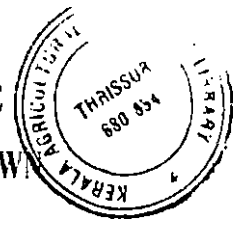

ROLE OF A PUTATIVE MALE PHEROMONE IN THE OVARIAN MATURATION OF THE FRESH WATER PRAWN *MACROBRACHIUM IDELLA* (HILGENDORF).

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

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DEPARTMENT OF FISHERY BIOLOGY

COLLEGE OF FISHERIES

PANANGAD, COCHIN

2001

Dedicated To

God And My Beloved Parents

DECLARATION

I hereby declare that this thesis entitled " ROLE OF A PUTATIVE MALE PHEROMONE IN THE OVARIAN MATURATION OF THE FRESH WATER PRAWN *MACROBRACHIUM IDELLA* (HILGENDORF)." is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or society.

Patil

Panangad

27/11/2001

Patil Chandrakant Sitaram

99-14-04

CERTIFICATE

Certified that this thesis entitled "ROLE OF A PUTATIVE MALE PHEROMONE IN THE OVARIAN MATURATION OF THE FRESH WATER PRAWN *MACROBRACHIUM IDELLA* (HILGENDORF)." is a record of research work done independently by Mr. Patil Chandrakant Sitaram under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Introduction

1. INTRODUCTION

The significance of freshwater prawns belonging to genus *Macrobrachium* (family - Palaemonidae) as candidate species for aquaculture has increased during the last decade (Jayachandran, 2001). The devastating viral infection in marine prawns has forced many entrepreneurs to grow freshwater prawns, which are disease resistant, as a substitute crop in many coastal districts of India. There is immense scope for development of freshwater prawn culture by improving the technology for seed production and scientific farming of established species and also by including new species.

The genus *Macrobrachium* Bate, 1868 includes the most important cultivable freshwater prawns. Though, there are more than 150 species reported under this genus, the aquacultural potential of only a few species, such as *Macrobrachium rosenbergii* (de Man), *M. americanum* Bate, *M. malcolmsonii* (H. Milne Edw.), *M. carcinus* (Linnaeus), *M. acanthurus* (Wiegmann) and *M. tenellum* (Smith) has been investigated. There are so many other medium sized species, the aquaculture of which may be biologically and commercially feasible.

M. idella, commonly known as slender river prawn is a medium sized species, which enjoys a wide distribution from East Africa, Madagascar, India, Indonesia to Malayan Archipelago (Holthuis, 1950; 1980). It is commercially important in Kerala waters (Jayachandran, and Joseph, 1992).

For successful farming of any organism, seed availability is a major limiting factor. In the case of *M. idella*, there does not exist wild collection of seeds. Therefore, acquiring of knowledge of optimum conditions for larval

development is of paramount importance. Though the technology for hatchery production of larvae of giant freshwater prawn has been more or less perfected, it remains unattended in the case of *M. idella*.

Sex pheromones are reported to be present in many crustaceans which have chemokinetic, chemotaxic and releaser effects (Dunham, 1978). The discovery of a male pheromone with primer effect on ovarian maturation and ovulation has added an important external factor to the mechanisms regulating reproduction in freshwater prawns. Growth and reproduction, the two major but opposite energy demanding physiological processes are very closely related in the freshwater prawns, unlike as in many other decapod crustaceans.

In freshwater prawns, the final ovarian maturation is coinciding with a preovulatory moult facilitating courtship and spermatophore transfer.

The process of ovarian maturation in decapod crustaceans is reported to be under a dual control mechanism, viz; a gonad inhibiting hormone (vitellogenesis inhibiting hormone) secreted from the X-organ-sinus gland system and a gonad stimulating hormone secreted by brain/thoracic gland (Adiyodi and Subramoniam, 1983). The relative importance of these two mechanisms varies among the different species. Thus, ablation leads to speedy ovarian maturation in some, whereas it has negative effects in others. The release of these neurohormones is now known to be under neural control.

Studies in these lines are very few in *Macrobrachium* spp. The present study will be scientifically useful, as it will help to unravel the role of male pheromone in ovarian maturation and ovulation in females. This is also an aspect hitherto

unrecognised in hatchery operations. The knowledge thus acquired may help to potentiate reproduction and/or extend the breeding period of freshwater prawns. Hence the present study has got great relevance in aquacultural practices particularly in hatchery operations.

Review of Literature

2. REVIVEW OF LITERATURE :

2.1. Hormonal & pheromonal regulation of reproduction in shrimps/prawns and other decapod crustaceans

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

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

Although many observations have been made on the inhibition of reproductive maturation by eyestalk hormone(s), recent research has focused mostly on brain, thoracic ganglion, ovary and mandibular organ and their functions which are closely related with the release of gonad stimulatory factors or hormone(s) (Yano, 1992a). As with ovarian maturation, it has long been suspected that vitellogenesis in crustaceans is controlled by two antagonistic hormones. In penaeids, gonad inhibiting hormone (GIH) secreted from the XO-SG complex inhibits vitellogenesis and gonad stimulating hormone (GSH) secreted from the thoracic ganglion and brain stimulates vitellogenesis (Yano, 1992a).

2.1.1. Gonadal maturation

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

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

2.1.2. Inhibitory factors of gonad maturation

2.1.2.1. Gonad inhibiting hormone (GIH)

In decapods, it is known that the removal of eyestalk induces ovarian activity (Adiyodi and Adiyodi, 1970). The existence of 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 in *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 ovarian size which precedes the breeding season may be inhibited by injection of extracts of whole eyestalk or sinus gland or medulla terminalis ganglionic X organ (MTGX) (Carlisle, 1953). Knowles and Carlisle (1956) took these results as evidence for existence of an ovary inhibiting hormone (OIH). Eyestalk ablation according to them removes an inhibition, which is preventing ovarian growth, thus leading to rapid uninhibited proliferation of the ovarian

tissue, which may increase several folds in a month. Conversely, injection of eyestalk extract supplies the inhibitor which keeps the ovary in check.

Though Carlisle (1954) proposed specificity of GIH, Adiyodi and Adiyodi (1970), quoting Otsu (1963), Carlisle (1953), Payen *et al.*(1967) and Juchault and Legrand (1967) argued that OIH and testis inhibiting hormone (TIH) are not different from one another and termed the inhibitory hormone in both sexes as GIH.

Quackenbush (1991) agrees to this argument and Chang (1992) finds little reason to suspect that OIH and Vitellogenesis inhibiting hormone (VIH) mentioned by various workers are different from that of GIH. GIH appears to be present not only in adults, but also in immature stages as well. In *Potamon dehaani*, eyestalks of immature crabs of both sexes have been stated to contain the hormone (Otsu, 1963). When vitellogenesis is already in full swing, eyestalk ablation does not perceptibly accelerate ovarian growth, suggesting that during this period the synthesis of GIH and /or its release from eyestalk into general circulation may be very low; the possibility that this may be related to an optimum is not ruled out (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 in moulting, as in juveniles and some adults, but in premature yolk deposition in the ovary, both during the non-breeding season and in certain species like *Paratelphusa hydrodromous* (see Gomez, 1965) and *Scylla serrata* (see Rangneker and

Deshmukh, 1968) even in prepubertal stages. Adiyodi (1980) suspects that MIH and GIH represent a single hormone say growth restraining hormone (GRH) that exercises its influence on two 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 individual, season and other factors (Adiyodi, 1985; Quackenbush, 1986; Fingerman, 1987). The production of GIH has been shown to be seasonal and is responsible for the period of ovarian rest during the non-breeding season (Adiyodi and Adiyodi, 1970; Bomirski and Kelk, 1974; Kelk-Kawinska and Bomirski, 1975).

