary *et al.*, 1976)

All the rematuration tanks were checked four times a day (morning, noon, evening and night) for presence of exuviae. Moulting frequency was recorded individually on the basis of presence of exuviae in the rematuration tanks for both ablated and unablated females for the period of 60 days.

3.8.2 Spawning frequency: (Murugadass *et al.*, 1988)

Rematuration tanks were checked four times a day (morning, noon, evening and night) for presence of berried female. Spawning frequency was recorded individually on the basis of presence of berried female in the rematuration tanks for both ablated and unablated females for the period of 60 days.

3.8.3 Larval hatch fecundity: (Ang and Kok, 1991)

Hatching tanks were observed twice a day (morning and night) for presence of larvae. Larval hatch fecundity was recorded for first and second time hatching in both ablated and unablated prawns individually. Larval hatch fecundity was counted by collecting the hatched larvae into plastic bucket from hatching tank. The volume of water in the bucket was brought down to 2 litres. The larval hatch fecundity was estimated from the average of three samples taken after stirring the water to ensure uniform distribution.

3.8.4 Incubation period:

Incubation period in number of days was recorded individually for eyestalk ablated and unablated berried females (Rao, 1986; Bijulal, 1994).

3.8.5 Ovarian index (OI):

The ovarian index was calculated for each week interval for both eyestalk ablated and unablated female prawns using the following formula (Adiyodi and Adiyodi, 1983).

$$OI = \frac{\text{Wet weight of ovary}}{\text{Wet weight of body}} \times 100$$

For this purpose, the animal was first weighed using a Contech (Model CB 120) Electronic Balance. Then the ovary was dissected out and weight of the ovary noted. For each week interval OI was calculated from the average of 2 ovaries from one week interval time.

3.8.6 Microscopic observation of ovarian development:

Microscopic observation of ovarian development was done by histological study in both ablated and unablated female prawn at each week interval. The oocyte diameter was measured from the slide using calibrated ocular micrometer (ERMA, Japan) (Humason, 1982).

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3.9 STATISTICAL DESIGN AND ANALYSIS:

In the experiment to find out the effect of eyestalk ablation on moulting frequency, spawning frequency, larval hatch fecundity and incubation period, student's 't'- test has been used for the comparison between ablated and unablated groups. In the case of comparison of larval hatch fecundity after first time hatching and second time hatching of both ablated and unablated female prawns paired t-test has been used. Range and mean values were calculated for observations on water quality parameters.

Results

4. RESULTS

The effect of eyestalk ablation on moulting frequency, spawning frequency, larval hatch fecundity, incubation period, ovarian development and ovarian index in spent females of *M. rosenbergii* was evaluated using standard procedures. The details of the observations made during the study are presented below.

4.1 EXPERIMENT TO STUDY THE EFFECT OF EYESTALK ABLATION ON MOULTING FREQUENCY, SPAWNING FREQUENCY, LARVAL HATCH FECUNDITY AND INCUBATION PERIOD OF SPENT FEMALE OF *M. ROSENBERGII*

The data of the experiment to find out the effect of eyestalk ablation on moulting frequency, spawning frequency, larval hatch fecundity and incubation period in spent female prawns for 60 days period are given below. The experiment was conducted using 28 numbers of spent females of *M. rosenbergii*.

4.1.1 Effect of eyestalk ablation on moulting frequency

The moulting frequencies in ablated and unablated spent females of *M. rosenbergii* are presented in Table 3.

The highest moulting frequency noticed in ablated female was 3 and 2 in the case of unablated during the experimental period. The average number of moults was 1.93 per female in the case of ablated and 1.50 in unablated spent females of *M. rosenbergii*. Comparison of average moulting frequency by student's t-test showed that there is significant increase in moulting frequency after eyestalk ablation.

Table 3. Moulting frequency in eyestalk ablated and unablated spent females of *M. rosenbergii* for 60 days

Sr. No.	Ablated				Unablated			
	Size of animal		MF*	Average MF	Size of animal		MF*	Average MF
	Length (cm)	Weight (g)			Length (cm)	Weight (g)		
1	15.5	42.0	2		14.5	35.5	2	
2	16.5	35.5	2		16.5	42.0	1	
3	14.5	39.0	3		15.5	43.5	2	
4	15.5	42.0	2		16.0	36.5	1	
5	14.5	35.0	2		14.5	35.0	2	
6	16.5	44.0	2		16.6	36.0	2	
7	14.5	39.0	2	1.93	15.5	38.0	2	1.50
8	15.5	40.5	2		14.5	35.0	1	
9	16.0	38.5	1		14.5	35.5	2	
10	15.5	38.0	2		17.0	45.0	1	
11	16.0	43.5	2		16.5	42.0	1	
12	15.0	42.5	2		15.5	40.0	1	
13	14.5	40.0	1		14.5	39.0	2	
14	15.5	39.0	2		15.6	43.5	1	

* MF-Moulting frequency

4.1.2 Effect of eyestalk ablation on spawning frequency

The spawning frequencies of eyestalk ablated and unablated spent females of *M. rosenbergii* are presented in Table 4.

Table 4 showed that the average number of spawns was 1.71 in the case of ablated ones and 1.28 in unablated spent females of *M. rosenbergii*. Student's t- test showed that there is significant increase in average spawning frequency after eyestalk ablation of spent female of *M. rosenbergii*. In the present study it was observed that in the case of ablated spent females 89% of the moults were berried and 11% were neuter while in the case of unablated spent females 86% of moults were berried and 14% were neuter. Thus it could be seen that eyestalk ablation not only increased moulting frequency but also frequency of berried moults in spent females of *M. rosenbergii*.

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Table 4. Spawning frequency in eyestalk ablated and unablated spent females of *M. rosenbergii* for 60 days

Sr. No.	Ablated				Unablated			
	Size of animal		SF*	Average SF	Size of animal		SF*	Average SF
	Length (cm)	Weight (g)			Length (cm)	Weight (g)		
1	15.5	42.0	2		14.5	35.5	2	
2	16.5	35.5	2		16.5	42.0	1	
3	14.5	39.0	2		15.5	43.5	1	
4	15.5	42.0	2		16.0	36.5	1	
5	14.5	35.0	1		14.5	35.0	1	
6	16.5	44.0	2		16.6	36.0	2	
7	14.5	39.0	2	1.71	15.5	38.0	1	1.28
8	15.5	40.5	2		14.5	35.0	1	
9	16.0	38.5	1		14.5	35.5	2	
10	15.5	38.0	2		17.0	45.0	1	
11	16.0	43.5	1		16.5	42.0	1	
12	15.0	42.5	2		15.5	40.0	1	
13	14.5	40.0	1		14.5	39.0	2	
14	15.5	39.0	2		15.6	43.5	1	

*SF- Spawning frequency

4.1.3 Effect of eyestalk ablation on larval hatch fecundity

Larval hatch fecundity recorded during first time and second time hatching of eyestalk ablated and unablated spent females of *M. rosenbergii* are presented in Table 5 and Table 6 respectively.

The average larval hatch fecundity per female of *M. rosenbergii* was 21923 during first time hatching (before ablation) and 17423 during second time hatching (after ablation) for ablated group. In case of unablated group the average larval hatch fecundity was 21410 during first time hatching and 16147 during second time hatching. From the averages of larval hatch fecundity per female, it could be seen that there is reduction of larval numbers during second time hatching in both ablated and unablated spent female of *M. rosenbergii*. Pair-wise comparison of larval hatch fecundity by paired t-test showed that there is significant decrease in the larval hatch fecundity during second time hatching in both eyestalk ablated and unablated spent female of *M. rosenbergii*.

Comparison of larval hatch fecundity by using student's t-test showed that there is no significant difference in larval hatch fecundity of ablated and unablated spent female of *M. rosenbergii* during second time hatching.

4.1.4 Effect of eyestalk ablation on incubation period

The incubation period of eyestalk ablated and unablated spent females of *M. rosenbergii* are presented in Table 7.

The incubation period ranged from 15 to 20 days with an average of 18.8 ± 1.53 days in ablated spent female while it ranged from 18 to 21 days with an average of 19.6 ± 0.76 days in unablated spent female of *M. rosenbergii*.

Student's t-test showed no significant difference in incubation period of eyestalk ablated and unablated spent females of *M. rosenbergii*.