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

GIH is thought to exert its effect directly on the ovary and hepatopancreas *in vivo* since eyestalk extract inhibits protein synthesis by cultured ovaries (Paulus and Laufer,1987; Quackenbush,1989; Yano,1992a). The fact that cyclic AMP can mimic this inhibition suggests its function as an

intermediate (Eastman-Reks and Fingerman, 1984). The putative target tissue of the GIH probably responds to eyestalk ablation by rapid increase in biosynthetic activity of yolk proteins (Quackenbush, 1989).

Primary action of GIH in females apparently occurs during the secondary vitellogenesis, the time when ovary increases dramatically in size due to synthesis and uptake of yolk proteins produced in either follicle cells or extra ovarian sites (Quackenbush, 1991; Chang, 1992). But in *P. hydrodromous* there is evidence to show that GIH principally inhibits the primary vitellogenesis in these crabs, however, its effects on secondary vitellogenesis is far from impressive (Kurup and Adiyodi, 1980). Alternatively GIH may have non-ovarian target or in fact there may be more than one eyestalk factor which inhibit ovarian growth (Laufer *et al.*, 1992).

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

Kallen and Meusy (1989) have advanced the theory that GIH is similar in structure and not different from crustacean hyperglycemic hormone (CHH). There are indications that moult inhibition and hyperglycemic activity are associated with the same peptide as demonstrated in the lobster *H.americanus* (see Chang *et al.*, 1990; Soyez *et al.*, 1991) and in the shore crab, *Carcinus maenas* (see Webster and Keller, 1986). In a similar immunological study in the lobster, indications are that GIH and CHH share common antigens (Subramonium and Keller, 1993).

2.1.2.2. Other inhibitory factors:-

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

It is well known that biogenic amines release peptide neurohormones from neuroendocrine structures in several crustaceans (Fingerman, 1985). Certain biogenic amines (Octopamine and Serotonin) inhibited methyl farnesoate synthesis in *Libinia emarginata* (Homola *et al.*, 1989). Serotonin has been found to induce the release of GIH from isolated eyestalk (Mattson and Spaziani, 1985). Thus 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 farnesoate in *Procambarus clarkii*. Quackenbush and Herrnkind (1983) reported

that partially purified GIH could not be separated from PDH. Thus in some cases the functions of pigment dispersal and gonad inhibition may be performed by the same or similar molecules (Laufer *et al.*, 1987 b).

2.1.3. Stimulatory factors for control of reproduction:-

2.1.3.1. Gonad stimulating hormone (GSH).

A second decapod reproductive neurohormone is found in the brain and thoracic ganglion which acts to stimulate ovarian development in shrimps, crabs and lobsters. The concept of 'bihormonal system' was first proposed by Otsu (1960, 1963). Otsu (1963) after working with *Potamon dehaani* and Yano and Wyban (1992) with *P. vannamei* suggested its existence because eyestalk ablation caused precocious ovarian growth in adult, but not in juvenile. This led them to reason that not only is the absence of GIH required for ovarian growth, but the presence of a stimulatory homone is also necessary. Otsu (1963) also observed that implantation of adult thoracic ganglion was effective in triggering maturation of ovary in eyestalk ablated juveniles. The experiments of Hinsch and Bannett (1979) using *Libinia emarginata*, Gomez (1965) using *P. hydrodromous* with both brain and thoracic ganglion and Takayanagi *et al.* (1986 a) using the shrimp, *Paratya compressa* also proved that GSH from thoracic ganglion has got a role to play in ovarian maturation. Extract of thoracic ganglion of reproductive *Uca pugilator* stimulates ovarian growth in adult crabs (both intact and ablated) while thoracic ganglion extract from non-reproductive crabs has no effect on normal crabs and actually inhibited ovarian growth in ablated crabs (Eastman- Reks and Fingerman, 1984). Nagabhushanam *et al.* (1988) found that GSH from brain was more effective than that from the thoracic ganglion in stimulating ovarian growth

in *Macrobrachium kistnensis*. Yano and Wyban (1992) proposes a GSH – releasing hormone (GSH-RH) from brain of the lobster *H. americanus* which stimulated ovarian maturation in *P. vannamei*. Yano (1992b) speculates that in immature females the ovarian stimulating principle is absent or not yet functioning. Yano, (1992b) found that thoracic ganglion extract prepared from maturing females is effective in increasing serum vitellogenin in *P. japonicus* and suggested that GSH also stimulates vitellogenin synthesis and/or secretion into the blood in penaeid shrimp. He further noted that brain extract prepared from maturing females induced vitellogenin secretion in *P. japonicus* suggesting a brain hormone that stimulates release of GSH in penaeid shrimps.

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

The role of GSH appears to be dual in that it promotes oocyte growth and prevents Y-organ (YO) activity; the latter is accomplished either directly or

indirectly by raising the level of MIH and/or lowering the level of GIH (Adiyodi and Adiyodi, 1970).

2.1.3.2. Juvenile hormone (JH) :-

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

reviewing the literature in this field Subramonium and Keller (1993) proposed methyl farnesoate as the crustacean juvenoid probably involved in the stimulation of vitellogenesis and farnesoic acid as a pre-hormone which could undergo conversion to methyl farnesoate or even JH III in the target tissues.

Landau *et al.* (1989) noticed that red pigment concentrating hormone (RPCH) significantly stimulates the rate of inhibited synthesis of methyl farnesoate by the mandibular organ of the crayfish, *P. clarkii*. Experiments of Laufer and Landau (1991) also indicated that RPCH has mandibular organ stimulating activity in *P. clarkii* and *Libinia emarginata*.

2.1.3.3. Steroid hormones

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

2.1.3.3.1. Ecdysteroids (ECDs):-

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

Crustaceans resemble insects in that MH secreted by the Y organ is not required for the maintenance of the gonad once puberty is attained (Adiyodi, 1969). There is now a growing body of evidence to suggest that in insects and crustaceans, ECDs which are primarily MH (Adiyodi and Adiyodi, 1970) are also there in adult life to stimulate the ovarian growth (Adiyodi, 1980). Prepubertal growth and development of gonads appear to be part of the normal genetically determined growth process and ECDs may have a role in post pubertal development (Adiyodi, 1985). Lachaise and Hoffman (1977) were successful in detecting several ECDs, especially ecdysone in ovaries of crab, *C. maenas* whose titre in ovary registered a gradual increase with the progress of vitellogenesis, with peak levels detected on termination of the process. ECDs may be synthesized or get accumulated in the gonads (Young *et al.*, 1993). Experiments have shown that Y organ removal can result in either stimulatory or inhibitory effect on vitellogenesis depending on species, age and stage in the moult stage and reproductive cycle (Chang, 1992). Thus though ECDs are primarily moulting hormones, they may also have secondary effect on reproduction (Young *et al.*, 1993).

2.1.3.3.2. Other Steriods:-

Evidence is accumulating none the less, from scattered works suggesting that the crustacean ovary might play a role in the biosynthesis of steriod hormone(s). Lisk (1961) confirmed the estrogenic compound reported by Donahue (1957) in *H. americanus* to be 17-β estradiol. Subsequently, Teshima and Kanazawa (1971) have found that the ovaries of *Portunus trituberculatus* possess the enzymes involved in the conversion of progesterone to 17a-

hydroxyprogesterone, testosterone and deoxycorticosterone. Kanazawa and Teshima (1971) detected progesterone and testosterone in the ovaries of *Panulirus japonicus*. Estrogen was detected in the ovaries of the shrimp *Parapenaeus fissurus* (Jeng *et al.*, 1987), *Nephros norvegicus* and *P.monodon* (Fairs *et al.*, 1989; 1990) and *Pandalus kessleri* (Quinitio *et al.*, 1991). A number of steriods including testosterone, progesterone, and pregnenolone have been identified in the gonads and serum of the crayfish *Astacus leptodactylus* and the lobster *H.americanus* (Burns *et al.*,1984; Ollivier *et al.*,1986) and the shrimps, *N.norvegicus*, *P.monodon* and *Pandalus kessleri* (Fairs *et al.*,1989;Young *et al.*,1992; Quinitio *et al.*, 1991) and the crab *C.maenas* (Hazel,1986). Couch *et al.* (1987) detected significant levels of estradiol and progesterone in the mandibular organ of *H. americanus*.

2.1.3.4. Other Factors

Tensen *et al.*,(1989) found a stimulatory effect on oocyte growth of the shrimp, *Palaemonetes varians* by a peptide of *H.americanus* which is similar to that of Crustacean Hyperglycaemic Hormone (CHH). With regard to the possible existence of such an ovary stimulating hormone in the sinus gland, it will be interesting to know whether this peptide works antagonistically to GIH or synergistically with the putative GSH (Subramoniam and Keller, 1993)

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

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

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

2.2. Breeding Biology of *Macrobrachium*

Understanding the reproductive biology of an animal is vital for proper animal management. With the advent of intensive aquaculture of useful marine and fresh water invertebrates such as prawns, crabs and molluscs, there is an increased need for a basic knowledge of the reproductive process of these invertebrates. An experimental approach to problems involving the extrinsic and intrinsic factors controlling reproduction has become highly necessary.

Although fairly good descriptions on the various aspects of the reproductive biology of the penaeid prawns are available, the same is comparatively scanty regarding the Palaemonids (Jayachandran, 1984). The only important contributions are those of Menon (1938) who first recorded the larvae of *M.rosenbergii* from brackish water areas; Mary John (1957) made a detailed study of the bionomics, life history and economics of *M.rosenbergii* with a detailed analysis of its migration to the feeding grounds and from these back to the breeding grounds in the brackish water areas of the Vembanad lake,

Rajyalakshmi (1961,1980) studied the maturation and breeding in estuarine prawns, *M.rosenbergii*, *M.mirabile* and *M.malcolmsonii* in the Hooghly estuary. Raman (1964,1967) while studying the various aspects of the biology of *M. rosenbergii* at Pamba river, Kerala, reported on the breeding period and the location of its nursery ground in the lower reaches of the Pamba river. Rao (1967) investigated the breeding biology of *M.rosenbergii* of the Hooghly estuary and Subramonyam (1975) studied the breeding behaviour, breeding period and spawning in *Palaemon styliferus* from the Godavari estuarine system.

2.2.1. Size at first maturity :-

Jayachandran (1984) showed that no mature prawns of *M. idella* were found in length groups less than 41 mm and only a few in the 41 to 55mm length groups. The length at which 50% of the prawn attains maturity is 56.00 – 60.00 mm length group and that is regarded as the length at first maturity. Therefore, it may be considered that the minimum length at first maturity of female of this species is at about 56 mm, total length.

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) and in Kerala one year (Raman, 1967) to attain maturity. Rao (1967) recorded the mean size of 155mm, as the maturity size in Hooghly estuary. Goorah and Parameswaran (1983) recorded 118 mm and 20 gm (5 – 7 months old) as the smallest size of berried females in ponds at Mauritius. The size at first maturity of *M. malcolmsonii* is 41 mm according to Ibrahim (1962) and 40 – 50 mm according to

Sankolli and Shenoy (1980). Rajyalakshmi (1980) has reported that the size at first maturity of this species in river Gaodvari is smaller compared to Hooghly estuary. In *M. acanthurus* sexual maturity is attained at a size of 40 mm (Berber; 1984). Inyang (1984) recorded a size of 30 mm, as the maturity size of *M. felicinum* in Africa. The size at first maturity of *M. tenellum* in Mexico is 74 mm (Arroya *et al.*, 1982).

2.2.2. Breeding Season :-

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

The breeding season in the case of *M. rosenbergii* varies from region to region and depends on the habitat. In the Hooghly estuary, it extends from December to July, i.e. winter and summer months (Rajyalakshmi, 1961, Rao, 1967). In Kerala its breeding season is during August to December with a peak in October to November (Raman, 1967). *M. malcolmsonii* in river Godavari has a prolonged breeding season extending from April to November according to Ibrahim (1962), with two spawning peaks, one in June and again during August to October and according to Rajyalakshmi (1974), May to October with peak in July – September.

Palaemon idea in Vellayani lake, has been reported to have a long breeding season, extending from September to January, i.e. covering the North-East monsoon months (Jayachandran, 1984). Similar observation was made in case of *Macrobrachium amazonicum* (Romero, 1982), *M. tenellum* (Arroya *et al.*,

1982) and *M. felicinum* (Inyang, 1984), in which the breeding season coincides with the rainy season. The breeding season of *M. idella* extends from August to December / January in Vellayani lake, Trivandrum (Jayachandran, 1984). *M. scabriculum* breeds throughout the year (Jayachandran and Joseph, 1989). Jayachandran and Joseph (1989) reported that the breeding season of *M. equidens* as from August to January/February while Pillay (1990) noted it as August to November.

2.2.3. Moulting and reproduction in females : -

In freshwater prawns ovarian maturation proceeds through intermoult and culminates in prespawning moult (Narayanan and Adiyodi, 1992). This prespawning 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. According to Joshi and Diwan (1992) the ideal time for copulation is between 2.5 – 4 hours after prespawning moult as evidenced by the success in artificial insemination during this period in *M. idella*. The female exoskeleton is likely to harden, resulting in failure of insemination after this period. Male deposi ts sperms on the ventral side in between the second and fourth periopods of females. The production of pheromone during the time of spawning moult by the female serves in attracting males.

2.2.4. Spawning :-

Spawning closely follows mating, but neither mating nor presence of a mating partner is a prerequisite for oviposition in species of *Macrobrachium* such as *M. australience* (Lee and Fielder, 1982); *M. idella* (Narayanan and Adiyodi,

1992) and *M. rosenbergii* (Wickins and Beard, 1974). In. *M. idella* spawning occurs 10-12 hours after moulting of females. (Narayanan and Adiyodi, 1992).