Table 7. Incubation period of eyestalk ablated and unablated spent females of *M. rosenbergii*

Sr. No.	Incubation period (days)	
	Ablated	Unablated
1	15	19
2	19	18
3	20	20
4	19	21
5	17	20
6	18	20
7	20	19
8	19	19
9	20	19
10	20	20
11	19	20
12	20	19
13	20	20
14	17	20

4.2 EXPERIMENT TO STUDY THE HISTOLOGY OF THE OVARIAN DEVELOPMENT DURING THE REMATURATION OF EYESTALK ABLATED AND UNABLATED SPENT FEMALE OF *M. ROSENBERGII* AT WEEKLY INTERVALS

In the experiment to study the histology of the ovary, histological sections of the ovaries of ablated and unablated *M. rosenbergii*, were taken starting from spent ovary to the ripe ovary at weekly intervals in order to study the histological changes of the ovary during rematuration.

4.2.1 Histology of ovary in *M. rosenbergii*

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

I) Oogonial stage

These are small, spherical, basophilic cells with large and round nuclei (Plate 3). These cells are characterized by a prominent nucleus containing chromatin granules and a thin layer of cytoplasm. They are lacking stainable yolk material. In the immature ovary, oogonial cells are highly aggregated near the germinal zone. Oogonia develops into previtellogenic oocyte. Oogonial cells have a diameter ranging from 30 to 35 μ. The average nuclear diameter is 15 μ.

II) Pre-vitellogenic oocytes

A large amount of basophilic cytoplasm is acquired by previtellogenic oocytes (Plate 3). Yolk formation has not yet begun. Large dense active nucleus with prominent nucleolus is present. The previtellogenic oocytes are surrounded partly by follicle cells. The average nuclear diameter is 19 μ. This oocyte has a diameter ranging from 43 to 66 μ. This stage corresponds to maturity stage I.

III) Vitellogenic oocytes

This is the synthetic phase of the oocytes in which yolk synthesis took place. This stage is marked by considerable changes in nucleus, nucleolus and ooplasm, and is divided in to three sub stages.

a) Early vitellogenic oocytes

These are spherical in shape with conspicuous nucleus and nucleolus (Plate 4). Small yolk droplets appeared in a single concentric layer at the periphery of the ooplasm, which stained purple to black with Mayer's Haemalum. Small round follicular cells appeared completely around the oocytes (Plate 8A). The average diameter of nucleus is 26 μ. Oocyte has a diameter ranging from 70 to 92 μ. This stage corresponds to maturity stage II.

b) Vitellogenic oocytes

The oocytes further increased in size. Small unstainable vacuoles appeared in the ooplasm, which later fused together to form large unstainable yolk vesicles (Plate 5). Small eosinophilic yolk granules started accumulating in the peripheral region of the oocortex. Many yolk vesicles were found compared to earlier stage. Follicle cells completely surround the oocytes. Nucleus appeared inconspicuous. The nucleur diameter is 39 μ. Oocyte has a diameter ranging from 95 to 110 μ. This stage corresponds to maturity stage III.

c) Late vitellogenic oocytes

Yolk droplets converted into yolk vesicles, which are strongly eosinophilic (Plate 6). Most of the ooplasm including the perinuclear region was occupied by yolk platelets (Plate 8B). The entire ooplasm thus become acidophilic. The follicular layer becomes thinner. The oocyte diameter ranged from 211 to 239 μ. This stage corresponds to maturity stage IV (maturing).

IV) Matured oocytes

The oocyte attained their utmost size in this phase (Plate 7). The ooplasm is completely filled by yolk vesicles (yolk platelets). The germinal vesicle disappears and ova increase enormously in size. No follicle cells were seen around them and they are surrounded by a vitelline envelope. This oocyte has a diameter up to 380 to 413 μ. This stage corresponds to maturity stage V (ripe).

4.2.2 Effect of eyestalk ablation on ovarian development in spent females of *M. rosenbergii*.

Comparison of ovarian development of ablated and unablated spent female of *M. rosenbergii* after each week of development is described below.

1) Spent ovaries

Spent ovaries were characterized by partially empty cordons after oviposition (Plate 2).

2) Ovaries of female bearing eggs after one week of development

The largest oocytes in the female, which bear eggs, were observed in previtellogenic stage in both ablated (Plate 3A) and unablated (Plate 3B) prawns after one week of development. The mean

size of the oocyte was 60.02 ± 6.15 μ in ablated and 52.03 ± 7.53 μ in unablated.

3) Ovaries of female bearing eggs after two weeks of development

The largest oocytes in females bearing eggs were in early vitellogenic stage in both ablated (Plate 4A) and unablated (Plate 4B) after two-weeks of development. The mean size of oocyte in ablated and unablated was 82.11 ± 9.63 μ and 75.02 ± 5.04 μ respectively.

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4) Ovaries of female after three weeks of development
   (immediately after hatching)

In ablated (Plate 5A) and unablated (Plate 5B) females the oocytes were at the vitellogenic stage after three weeks of development. The mean size of oocyte was 103.01 ± 7.53 μ and 96.03 ± 1.73 μ respectively for ablated and unablated.

5) Ovaries of female after four weeks of development
   (one week after hatching)

In ablated (Plate 6A) and unablated (Plate 6B) females of *M. rosenbergii* after four weeks of development the oocytes were at late vitellogenic stage. The mean size of oocyte in ablated and unablated was 235.00 ± 4.53 μ and 223.21 ± 12.03 μ respectively.

6) Ovaries of female after five weeks of development
   (two weeks after hatching)

Ripe oocytes were observed in ablated (Plate 7A) and unablated (Plate 7B) female of *M. rosenbergii* after five weeks of development. No follicle cells were seen around the ripe oocytes. The oocytes were

surrounded by a vitelline envelope. The mean size of the oocyte was 401.03 ± 12.53 μ in ablated and 391.01 ± 10.30 μ in unablated prawn.

From the histological observations of the ovaries of female after each week of development, it was observed that similar oocytes were noticed in eyestalk ablated and unablated prawn after each week of development. However, it is observed that the size of the oocytes in ablated ones after each week of development is slightly higher than the unablated ones. This indicates that eyestalk ablation enhances the ovarian development. The present study delineates that the development of oocytes in the ovary was continued when the female is bearing the eggs between the pleopods.
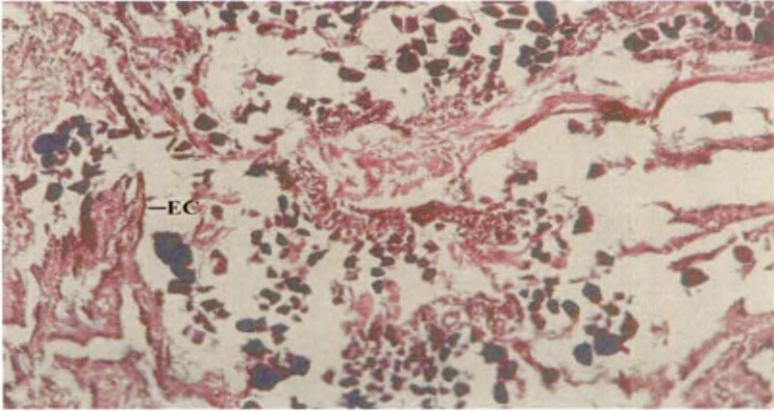
(A)
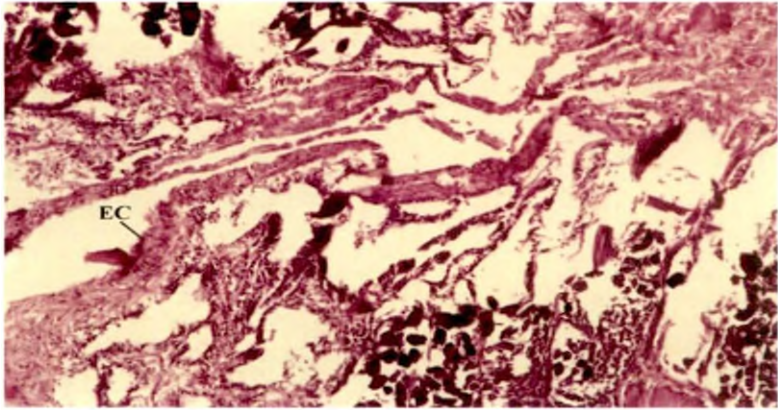
(B)

Plate 2. Photomicrographs of spent ovaries of *M. rosenbergii* showing empty cordons (EC). Mayer's Haemalum and Eosin Y staining 6-8 μ sections x 100

(A) Spent ovary of Ablated (B) Spent ovary of Unablated
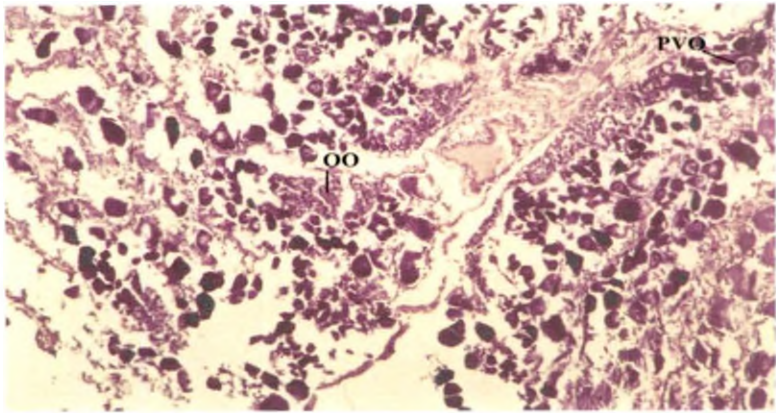
(A)
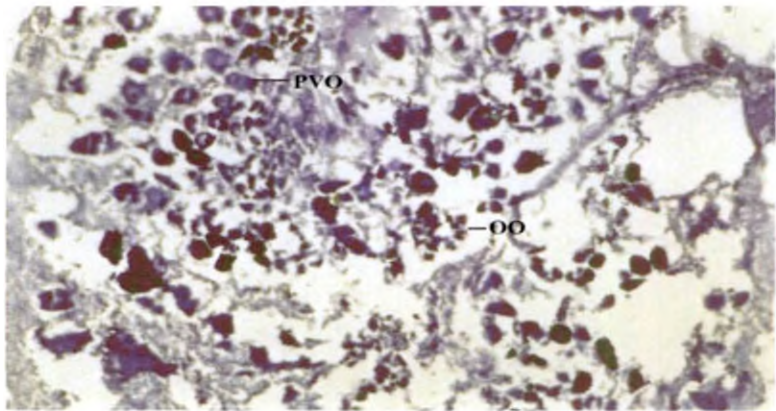
(B)

Plate 3. Photomicrographs of ovaries of female *M. rosenbergii* bearing eggs after one week of development showing Previtellogenic oocyte (PVO) and Oogonia (OO). (Maturity stage I) Mayer's Haemalum and Eosin Y staining 6-8 μ sections x 100

(A) Ablated   (B) Unablated
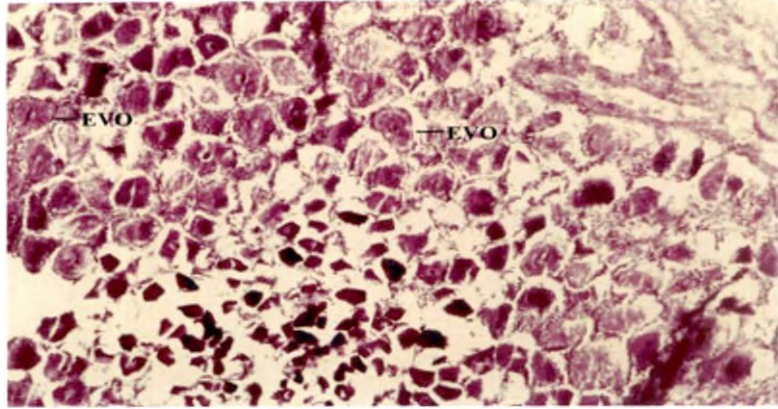
(A)
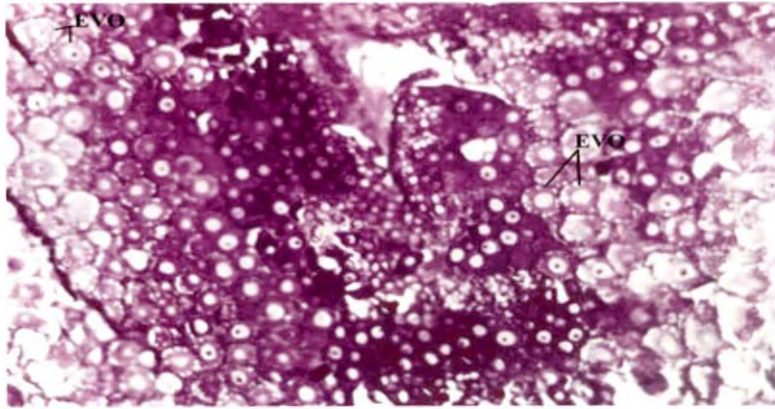
(B)

Plate 4. Photomicrographs of ovaries of female *M. rosenbergii* bearing eggs after two weeks of development showing early vitellogenic oocyte (EVO) (Maturity stage II). Mayer's Haemalum and Eosin Y staining 6-8 μ sections x 100

(A) Ablated   (B) Unablated
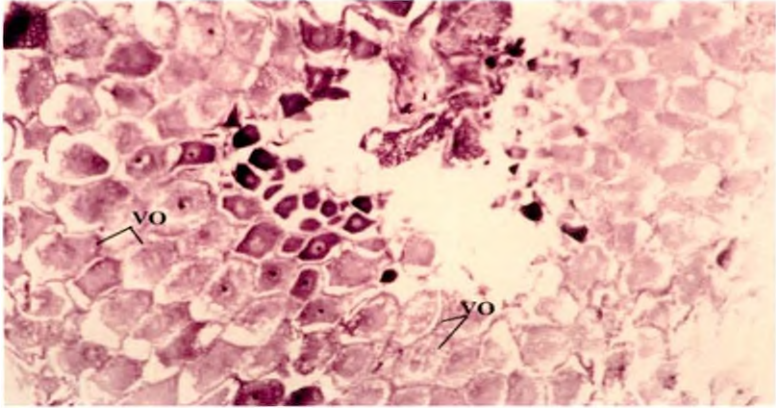
(A)
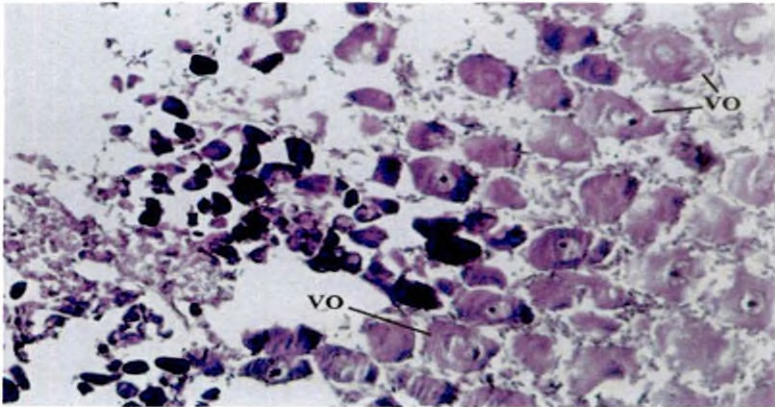
(B)

Plate 5. Photomicrographs of ovaries of female *M. rosenbergii* after three weeks of development (immediately after hatching) showing Vitellogenic oocyte (VO) (Maturity stage III). Mayer's Haemalum and Eosin Y staining 6-8 μ sections x 100

(A) Ablated   (B) Unablated
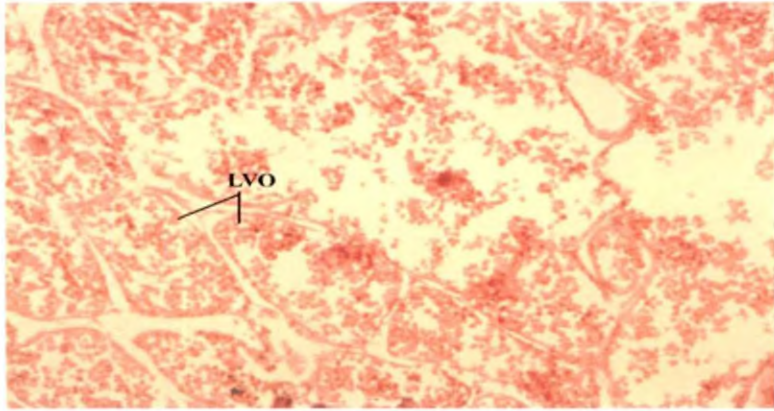
(A)
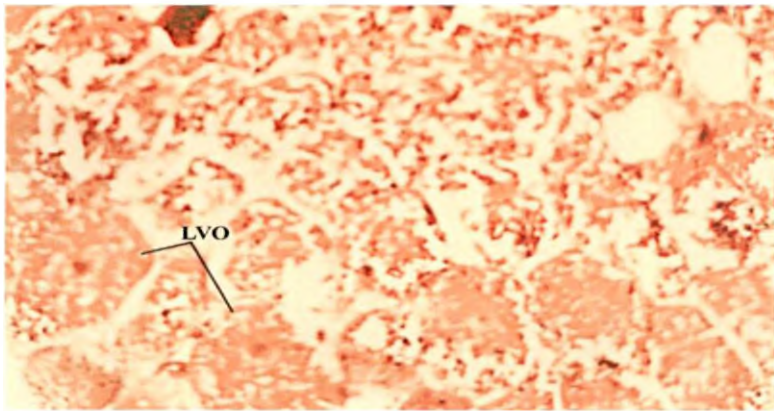
(B)