In many palaemonids although oviposition may occur even without mating, such eggs fall off after 2 or 3 days. Brachyurans such as *Gecarcinus lateralis* (Klassen, 1975) and *Paratelphusa hydrodromous* (Krishnakumar, 1985) are a step ahead in conditioning mating as a prerequisite to spawning thereby successfully avoiding the otherwise wasteful process of laying unfertilized eggs. The caridean shrimp, *Caridina natarajani* also fails to spawn in case there is no mating, thereby avoiding the laying of unfertilized eggs (Thampy, 1972).

According to Wickins and Beard (1974) in palaemonids, ovarian growth and somatic growth are tuned as synergistic and specific stages in oogenesis programmed strictly in relation to specific stages of moult cycle. Narayanan and Adiyodi (1992) reported that each stage of ovarian growth corresponds with a specific stage in moulting cycle in *M. idella*. However in penaeids, ovarian growth and somatic growth are reported to be antagonistic processes.

In many fresh water prawns including *M. idella* in conditions of stress like handling and overcrowding females are shown to skip an ovarian cycle during an ecdysial cycle (Narayanan and Adiyodi, 1992).

2.2.5. Fecundity : -

Dobkin (1969) stated that low fecundity is characteristic of palaemonids. Fecundity of *M. rosenbergii* has been worked out by many investigators and it has been reported to be of the order of 7,000 – 1,11,400 (Rajyalakshmi, 1961); 60,000

– 1,00,000 (Ling, 1964); 1,39,000 – 5,03,000 (Raman, 1967); 22,552 – 1,09,491 (Goorah and Parmeswaran, 1983). Ibrahim (1962) recorded a fecundity of 5,463 – 63,080 for *M. malcosonii* of the size 54 – 164 mm in Godavari river. Sankolli and Shenoy (1980) has reported the fecundity of *M. malcolmsonii* to be of the order of 3,400 – 68,000.

Compared to the above two species, *M. idella* is a low fecund freshwater prawn. Jayachandran (1984) reported it to be of 6,089 and 29, 773 for prawns having a length of 68mm and 92 mm respectively, the number of ova per gram body weight ranging from 1450 to 5920.

Based on the number of eggs produced per year, Cabrera - Jimenez *et al.* (1979) divided various species coming under the genus *Macrobrachium* into low fecundity species, such as *M. acanthurus* (52,000 eggs/year), *M. tenellum* (70,000 eggs/year) and *M. rosenbergii* (1,12,000 eggs/year) and high fecundity species, such as *M.americanum* (9,00,000 eggs/year) and *M. carcinus* (10,50,000 eggs/year).

The intermittent spawning habit of *Macrobrachium* has been observed by many workers (Ling, 1962 in *M. rosenbergii*; Pillai and Mohamed, 1973 in *M. idella*; Inyang, 1984 in *M. felicinum*). There is no study in *Macrobrachium* regarding the variation in fecundity, in each successive breeding.

2.2.6. Sex ratio

The study of the sex ratio of *M. idella* reveals that the males outnumbered the females in a given locality in a year. The ratio being 1: 0:44 (Jayachandran,

1984). Ibrahim (1962) while working on the fishery and biology of freshwater prawn, *M. malcolmsonii* in the river Godavari, observed that males constituted 61.9%. Raman (1964) recorded a higher percentage of males of *M. rosenbergii* in river Pamba, Kerala, whereas in *P. styliferus* the females almost always outnumbered males in the commercial catches with a male: female ratio of 1 : 11.4 in the Godavari estuarine system (Subramonyam, 1975).

In *M. idella* the male outnumbered females and the males are bigger in size (Jayachandran, 1984). The males of *M. rosenbergii* are larger than females and males predominate in the habitat (Raman, 1967). The males of *M. malcolmsonii* are bigger in size than females and the ratio is in favour of the males (Rajyalakshmi, 1980). In *M. lamarrei*, the sex ratio of male to female is 43 : 57 and surprisingly for palaemonids in *M. lamarrei* the females are larger than males (Koshy, 1969). The above facts undoubtedly seem to suggest that the sex which outnumbers the other has also a tendency to be bigger in size.

2.3. Classification of maturity stages in *M. idella*

Classifiaction of the maturity stages in *M. idella* was done based on the pattern of classification adopted by Rajyalakshmi (1961, 1980) for *M. rosenbergii*, *M. mirabile* and *M. malcolmsonii*.

Seven arbitrary stages have been defined based on the size of ovary in relation to carapace cavity, colour of the ovary after preservation (as colour after preservation shows a clear difference between the stages than fresh ovaries that are uniformly green coloured), microscopic structure and size of the ova. The maturity stages are – Stage I (immature virgin); Stage II (maturing I); Stage III

(maturing II); Stage IV (maturing III); Stage V (mature I); Stage VI (mature II or ripe); Stage VII (spent) (according to Jayachandran, 1984).

2.4. Sex pheromones in Crustacea

It is well known that pheromones are substances, released by an organism, that influence a definite behaviour or developmental process in other organisms of the same species (Karlson and Luscher, 1959). The existing literature on crustacean sex pheromones has accepted three different behavioural assays as admissible evidence for a sex pheromone. These are best described as: (a) chemokinetic reactions (b) chemotaxic reactions; and (c) releaser reactions. Chemokinetic reactions are defined as changes in the speed of movement (orthokinesis) and /or rate of turning (klinokinesis) produced by chemical stimulation. Chemotaxic reactions are defined as movements oriented directly toward or away from a source of chemical stimulation; and, finally, releaser reactions are defined as stereotyped patterns of motor movement (e.g. courtship behaviour) elicited by a chemical stimulus alone or in combination with other stimuli (Dunham, 1978). Among decapods, studies on sex pheromones have been extensively done on *H. americanus, P. homarus, Jasus lalandii and Procambarus clarkii.* Two general assumptions emerged from these. Firstly mating is a moult-related phenomenon. Secondly, the post-moult female releases a pheromone to attract males and elicit the appropriate courtship and mating behaviour (see Dunham, 1978).

Kittredge and Takahashi (1972) suggested that crustecdysone, the crustacean moulting hormone also functions as a sex pheromone which attracts a

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

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

Regarding the chemical nature, Christofferson's work on the urine of *Portunus sanguinolentus* revealed that the active component as a heat stable substance extractable with organic solvents like ether and alcohol.(see Dunham,1978). Chemical nature of releaser pheromones have been reported by various workers. In *Paratelphusa hydrodromous*, Sundara Rajulu *et al.* (1973)

reported it to have the narture of 5 hydroxy tryptamine and in *Procambarus clarkii*, Ameyaw-Akumfi and Hazlett (1975) recorded it to be a carbohydrate. Sarojini *et al.*, (1982) reported the sternal gland pheromone in *M. kistnensis* to have histochemical properties of a glycoprotein. The situation here appears to be similar to that in fishes. The spawning pheromone in the herring *Clupea harengus pallasi* is present in the milt and mature male testis extracts and chemically have properties similar to that of polar steroids, prostaglandins, or their conjugated forms (sulphates/glucuronide) (Sherwood *et al.*, 1991). In the African catfish *Clarius gariepinus* seminal vesicle fluid contains the male sex pheromone and steroid glucuronides have been detected. 17 α - hydroxy progesterone glucuronide has been found to be the most potent odorant (Lambert and Resink, 1991).

Takayanagi *et al.*,(1986 b), reported that in the female shrimp, *Paratya compressa*, reared in the absence of males, ovarian development was usually delayed, whereas it occurred normally in the females reared with mature males. Ovarian development also occurred in shrimps reared in water that contained an extract of testis or vas deferens, which suggested that male shrimps of this species secreted an ovary stimulating pheromone which accelerated ovarian development. This was the first report of a primer pheromone in Crustacea. Similar results were also obtained by Nagabhushanam *et al.*, (1989) in *Macrobrachium kistnensis.* This pheromone is probably synthesized by testis and transported to vas deferens. Boiling did not affect its activity in *P. compressa*, but not so in *M. kistnensis.* In these two species, this is essential for normal ovarian maturation (for initiation of vitellogenesis). Jayachandran and Jose (1993) reported that in females of *Macrobrachium idella* reared in isolation, ovaries failed to develop beyond

maturation stage IV. The females also suffered from severe hypophagia. Unilateral eyestalk ablation did not help to overcome the ill effects of male deprivation. These results pointed towards a similar male pheromone in *M. idella*, which was essential for normal ovarian maturation and survival during the post breeding season.

Ovarian maturation in crustaceans in reported to be under a dual control mechanism (Adiyodi and Subramoniam, 1983). Accordingly, a gonad inhibiting hormone (GIH) is secreted by the X-organ-sinus gland complex and a gonad stimulating hormone (GSH) is secreted by brain/thoracic gland. The ovarian maturation is under the control of these two antagonistic factors and the relative importance of these two mechanisms varies from species to species (Subramoniam *et al.*, 1999). The GIH is now rechristened as Vitellogenesis inhibiting hormone (VIH) and in *Homarus americanus* its complete amino acid sequence has been worked out (Subramoniam *et al.*, 1999). The characterisation of GSH is yet to be materialised. The male pheromone may be affecting this neuroendocrine control machanism. Brain and thoracic ganglion extracts were able to induce vitellogenesis in isolated females of *P. compressa* and *M. kistnensis* (in the absence of male pheromone), showing that the male pheromone is operating through the neurohormones (Takayanagi *et al.*, 1986a and Nagbhushanam *et al.*, 1988).

Materials and Methods

3. MATERIALS AND METHODS

The experiments were conducted during January to September 2001.

3.1 Experimental Animals :

Adult specimens of *Macrobrachium idella* (both male & female) were collected from different localities in Cochin backwaters. All animals were reared in big fiberglass tanks before experiment. The size range (total length) of the experimental animals was 71 – 93 mm for males and 51 – 62 mm for females.

3.2 Feed :

Animals were fed with two different kinds of feed, dried clam meat and pelleted feed.

Fresh clam meat was purchased from local market, washed several times with water and then dried in dryer at 60^0C for 12 hrs. The proximate composition of clam meat is as follows (as reported by Sebastian, 1970).

Protein	-	14.4%
Fat	-	7.8%
Ash	-	2.9%
Calcium	-	1.01%
Phosphorus	-	0.48%
Iron	-	0.06%
Moisture	-	73%

The pelleted feed was prepared with the following ingradients (as reported by Sherief, 1989).

Ingredients	Percentage
1) Clam meat	- 40
2) Ground nut oil cake	- 25
3) Wheat bran	- 25
4) Tapioca	- 10

3.3 Feeding :

Feed was given *ad libitum*. Feeding was done twice a day, in the morning after removing the left over feed and in the evening. Clam meat and pelleted feed were given alternatively.

3.4 Experimental Procedure :

3.4.1. Biological Observations

3.4.1.1 Histological examination of the ovary

For histological examination, the ovary of *M.idella* in each stage of maturity was fixed in Bouin's fluid and embedding was done in paraffin wax (congealing point, 58 - 60^{0}c). Sections were cut at 6-8µm thickness using a Cambridge rocking microtome. Conventional slide procedure was used. Staining was done with Delafield's haematoxylin and eosin. Mounting was done with DPX mountant.

Composition of Bouin's fluid :-

Picric acid (saturated aqueous solution)	- 75 ml
Formalin (38-40% formaldehyde solution)	- 25 ml
Glacial acetic acid	- 5 ml.

For advanced stages of maturity (stage V and stage VI) Smith's fluid was also used as a fixative.

Composition of smith's fluid :-

Potassium bicarbonate	-	5.0 gm
Formalin	-	10 ml
Distilled water	-	88 ml
Glacial acetic acid	-	2.5 ml

3.4.1.2 The ovarian index (OI)

The ovarian index (OI) was calculated for each maturity stage using the following formula.

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

For this purpose, the animal was first weighed using a Contech (Model CB 120) Electronic Balance. Then the ovary was dissected out and weighed and the stage of the ovary noted. For each stage, OI was calculated from 5 ovaries and the average taken.

3.4.1.3 Ova diameter measurement

Microscopic measurement of oocytes was done in each stage to find out the mean oocyte diameter with standard deviation, characteristic of each stage.

Ova diameter measurements were taken from the anterior, middle and posterior regions of the ovary to study the progress of the development of ova

throughout the ovary following the method adapted by Clark (1934) and Prabhu (1956). First the ovary was dissected out, cleared off all extra tissue and kept in Gilson's fluid for better separation of eggs. Random samples of about 300 ova of each stage of maturity were measured from each ovary. Three prawns with ovaries of the same maturity stage were utilized for the measurement of ova diameter. Calibrated ocular micrometer (ERMA, Japan) was used for this.

Composition of Gilson's fluid

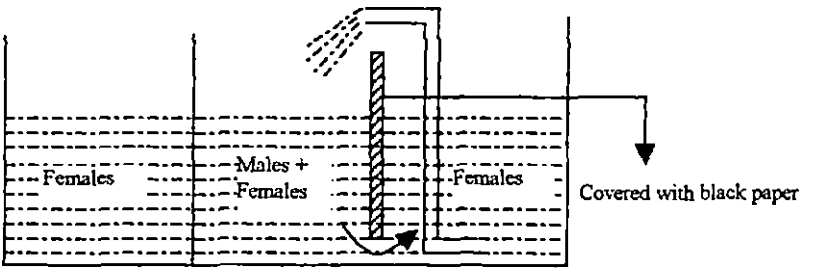
60% alcohol	-	100 ml
Distilled water	-	880 ml.
80% Nitric acid	-	15 ml
Glacial acetic acid	-	18 ml
Formalin	-	20 ml

3.4.2. Experimental Observations

3.4.2.1 First experiment

Ovarian growth was monitored in 3 groups of animals maintained in 3 adjacent glass chambers (1.5 × 1 × 1 feet each) with continuous aeration (see Fig. 1). First group of animals in the first chamber consisted of only females, but these had visual contact with the second group in the second chamber containing males and females. The third group in the third chamber consisted of only females with no visual contact with the second group (glass wall covered by black paper), but had a common water circulation with the second chamber and could be thus exposed to the putative male pheromone. The duration of the experiment was 48 days (maximum period of two maturation cycles).