Plate 6. Photomicrographs of ovaries of female *M. rosenbergii* after four weeks of development (one week after hatching) showing Late vitellogenic oocyte (LVO) (Maturity stage IV). Mayer's Haemalum and Eosin Y staining 6-8 μ sections x 100

(A) Ablated   (B) Unablated

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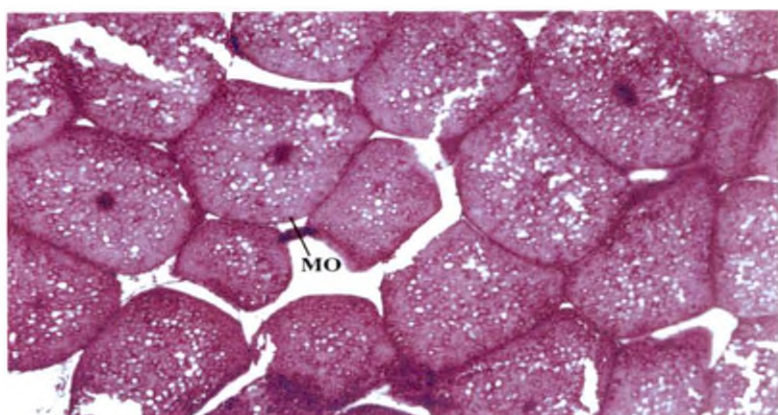
(A)
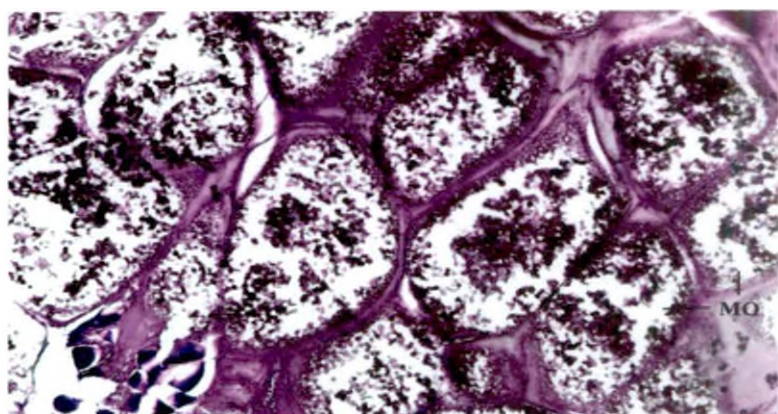
(B)

Plate 7. Photomicrographs of ovaries of female *M. rosenbergii* after five weeks of development (two weeks after hatching) showing Matured oocyte stage (MO) (Maturity stage V). Mayer's Haemalum and Eosin Y staining 6-8 μ sections x 100

(A) Ablated   (B) Unablated
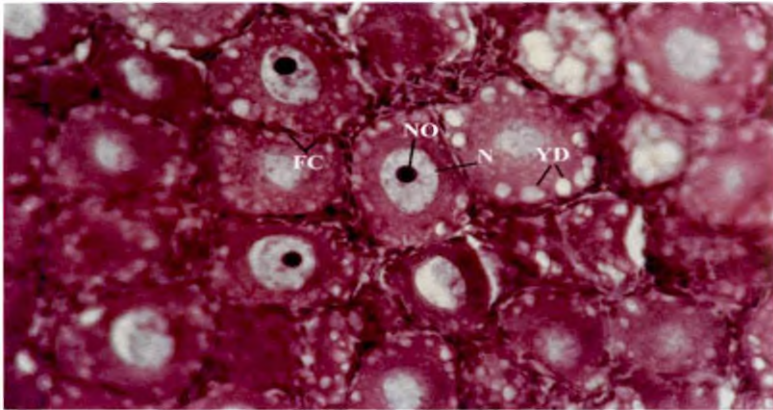
(A)
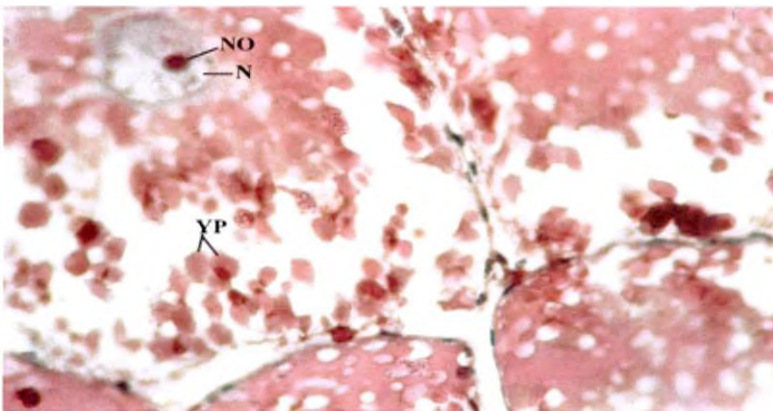
(B)

Plate 8. Photomicrographs of ovaries of _M. rosenbergii._ Mayer's Haemalum and Eosin Y staining 6-8 μ sections x 400.

A) Photomicrograph of ovary in Maturity stage II, showing early vitellogenic oocyte with nucleus (N), nucleolus (NO), yolk droplets (YD) and Follicle cells (FC)

B) Photomicrograph of ovary in Maturity stage IV showing late vitellogenic oocyte with nucleus (N), nucleolus (NO) and yolk platelets (YP)

4.3 EFFECT OF EYESTALK ABLATION ON OVARIAN INDEX (OI)

The average ovarian indices (OI) from spent ovary to ripe stage at each week interval for eyestalk ablated and unablated spent female of *M. rosenbergii* are given in Table 8.

Table 8. **Ovarian index of eyestalk ablated and unablated spent females of *M. rosenbergii***

Weeks	Average Ovarian Index (%)	
	Ablated	Unablated
0 (Spent)	0.43	0.42
I	0.45	0.44
II	0.54	0.47
III	0.64	0.63
IV	3.71	3.51
V	9.20	9.10

The progressive increase of OI from week 0 to V for ablated and unablated spent females of *M. rosenbergii* are graphically presented in Fig.1. Generally, higher ovarian index value was observed after fourth and fifth week in both ablated and unablated *M. rosenbergii*. It was lowest in spent stage (0.43 in ablated and o.42 in unablated) and highest in the fifth week (9.20 in ablated and 9.10 in unablated). It could be seen from the Table 8 and Fig. 1 that in all the five weeks of observation ovarian index of ablated spent female of *M. rosenbergii* are higher.

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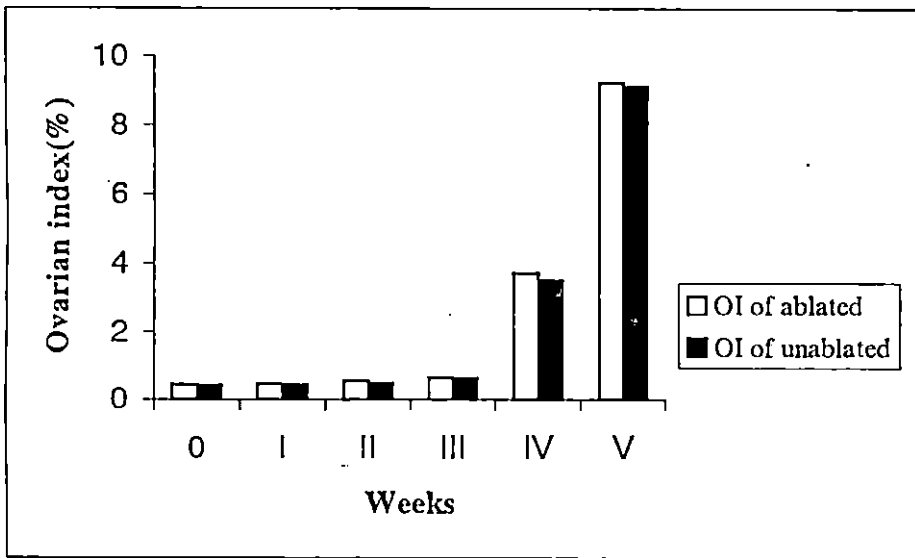
Fig. 1. The progressive increase of ovarian index (OI) in ablated
and unablated spent females of *M. rosenbergii*

4.4 OBSERVATIONS OF WATER QUALITY PARAMETERS

The water quality parameters such as temperature, pH, dissolved oxygen noted during the study period are given in the Tables 9,10 and 11 respectively.