Fig. 1 The arrangement of the glass chambers.



Ovarian stage of each specimen was recorded daily and the appearance of berry in the last 3 weeks noted as the end point. The results were analysed statistically and Fisher's exact test was used for comparison of the stages between the glass chambers.

3.4.2.2 Second experiment

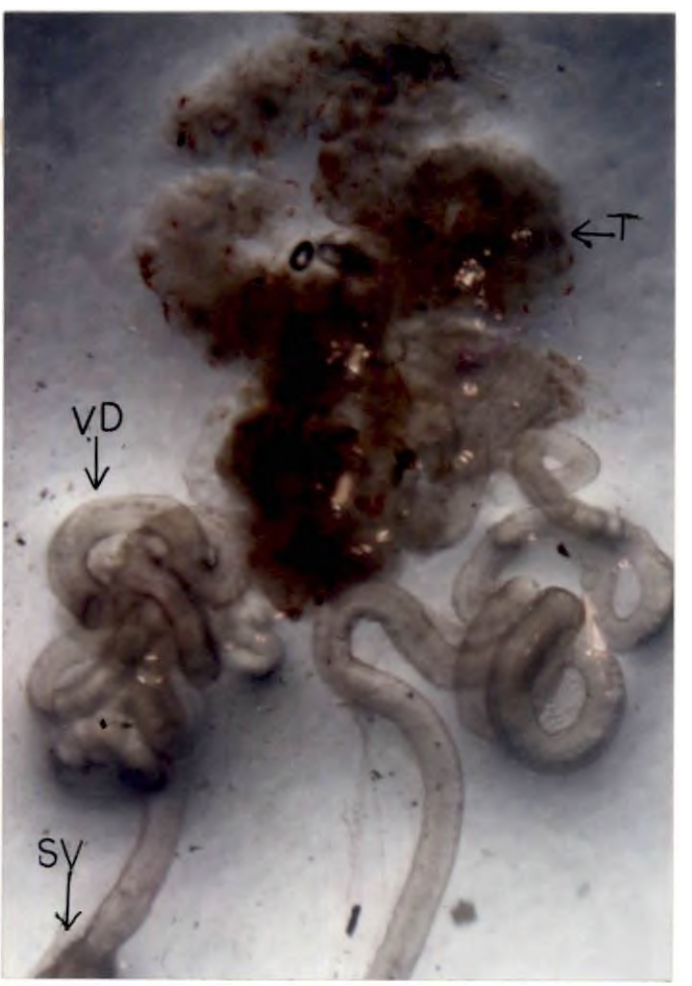
Three groups of females (two experimental and one control) were kept in isolation for 6-7 weeks till their ovaries had arrested maturation at stage IV. Further the two experimental groups were treated either with Testis (T) extract or Vas deferens (VD) extract and the control group with dorsal flexure muscle (M) extract to find out the possible source of male pheromone. The advancement to berried condition was taken as the end point of the experiment.

This experiment was continued for 24 days with replenishment of extracts at 4 days interval. Ovarian maturation was monitored daily and full maturation and ovulation was indicated by presence of berry in the females.

Testis, vas deferens (plate 1) and muscle (dorsal flexure muscle of the abdomen), removed from mature males were kept in ice and homo-genized in glass distilled water for 15min. at 0^0C. Each tissue extract was then centrifuged at 5000 rpm for 10min in a cold centrifuge (model MB-20

Plate 1

The position of testis (T), vas deferens (VD) and seminal vesicle (SV).×10



Superspeed Refrigerated Centrifuge) and the supernatant was added to the tanks at a concentration of 1 organ /animal /2.5 lits. of water.

3.4.2.3 Third experiment

Four groups of isolated females with maturation arrested ovaries similar to those in second experiment were used in this experiment. Here Testis (T) and Vas deferens (VD) extracts were pooled and further subjected to charcoal extraction or boiling as given below. In this experiment the four groups were charcoal extracted T & VD, Boiled T & VD, Untreated T & VD and Muscle (control). All the conditions were similar to that in second experiment.

In case of charcoal mixed extracts, 0.10 g of activated charcoal procured from Nice Chemicals was added with 10 ml of the extracts (both testis and vas deferens). Then this charcoal mixed extract was vortexed for 5min. Then it was centrifuged in cold centrifuge as mentioned above. If charcoal particles were still visible, the extract was filtered through Whatman No. 1 filter paper and then added to tanks.

In case of boiled extract, the extract of testis (T) and vas deferens (VD) was boiled for 15 min. in boiling water, then the extract was centrifuged in cold centrifuge as given above and supernatant was added to tanks.

In the case of untreated extract, plain extract of testis (T) and vas deferens (VD) (without any treatment) was centrifuged and supernatant added to tanks.

3.4.3. Experimental tanks

Second and third experiments were carried out in blue coloured plastic tanks (60 × 30 × 38 cm). Continuous aeration was provided in the tanks. Broken tiles were provided in all tanks to offer shelter for the weak shrimps during moulting and to reduce cannibalism. All the tanks were exposed to natural light in the *Macrobrachium* hatchery of the College.

3.4.4. Determination of water quality parameters

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

Temperature : 25.2 – 27.8 ^{0}c

pH : 6.5 – 7.3

3.5 Statistical design of the experiment.

The data of ovarian index was subjected to Completely Randomised Design (CRD) to differentiate between the maturity stages.

In the experiment to find out regulatory role of visual/chemical stimuli on ovarian maturation, the results were analysed statistically. Fisher's exact test was used for comparison of reproductive activity of animals between the glass chambers.

To find out the source of male pheromone, the results of three treatments with testis extract, vas deferens extract and muscle extract (control) were subjected to Fisher's exact test.

In the third experiment to find out the nature of the male pheromone, there were four treatments namely boiled tissue homogenate, charcoal extracted tissue homogenate, untreated tissue homogenate and muscle homogenate (control). Here both testis as well as vas deferens were taken together for making tissue homogenate. Here also results were analysed using Fisher's exact test.

Results

4. RESULTS

The results of present investigation can be conveniently categorized as :

4.1 Biological Observations :

4.1.1. Breeding biology of *M. idella*

4.1.2. Histological examination of ovary

4.1.3. Ovarian index (OI)

4.1.4. Ova diameter measurement

4.2 Experimental Observations :

4.2.1. Regulatory role of visual/chemical stimuli on ovarian maturation

4.2.2. Source of male pheromone.

4.2.3. Nature of male pheromone.

4.1 Biological Observations

4.1.1. Breeding biology of *M. idella* :-

4.1.1.1. Sexual dimorphism :

In. *M. idella* males are larger than the females. In males, second pair of pereiopods is excessively long and rather thick and genital aperture of males is situated on coxa of 5th pereiopod, while in females, second pair of pereiopods is shorter and slender and genital pore is situated at the coxa of 3rd pereiopod.

4.1.1.2. Reproductive organs :

The male reproductive system consists of a pair of testes, a pair of vas deferens and a pair of male genital pores. The testes consist of two elongated simple tubes situated in the posterior half of the cephalothorax above the hepatopancreas and below the heart. The vas deferens arises from the posterior region of the testis and divisible into an Anterior Vas Deferens, Mid Vas Deferens and a swollen Seminal Vesicle. The latter opens at the base of the coxa of the last walking leg.

The ovary is bilobed, flattened, leaf-like structure confined to the dorsal part of the cephalothoracic region. It curves under the heart rises up above the hepatopancreas forming an incipient 'S' – shape.

The minimum size at first maturity of this species is reported to be 56-60 mm.

The breeding season of this species extends from June to January in the Cochin backwaters. A female becomes berried more than once and hence is a multiple spawner.

4.1.1.3. Maturity stages :

Seven maturity stages have been distinguished in females based on ovary size in relation to carapace cavity, colour of ovary, microscopic structure and size of ova, adopting the already available classification. Details are given below,

Stages	Characteristic features
I	1/6 of carapace cavity, colourless (Immature virgin).
II	1/5 of carapace cavity , still colourless or slight yolk deposition starts (Maturing I)
III	1/4 carapace cavity, light green, ova visible (Maturing II)
IV	1/2 carapace cavity, more greenish (Maturing III)
V	3/4 carapace cavity, Deep greenish (Mature I)
VI	Completely fills carapace cavity; Deep green (Mature II or Ripe)
VII	Shrunken, 1/5 carapace cavity, slightly greenish (spent).

4.1.2. Histological examination of ovary

4.1.2.1. Oogonial stage :

Oogonial cells are small round or oval cells lie close to germogen. These cells are characterized by a prominent nucleus containing chromatin granules and a thin layer of cytoplasm. Later it transforms to primary oocyte stage (plate- 2).

4.1.2.2. Primary oocyte stage:

Primary oocyte stage is characterized by enlarged cells containing a large nucleus with chromatin granules and large nucleolus. The cytoplasmic content has increased (plate- 2).

4.1.2.3. Previtellogenic oocytes:

This stage can be divided into two sub stages, viz., Previtellogenesis I &II.

Previtellogenesis – I : Oocytes enlarge in size. Large dense active nucleus with prominent nucleolus is present. At a later stage the nucleus becomes enlarged and less dense called germinal vesicle. Cytoplasm is clear and non granular and its ratio increases. Oocyte grows to a diameter of 80 μm (plate – 2).

Previtellogenesis- II : In this stage the oocytes are either round, oval or polygonal in shape. The germinal vesicle is large and occupies a major part of the oocyte. At this stage, two nucleoli are seen inside the nucleus.

All these stages correspond to maturity stage I.

4.1.2.4. Vitellogenic oocytes :

Vitellogenesis I : The characteristic feature of this stage is the appearance of yolk vesicles in the ooplasm as a single concentric layer at the periphery. The germinal vesicle is prominent. Oocytes are seen arranged in two zones. A zone consisting of avitellogenic oocytes of oogonial and primary oocyte stages are concentrated around germogen and another of vitellogenic oocytes with yolk vesicles. Oocyte has a diameter up to 90 μm (plate – 3). This stage corresponds to maturity stage II.

Vitellogenesis II : This stage is characterized by the appearance of yolk platelets in between yolk vesicles in the periphery. The fully formed yolk platelets first form a layer in the periphery of ooplasm and then start filling the entire cytoplasm. These are strongly acidophilic. Oocyte has a diameter up to 95 μm. Follicular cells are prominent with darkly stained nuclei forming a broad band surrounding the oocytes (plate – 4). This stage corresponds to maturity stages III (maturing II) and IV (maturing III).

Vitellogeneis -II, Mature I : In this stage the accumulation of yolk platelets increases considerably. Ova increase in size. Germinal vesicle becomes smaller. The follicular layer becomes thinner. Ova diameter increases upto 230 µm (plate – 5). This corresponds to maturity stage V.

Vitellogenesis – III, Mature II (Ripe) : The ooplasm is completely filled by yolk platelets. The germinal vesicle disappears and ova increases enormously in size. Ova diameter increases up to 400 µm (plate - 6). This stage corresponds to maturity stage VI.

4.1.2.5. Maturation arrested ovary of isolated female :

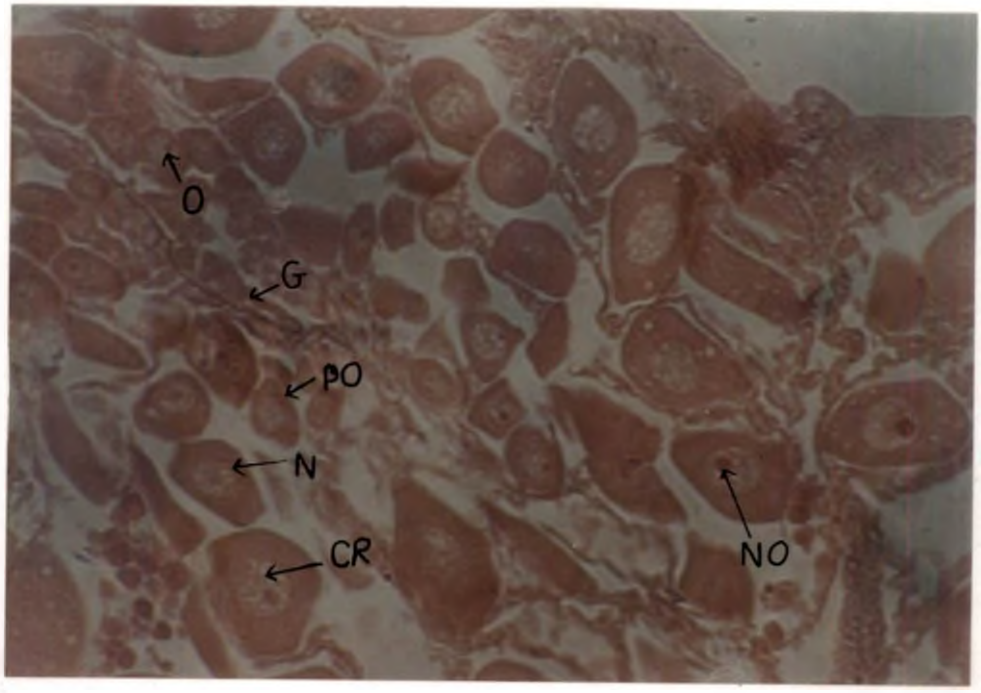
The ovary fills ½ carapace cavity, which corresponds to stage IV. But the germinal vesicle has shrunken chromatin and ooplasm with spaces similar to that seen in a resorptive ovary. (Plate – 7). Yolk platelets are absent. It gives the picture of an oocyte, which could not sustain vitellogenesis.

Plate 2

Photomicrograph of ovary in maturity stage I, showing germogen (G), oogonia (O), primary oocytes (PO), nucleus (N), nucleolus (NO) and chromatin reticulum (CR). Haematoxylin and Eosin staining 6 – 8 μ sections ×300.

Plate 3

Photomicrograph of ovary in maturity stage II, showing previtellogenic oocytes (PVO), vitellogenic oocytes (VO), yolk vesicle (YV), germinal vesicle (GV), follicular layer (F) and nucleolus (NO). Haematoxylin and Eosin staining 6 – 8 μ sections ×300.