Table 9. Water temperature in the experimental tanks during the study period

Weeks	Temperature (°C)	
	Range	Mean
1	24.60-26.50	25.46
2	24.50-27.80	25.96
3	24.30-27.10	25.54
4	24.50-28.10	26.04
5	24.30-26.40	25.39
6	23.10-28.10	25.84
7	24.00-26.30	25.31
8	23.80-27.10	25.11
9	25.30-26.30	25.83

Table 10. pH values observed in the experimental tanks during the study period

Weeks	pH	
	Range	Mean
1	6.5-8.0	7.36
2	6.5-8.5	7.36
3	7.0-8.5	7.50
4	6.5-8.5	7.17
5	7.0-8.0	7.43
6	7.0-7.5	7.29
7	6.5-8.0	7.36
8	7.0-8.0	7.43
9	7.5-8.5	8.00

Table 11. **Dissolved oxygen content in the experimental tanks**
during the study period

Weeks	Dissolved oxygen (ppm)	
	Range	Mean
1	6.13-6.75	6.47
2	6.34-7.30	6.76
3	6.32-7.80	7.00
4	6.28-8.10	7.71
5	6.13-7.53	6.64
6	6.23-8.10	7.37
7	6.23-8.25	6.80
8	6.03-7.93	6.81
9	6.07-7.10	6.58

It could be seen from the Tables 9, 10 and 11 that the temperature ranged from 23.1 to 28.1 °C, pH from 6.5 to 8.5 and dissolved oxygen from 6.03 to 8.25 ppm. All the parameters were more or less within the optimum levels needed for the prawn *M. rosenbergii*.

Discussion

5. DISCUSSION

5.1 EFFECT OF EYESTALK ABLATION ON MOULTING FREQUENCY

The experiment conducted to evaluate the effect of eyestalk ablation on moulting frequency in spent females of *M. rosenbergii* revealed that eyestalk ablation significantly enhances the moulting frequency. It could be seen from the result of the present study that the average number of moults was 1.93 per female in the case of ablated ones whereas it was 1.50 in unablated ones for the period of 60 days (Table 3). Comparison of moulting frequency by student's t- test showed that there is significant difference in the moulting frequency of eyestalk ablated and unablated spent female of *M. rosenbergii*. This revealed that there is significant increase in moulting frequency after eyestalk ablation in spent female of *M. rosenbergii*. This result is in agreement with the known fact that eyestalk ablation in crustaceans enhances either precocious moulting or gonadal development (Adiyodi and Adiyodi, 1970; Caillouet, 1973; Arnstein and Beard, 1975; Emmerson, 1980,1983).

Huang *et al.* (1981) studied the effect of eyestalk ablation on growth and moult of *M. rosenbergii* and found shortening of moult interval in ablated individuals. Kumari and Pandian (1987) reported that eyestalk ablation accelerated moulting frequency in both sexes of *M. nobilii*. They observed that in 330 days experiment ablated female prawns undertook nine moults as adults while nonablated prawns undertook four moults as adults. Murugadass *et al.* (1988) reported higher moulting frequency in destalked males and females of *M. malcolmsonii* as compared to control. They observed that moulting frequency was 14 nos in destalked females as against 12 nos in control females while in the case of male prawn moulting frequency was 13

nos in destalked ones and 11 nos in control ones during the 240 days experimental period.

Inducement of precocious moulting in male prawn of *M. rosenbergii* through eyestalk ablation has been reported by Chakravarty (1992). He recorded higher moulting frequency in bilaterally ablated males of *M. rosenbergii* compared to unilaterally ablated and unablated ones during a period of 130 days. Bijulal (1994) observed enhancement of moulting frequency in ablated female prawn of *Macrobrachium equidens* during 60 days experiment.

Soundarapandian *et al.* (1995) reported frequent moulting in ablated female prawns of both *M. rosenbergii* and *M. malcolmsonii* than controls. They noticed higher moulting frequency in small sized females than in larger ones. Sanjeeviraj *et al.* (1997) studied the effect of eyestalk ablation on enhancing moulting frequency in *M. rosenbergii* and observed that moulting frequency was more in smaller size group than bigger size group. Okumura and Aida (2001) reported that eyestalk ablation caused shortening of moulting interval and increase in haemolymph ecdysteroid levels in *M. rosenbergii*.

Sudarsanam *et al.* (1990) reported that ablated shrimps had higher individual moulting rate compared to non ablated ones in *Penaeus monodon*. Similar results were reported by Emmerson (1983) in *P. monodon* and by Petriella and Diaz (1987) in *Artemesia longinaris*.

An enhancement of moulting frequency observed in the present investigation is in agreement with the result reported by Huang *et al.* (1981), Chakravarty (1992), Soundarapandian *et al.* (1995), Sanjeeviraj *et al.* (1997) and Okumura and Aida (2001) in the same species. The result of the present study conforms to the result reported in other *Macrobrachium* species by Kumari and Pandian (1987) in *M. nobilii*,

Murugadass *et al.* (1988) in *M. malcolmsonii* and Bijulal (1994) in *M. equidens.*　　　　　　o

Enhancement of moulting frequency of spent female of *M. rosenbergii* by eyestalk ablation as observed in the present study is also in agreement with the result obtained in penaeid prawns (Emmerson, 1983; Petriella and Diaz, 1987; Sudarsanam *et al.,* 1990).

The arrest of moulting activity during the incubation period of female *M. rosenbergii* as observed in the present study may be because of the presence of developing eggs in brood pouch, which are to be hatched. Bijulal (1994) made similar observations in female of *M. equidens.* The presence of developing eggs in the brood pouch delays moulting in crustacea (Schone, 1961). Scudamore (1948) working on crayfish found that brooding induced prolonged production of MIH in X-organ sinus gland complex. Kamaguchi (1971) made similar observations in freshwater prawn *Palaemon paucidens.* In the present study also because of the increase in MIH production during berried condition, moulting was arrested in female *M. rosenbergii.* It may be assumed that the release of larvae might be responsible for stopping the production of MIH and accelerating MH activity. The physiological and histological evidence suggests a high titre of MIH and a comparatively low titre of GIH in the blood during intermoulting period in reproducing individuals. The high titre of MIH is essential for reproduction, which it facilitates indirectly by restraining the Y-organ (Adiyodi, 1985).

Studies on the possible effect of eyestalk removal on moulting frequency in crustacean by Brown and Cunningham (1939) and Smith (1940) suggested that eyestalk contain moulting hormone which delays moulting. Similarly, Kulkarni and Nagabhusanam (1980) suggested that declining levels of the moult-inhibiting hormone (MIH) and gonad-stimulating hormone (GSH) in ablated *Parapenaeopsis hardwickii,*

which increases the moulting rate. Unilateral eyestalk ablation has been shown to decrease levels of MIH secreted from the sinus gland and increase the levels of ecdysteroids directly leading to higher moulting rate in crustaceans (Lachaise *et al.*, 1993).

In the present study, eyestalk ablation significantly enhanced moulting frequency of spent female of *M. rosenbergii*. Therefore, it can be inferred that eyestalk ablation might have reduced the level of MIH and there by increased the moulting frequency in ablated spent female of *M. rosenbergii* as suggested by Huang *et al.* (1981), Chakravarty (1992), Soundarapandian *et al.* (1995), Sanjeeviraj *et al.* (1997) and Okumura and Aida (2001).

5.2 EFFECT OF EYESTALK ABLATION ON SPAWNING FREQUENCY

The result of the experiment conducted to study the impact of eyestalk ablation on spawning frequency in spent females of *M. rosenbergii* revealed that eyestalk ablation significantly increased spawning frequency. From the result of the present study, it could be seen that the average number of spawns per female was 1.71 in the case of eyestalk ablated and 1.28 in unablated case for the period of 60 days (Table 4). Comparison of spawning frequency by using student's t-test showed that there is significant difference in the spawning frequency of ablated and unablated spent female of *M. rosenbergii*. This indicates that there is significant increase in spawning frequency after eyestalk ablation in spent female *M. rosenbergii*.

Murugadass *et al.* (1988) reported higher spawning frequency in ablated female *M. malcolmsonii* compared to unablated female *M. malcolmsonii*. They noticed that spawning frequency was 7 nos in ablated females compared to 5 nos in unablated ones during the 240 days experiment.

Yano and Wyban (1993) showed that eyestalk ablation increased spawning frequency in female *Penaeus vannamei* under tank culture conditions. Bijulal (1994) observed increase in spawning frequency after eyestalk ablation in female *M. equidens*. He reported 2.25 spawns per female in case of ablated ones compared to 1.625 in case of unablated ones during 60 days experiment. Experiment conducted by Racotta *et al.* (2000) revealed that the proportion of spawning females and spawning frequency was greater for ablated females than unablated females of *P. vannamei*.

In the present study it was observed that in ablated females 89% of the moults were berried and remaining 11% were neuter while, in the case of unablated females 86% of moults were berried and 14% were neuter. It may be assumed that eyestalk ablation not only increased moulting frequency but also frequency of berried moults. Similar result was reported by Murugadass *et al.* (1988) in female *M. malcolmsonii*. They observed that in control females only 40% moults were berried while 60% were neuter whereas in the case of destalked females 50% of moults were berried.

In reptantians, moulting and reproduction are antagonistic process on the other hand in natantians moulting and spawning are synergistic events (Pandian and Balasundaram, 1982). In the present study, spawning is closely related to moulting and occurred always in the post moult period as observed by Shyama (1987) in female *M. idella* and Bijulal (1994) in female *M. equidens*.

The increase in spawning frequency in ablated spent female of *M. rosenbergii* is in accordance with the results obtained by Murugadass *et al.* (1988) in *M. malcolmsonii*, Bijulal (1994) in *M. equidens* and in *P. vannamei* by Yano and Wyban (1993) and Racotta *et al.* (2000).

It can be inferred from the present study that eyestalk ablation in spent female of *M. rosenbergii* had reduced the level of gonad

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inhibiting hormone and might have produced conducive conditions for the gonad stimulating hormone and there by increasing the spawning frequency as suggested by Murugadass *et al.* (1988), Bijulal (1994), Yano and Wyban (1993) and Racotta *et al.* (2000).

5.3 EFFECT OF EYESTALK ABLATION ON LARVAL HATCH FECUNDITY

The result of the experiment conducted to study the impact of eyestalk ablation on larval hatch fecundity in female *M. rosenbergii* indicated that the effect of eyestalk ablation was insignificant in terms of larval hatch fecundity. The average larval hatch fecundity per female was 21923 larvae after first time hatching (before ablation) and 17423 larvae after second time hatching (after ablation) in ablated spent females of *M. rosenbergii* (Table 5).

In unablated spent females the average larval hatch fecundity was 21410 larvae after first time hatching and 16147 larvae after second time hatching (Table 6). Pair wise comparison of larval hatch fecundity by paired t –test showed that significant difference in larval hatch fecundity after first time and second time hatching of same animal in both ablated and unablated ones. The larval hatch fecundity was lower in second time hatching than first time hatching in same animal of both ablated and unablated ones. Comparison of larval hatch fecundity by using student's t – test showed that there is no significant difference in larval hatch fecundity of second time hatching of ablated and unablated *M. rosenbergii*.

No study related to comparison of larval hatch fecundity of eyestalk ablated and unablated prawns are available. However, many researchers studied the effect of eyestalk ablation on fecundity in prawns and shrimps.

Primavera *et al.* (1982) reported substantial increase in the number of eggs in *P. indicus* through eyestalk ablation. They reported an increase in fecundity with subsequent spawning in both unablated and ablated females of *P. indicus*. A marginal increase in egg production in ablated *P. indicus* has also been observed by Emmerson (1983). Nascimento *et al.* (1991) observed a significant increment in the number of eggs in *Penaeus schimitti*. Browdy and Samocha (1985a) reported that the size of average first spawn in the moult cycle of ablated *P. semisulcatus* was significantly larger than subsequent spawns but the percent hatch and the percent metamorphosis were not significantly different.

Though many studies on fecundity in *Macrobrachium spp.* are there (Ling, 1969; Patra, 1976; Malecha, 1983; Coasta and Wanninayake, 1986; Mathavan *et al.*, 1986; Manna and Rant, 1990; Ang and Kok, 1991; Udo and Epke, 1991), the effect of eyestalk ablation on fecundity is poorly defined.

Bijulal (1994) reported that fecundity did not show any significant variation among the ablated and unablated treatments in *M. equidens*. Yano and Wyban (1993) reported that mean numbers of eggs per spawning for ablated and unablated spawners were not significantly different in *P. vannamei*.

Murugadass *et al.* (1988) reported that *M. malcolmsonii* carried more number of eggs per clutch and egg production in ablated series was double when compared to control. Soundarapandian *et al.* (1995) too reported that ablated *M. rosenbergii* and *M. malcolmsonii* carried more clutches of eggs than control and number of egg per clutch was more in ablated than control. The increase in number of clutches and eggs per clutch in ablated prawns are in accordance with results obtained for other decapods as well (Santiago, 1977; Aquacop, 1977). Contrary to the above observations, lower fecundity was observed in

ablated *P. canaliculatus* (Choy, 1987). Thus it can be seen that there is considerable variations in response, as to the number of egg produced, among the different species, some showing substantial increase, some marginal and some others showing insignificant increase.

Das (2003) reported lower fecundity and hatching rate during the second time spawning in unablated female *M. rosenbergii*. The reduction in larval hatch fecundity during second hatching of ablated and unablated spent females of *M. rosenbergii* in the present study may be due to the lower hatching rate during the second spawning as reported by Das (2003).

5.4 EFFECT OF EYESTALK ABLATION ON INCUBATION PERIOD

The experiment conducted to evaluate the effect of eyestalk ablation on incubation period of eggs of *M. rosenbergii* revealed that the incubation period was not found to vary significantly among ablated and unablated treatments.

The average incubation period was 18.8 ± 1.53 with a range of 15 to 20 days in ablated female while in unablated females it was 19.6 ± 0.76 with a range of 18 to 21 days (Table 7). Comparison of incubation period between eyestalk ablated and unablated spent females of *M. rosenbergii* indicated that there is no significant difference in incubation period among ablated and unablated individuals.

No significant difference in incubation period of eyestalk ablated and unablated female of *M. equidens* was observed by Bijulal (1994). The result of the present study corroborates with the result obtained by Bijulal (1994) in *M. equidens*.

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5.5 HISTOLOGY OF OVARY IN *M. ROSENBERGII*

Five histological stages viz. previtellogenic oocyte, early vitellogenesis, vitellogenesis, late vitellogenesis and matured oocyte were observed in the present study. Oogonial cells were characterized by a prominent nucleus containing chromatin granules and a thin layer of cytoplasm. Previtellogenic oocyte was found with large dense active nucleus and prominent nucleolus. The previtellogenic oocytes were surrounded by follicle cells. Small yolk droplets appeared in a single concentric layer at the periphery of the ooplasm of early vitellogenic oocyte. In the vitellogenic oocyte, many yolk vesicles were found compared to early vitellogenic stage. In the late vitellogenic stage most of the ooplasm including the perinuclear region was occupied by yolk globules and follicular layer became thinner. The ooplasm of matured oocyte was completely filled by yolk vesicles (yolk platelets).

The characteristic features of the oogonial stage, previtellogenic oocytes, early vitellogenesis, vitellogenesis, late vitellogenesis and matured oocyte during the histological observation in the present investigation fully agree with the observation already made on the same species by O'Donovan *et al.* (1984) and Chang and Shih (1995).

In comparison to other crustaceans, yolk vesicles or lipid globules were clearly observed in the vitellogenic oocytes of *M. rosenbergii*, which were similar to those in *Pandalu kessleri* (Quinitio *et al.*, 1989) but different from those in *Penaeus monodon* (Bell and Lightner, 1988). No cortical rod was observed in the periphery of mature oocytes in *M. rosenbergii*, which is contrary to what has been found in the oocytes of *P. monodon* (Bell and Lightner, 1988). Follicle cells were round, larger and more obvious in the early vitellogenic oocytes (stage II) which consistent with the study by O'Donovan *et al.* (1984).

Charles and Subramoniam (1982) also identified five histological stages for *M. malcolmsonii* and *M. lamarrei*. On contrary to this, Jayachandran and Joseph (1988) and Patil (2001) reported seven histological stages in *M. idella*, whereas Sebastian (1993) reported six well-marked histological stages in *M. equidens equidens* and *M. equidens* Pillai namely, oognial stage, previtellogenesis I, II, vitellogenesis I, II, and degenerating stage.

5.6 EFFECT OF EYESTALK ABLATION ON OVARIAN DEVELOPMENT IN SPENT FEMALES OF *M. ROSENBERGII*

The result of the experiment conducted to study the impact of eyestalk abaltion on ovarian development of spent females of *M. rosenbergii* revealed that eyestalk ablation enhanced ovarian development. From the histological sections of the ovaries starting from spent ovary to the ripe ovary at weekly interval, similar development stages of oocytes were observed in both ablated and unablated spent female of *M. rosenbergii*. However, the size of the largest oocytes in ablated spent female of *M. rosenbergii* was slightly higher than the average size of the oocyte in unablated at each week of development. This indicates enhancement of ovarian development after eyestalk ablation.

After one week of development the largest oocytes were in previtellogenic stage in both ablated and unablated females and the average size of the oocyte in ablated and unablated were 60.02 ± 6.15 μ and 52.03 ± 7.53 μ respectively. After two weeks of development largest oocytes were in early vitellogenic stage and average size was 82.11 ± 9.63 μ in ablated and 75.02 ± 5.04 μ in unablated. Vitellogenic oocytes were observed after three weeks of development and the average size of the oocytes were 103.01 ± 7.53 μ in ablated and $96.03 \pm$

1.73 μ in unablated ones. Late vitellogenic stage was observed after four weeks of development and the average size of oocytes was 235.00 $\pm$ 4.53 μ in ablated and 223.21 $\pm$ 12.03 μ in unablated. After five weeks of development the largest oocytes were in ripe stage and the average size was 401.03 $\pm$ 12.53 μ in ablated and 391.01 $\pm$ 10.30 μ in unablated.

These results are in the agreement with the fact that eyestalk ablation in crustaceans enhances gonadal development (Adiyodi and Adiyodi, 1970; Caillouet, 1973; Arnstein and Beard, 1975; Emmerson, 1980,1983). Panouse (1943) first showed that the eyestalk ablation induced ovarian maturation in the shrimp, *Leander serratus* (Pennant). This was subsequently confirmed with other species including *P. monodon* (Primavera, 1978; Emmerson, 1983).

It has been proved that ablation accelerates gonadal maturation in certain species of penaeid crustacea and, in some cases, under culture conditions ova development takes place only after eyestalk ablation (Caillouet, 1973 in *P. orientalis*; Aquacop, 1977 in *P. aztecus* and *P. monodon*). Petriella and Diaz (1987) reported that unilateral eyestalk ablation accelerates gonadal maturation in Argentine prawn, *Artemesia longinaris* Bate. The induced maturation of freshwater prawns, *M. malcolmsonii* and *M. rosenbergii* through eyestalk ablation has been successfully carried out in a low cost experimental set up by Soundarapandian *et al.* (1995). The ovarian development of ablated and non ablated females of *Pleoticus muelleri* was compared by Diaz *et al.* (1997). They noticed that ovaries of eyestalk ablated female were in mature condition and those of control were in primary vitellogenesis stage at the end of the experiment.

Kulkarni *et al.* (1979) reported that there is significant difference in oocyte diameters of progesterone injected and control prawns of

Parapenaeopsis hardwickii, which indicated enhancement of ovarian development. In the present study also the oocyte diameter in the eyestalk ablated is slightly higher than unablated *M. rosenbergii* at each week interval.

Okumura and Aida (2001) revealed that the duration of ovarian development was shorter in destalked females than in control females of *M. rosenbergii*. They opined that as ablation is expected to remove source of vitellogenesis inhibiting hormone (VIH) and mandibular organ inhibiting hormone (MOIH), decreases in haemolymph levels of VIH and/or MOIH is likely to be a basis for the 100% occurrence of the reproductive moult cycle and the acceleration of ovarian development.

The present study indicates that the development of oocytes in the ovary was a continuous process when the female is bearing the eggs between the pleopods till it hatch. This observation is in the agreement with the observation of O'Donovan *et al.* (1984) in the same species. Similar observation has been reported by Fish and Preece (1970) and Salvat (1967). They described a sequence of broods in the amphipod genus *Bathyporeia* in which one set of embryo develops in the brood pouch while oogonia enlarge in the ovary. Hinsch (1968) also reported that in *Libinia emarginata* the females have a new egg mass in the brood chamber a short time after zoea release, and are able to produce three to four consecutive broods. Schone (1968) observed that in *Maja* spp. of crabs mate prior to zoea release and have a new egg mass in the brood pouch a short time after hatching of the larvae.

The neuroendocrine complex in the eyestalk consists of the medulla terminalis x-organ sinus gland complex. It produces an ovarian inhibiting hormone which when removed allows the ovaries to mature. The maturation is probably influenced by an ovary-stimulating hormone produced by brain and thoracic ganglion (Adiyodi and Adiyodi, 1970).

The slight increment in ovarian development of ablated spent female of *M. rosenbergii* in the present study may be due to the declining level of ovary inhibiting hormone in haemolymph after eyestalk ablation as suggested by Adiyodi and Adiyodi (1970).

In the present study, much influence of eyestalk ablation on ovarian development was not observed as the same type of oocyte stage observed in both ablated and unablated spent female of *M rosenbergii* at each week interval. It can be stated that GSH from the thoracic ganglion and brain might have suppressed the effect of GIH and there by influenced the ovarian development in the unablated one also as suggested by Otsu (1963) and Gomez (1965).

5.7 EFFECT OF EYESTALK ABLATION ON OVARIAN INDEX

In the present rematuration experiment effect of eyestalk ablation on ovarian index of spent female of *M. rosenbergii* was evaluated. Slight enhancement of the ovarian index was observed in ablated ones compared to unablated at each week interval.

The ovarian index progressively increases as maturity stages advances in spent females of *M. rosenbergii* for both ablated and unablated. The OI of spent ovary was 0.43 for ablated and 0.42 for unablated; after one week OI was 0.45 in ablated and 0.44 in unablated; while it was 0.54 for ablated and 0.47 for unablated after 2 weeks; After 3 weeks, 0.64 for ablated and 0.63 for unablated; 3.71 for ablated, 3.51 for unablated after 4 weeks; 9.20 for ablated, 9.10 for unablated after 5 weeks (Table 8).

Chang and Shih (1995) reported ovarian index of *M. rosenbergii* in different development stages from immature to mature ones. In stage I OI was 0.52 ± 0.04; while it was 1.17 ± 0.41 in stage II; 2.41 ± 0.36 in stage III; 5.77 ± 0.68 in stage IV; and 6.93 ± 0.066 in stage V. Ovarian

index has been variously used in assessing ovarian growth in penaeids (Lawrence *et al.*, 1979; Joshi, 1980).

Kulkarni *et al.* (1979) studied the effect of progesterone on ovarian maturation in marine penaeid prawn, *P. hardwickii* and the study revealed that a significant increase in the ovarian index and oocyte diameter after progesterone injection. In the present investigation the ovarian index of eyestalk ablated spent female of *M. rosenbergii* showed slight increase in value than it was observed for unablated ones after each week of development.

The present result is in agreement with the result of Diaz *et al.* (1997) in *Pleoticus muelleri*. They studied the GSI of ablated and unablated females of *Pleoticus muelleri* and found that gonad index of ablated prawn (3.471-4.471) was higher than control prawn (2.059-4.520).

In the present study the values of OI after each week of development were different from the OI values estimated by Chang and Shih (1995). Which may be due to limited numbers of female prawn used in the study.

5.8 WATER QUALITY PARAMETERS

All animals are known to select the most appropriate time for breeding. As such the fluctuations of water quality parameters have a definite influence in stimulating maturation and subsequent reproduction process (Primavera, 1985). Different water quality parameters such as temperature, dissolved oxygen and pH were maintained with the optimum range required for reproduction of *M. rosenbergii* during the experimental period.

The rematuration experiments were conducted in freshwater condition. This is in accordance with the result of various investigators. Wickins (1972), Venugopalan (1988) and Venugopalan and Thampy

(1992) have suggested that salinity range of 0-2 ppt as ideal for *M. rosenbergii*. The temperature in the tanks varied between 23.1 and 28.1 °C which is near to the range from 27 to 29 °C reported by many workers such as Uno *et al.* (1975), New and Singholka (1982), Sandifer and Smith (1985) as within the optimum range for *M. rosenbergii*.

The Dissolved oxygen levels of water in the experimental tanks varied between 6.03 and 8.25 ppm, which was also within the optimum range required for *Macrobrachium spp.* culture as reported by Subramoniam (1987). New and Singholka (1982) reported that an oxygen concentration of 75% saturation as optimum for *Macrobrachium spp.*

The pH of the water in the experimental tanks varied between 6.5 and 8.5. New and Singholka (1982) and Sandifer and Smith (1985) reported a pH range of 7.5 to 8.5 as optimum for culture of *M. rosenbergii*, whereas Susheela *et al.* (1992) reported a pH range of 6.0-7.5 in culture pond of *M. rosenbergii*.

From the results of rematuration experiment, it could be seen that eyestalk ablation significantly enhanced moulting frequency and spawning frequency but the effect was insignificant in terms of larval hatch fecundity and incubation period in spent female of *M. rosenbergii*. Insignificant increase in ovarian index and ovarian development was also observed in ablated spent female of *M. rosenbergii*.

It may be concluded from the present study that eyestalk ablation can be used as a rematuration technique in spent female of *M. rosenbergii* as it enhances the moulting frequency and spawning frequency significantly than that of unablated ones. But, further study is warranted especially during the non-breeding season to derive a specific conclusion.

Summary

6. SUMMARY

The objectives of the present study were to find out the role of eyestalk ablation on rematuration of spent female of *M. rosenbergii* in captive conditions and to study the histology of the ovary during rematuration of eyestalk ablated and unablated spent female of *M. rosenbergii*. In the rematuration experiment, the effect of eyestalk ablation on moulting frequency, spawning frequency, larval hatch fecundity, incubation period, ovarian development and ovarian index in spent female of *M. rosenbergii* was evaluated. The histological examination of the ovary during rematuration was also carried out in ablated and unablated spent female of *M. rosenbergii* at weekly intervals. The methodology, important results and conclusions of the study are as follows.

1. Single aged healthy berried females of *M. rosenbergii* were procured from a culture pond. Strong blue clawed (SBC) males of *M. rosenbergii* were collected from wild. The length and weight of the experimental animal was 145-170 mm and 35-45 g for females and 210-250 mm and 140-160 g for SBC males. The animals were fed with fresh clam meat *ad libitum*.

2. In the rematuration experiment to evaluate the effect of eyestalk ablation on moulting frequency, spawning frequency, larval hatch fecundity and incubation period, 40 healthy berried females were selected and individually stocked in round cement cisterns. Estimation of the larval hatch fecundity of the experimental animal was individually carried out during first time hatching. Eyestalk ablation was performed in 20 nos of spent females and remaining 20 nos were kept unablated. Ablated and unablated spent females were introduced separately in oval fiberglass tank along with male

at the ratio of 1male: 4female for rematuration. Observation on moulting frequency, spawning frequency, larval hatch fecundity and incubation period of ablated and unablated spent females were individually carried out for the period of 60 days.

3. The study of histology of the ovary during rematuration of the eyestalk ablated and unablated spent female prawns were carried out from spent ovary to the ripe ovary at each week of development. For histological study prawn from above experiment was taken out from both ablated and unablated group at each week interval and ovaries were dissected out and fixed in Bouin's fluid. Embedding was done in paraffin wax and sections were cut at 6-8 μ thickness. Conventional slide preparation procedure was used. The sections were stained with Mayer's Haemalum and Eosin Y. The oocyte diameter from the slide was measured using calibrated ocular micrometer.

4. In experiment to study the effect of eyestalk ablation on ovarian index 24 nos of spent female of *M. rosenbergii* were used. 12 nos of spent females were ablated and another 12 nos kept unablated. The ablated and unablated spent females were kept separately along with male for mating. The average ovarian index (OI) values from spent ovary to ripe stage at each week interval for eyestalk ablated and unablated spent female of *M. rosenbergii* was estimated.

5. The average number of moults was 1.93 per female in the case of ablated ones compared to 1.50 in unablated individuals. Comparison of moulting frequency by using student's t-test showed that there is significant difference in the moulting frequency of ablated and unablated spent female of *M. rosenbergii*. This indicates that there is significant increase in moulting frequency after eyestalk ablation.

6. The average number of spawns was 1.71 in eyestalk ablated ones compared to 1.28 in unablated individuals. Comparison of spawning frequency by using student's t-test showed that there is significant difference in the spawning frequency of ablated and unablated spent female of *M. rosenbergii*. This indicates that there is significant increase in spawning frequency after eyestalk ablation.

7. The average larval hatch fecundity per female was 21923 after first time hatching (before ablation) and 17423 after second time hatching (after ablation) for ablated group. In the case of unablated group the average larval hatch fecundity was 21410 after first time hatching and 16147 after second time hatching. Pair wise comparison of larval hatch fecundity by paired t-test showed that there is significant difference in larval hatch fecundity after first time and second time hatching of same animal of both ablated and unablated spent female of *M. rosenbergii*. Comparison of larval hatch fecundity by using student's t-test showed that there is no significant difference in larval hatch fecundity during second time hatching of ablated and unablated spent female of *M. rosenbergii*.

8. The incubation period was not found to vary significantly among ablated and unablated spent females of *M. rosenbergii*. The incubation period ranged from 15 to 20 days in ablated with an average of 18.8 ± 1.53 while in unablated these values ranged from 18 to 21 days with an average of 19.6 ± 0.76.

9. Five histological stages viz. previtellogenic oocytes, early vitellogenic, vitellogenic, late vitellogenic and matured oocyte were observed in the present study.

10. In the histological sections of the ovaries starting from spent ovary to the ripe ovary at weekly interval, similar type of oocytes were

observed in both ablated and unablated spent females of *M. rosenbergii*. However, the size of largest oocyte in ablated spent female of *M. rosenbergii* was slightly higher than the size of oocytes in unablated at each week of development.

11. Generally higher ovarian index was observed after fourth and fifth week of development in both eyestalk ablated and unablated spent female of *M. rosenbergii*. It was observed that ovarian index of eyestalk ablated spent female prawn was higher than unablated prawn at each week interval, which suggests impact of eyestalk ablation on ovarian development.

12. Different water quality parameters such as temperature, dissolved oxygen and pH were maintained within the optimum range required for reproduction of *M. rosenbergii* during the rematuration experiment.

13. From the results of rematuration experiment, it could be seen that eyestalk ablation significantly enhanced moulting frequency and spawning frequency but the effect was insignificant in terms of larval hatch fecundity and incubation period in spent female of *M. rosenbergii*. Insignificant increase in ovarian development and ovarian index was also observed in ablated spent female of *M. rosenbergii*.

14. It may be concluded from the present study that eyestalk ablation can be used as a rematuration technique in spent female of *M. rosenbergii* as it enhances the moulting frequency and spawning frequency significantly than that of unablated ones. But, further study is warranted especially during the non-breeding season to derive a specific conclusion.

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EFFECT OF EYESTALK ABLATION ON THE REMATURATION OF FEMALE *MACROBRACHIUM ROSENBERGII* (DE MAN) IN CAPTIVITY

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ABSTRACT OF THE THESIS

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ABSTRACT

The giant freshwater prawn, *Macrobrachium rosenbergii* is one of the commercially important species widely cultured throughout the tropics, subtropics and some parts of the temperate region. Non-availability of brood stock in sufficient numbers at appropriate time has been the greatest constraint for successful operations of prawn hatcheries. To avoid entire dependence on natural sources, development of brood stock of female prawns in captivity is essential. The objectives of the present study were to find out the role of eyestalk ablation on rematuration of spent female of *M. rosenbergii* in captive conditions and to study the histology of the ovary during rematuration of eyestalk ablated and unablated spent female of *M. rosenbergii*.

In the rematuration experiment the effect of eyestalk ablation on moulting frequency, spawning frequency, larval hatch fecundity, incubation period, ovarian development and ovarian index of spent female of *M. rosenbergii* was evaluated for 60 days. The histology of ovary in *M. rosenbergii* during rematuration was also studied. The analysis of the result was done with student's t-test and paired t- test.

A significant difference was observed in moulting frequency and spawning frequency of ablated and unablated spent female of *M. rosenbergii*. Larval hatch fecundity of ablated and unablated spent female of *M. rosenbergii* was not found to vary significantly. Significant difference was found in larval hatch fecundity after first time and second time hatching of both ablated and unablated individuals. The incubation period was not found to vary significantly among ablated and unablated. However, slight reduction in incubation period was observed in ablated ones.

Five histological stages of ovaries viz. previtellogenic stage, early vitellogenic, vitellogenic and late vitellogenic and matured oocytes were

observed. In the histological sections of the ovaries, starting from spent ovary to the ripe ovary at weekly interval, similar type of oocyte development stages were observed in both ablated and unablated. However, the size of largest oocyte in ablated was slightly higher than the size of oocyte in unablated at each week of development. It was observed in the present study that ovarian index of ablated spent female was higher than unablated at each week of development.

The rematuration experiment has shown that eyestalk ablation significantly enhanced moulting frequency and spawning frequency but the effect was insignificant in terms of larval hatch fecundity and incubation period in spent female of *M. rosenbergii*. Insignificant increase in ovarian development and ovarian index were also observed in ablated spent female of *M. rosenbergii*.