O
G
PO
N
CR
NO

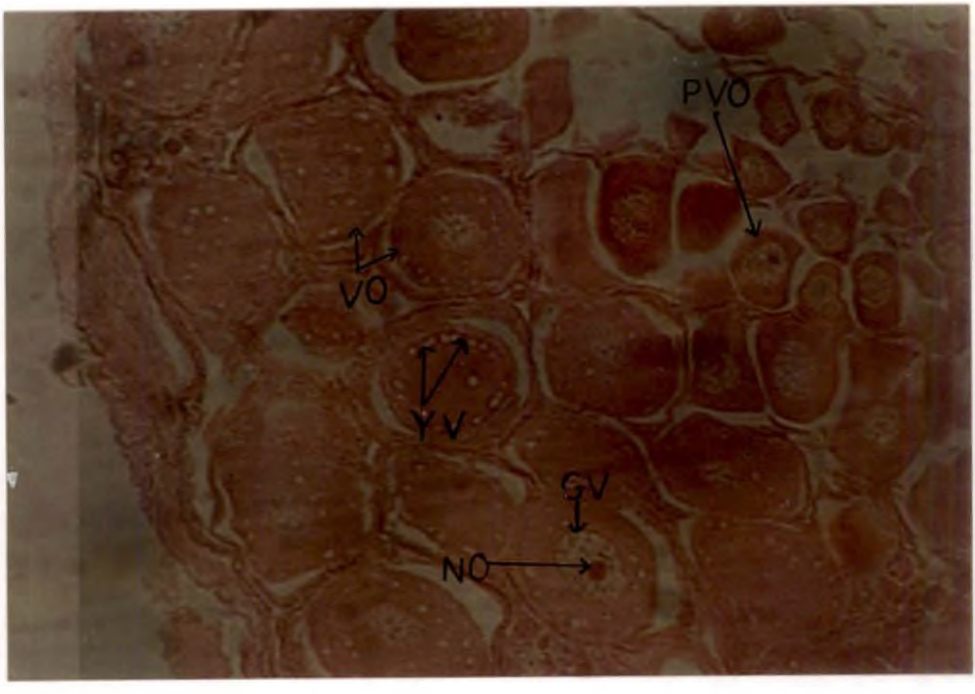
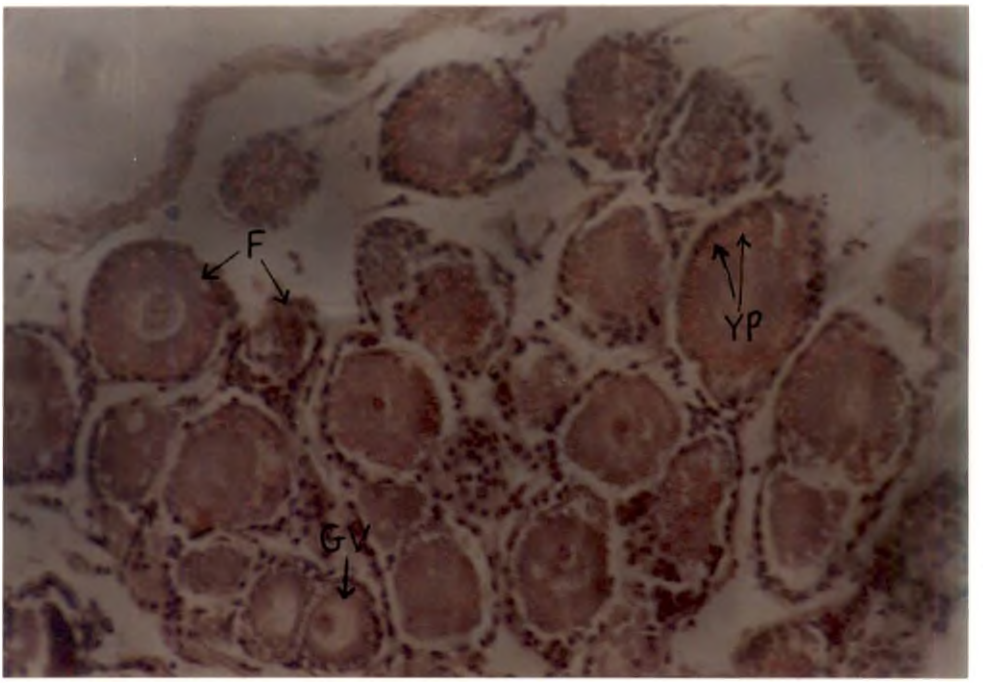

PVO
VO
YV
CV
NO

Plate 4

Photomicrograph of ovary in maturity stage IV, showing yolk platelets (YP), germinal vesicle (GV) and follicular layer (F). Haematoxylin and Eosin staining 6 – 8 μ sections ×300.

Plate 5

Photomicrograph of ovary in maturity stage V, showing yolk platelets (YP), germinal vesicle (GV), vitellogenic oocytes (VO) and previtellogenic oocytes (PVO). Haematoxylin and Eosin staining 6 – 8 μ sections ×150.


F
YP
GV

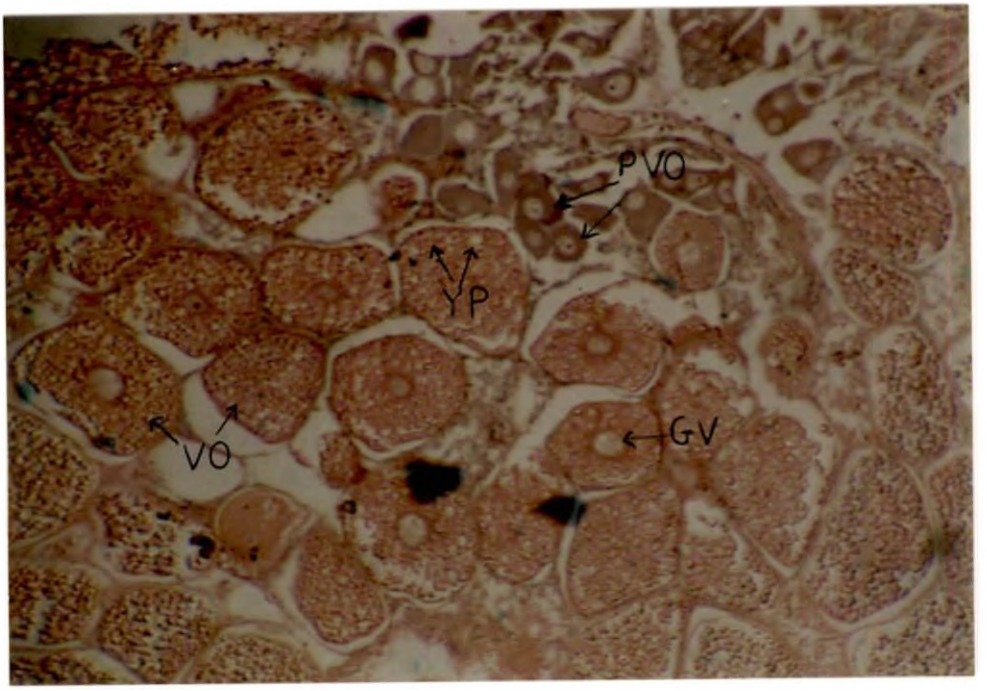
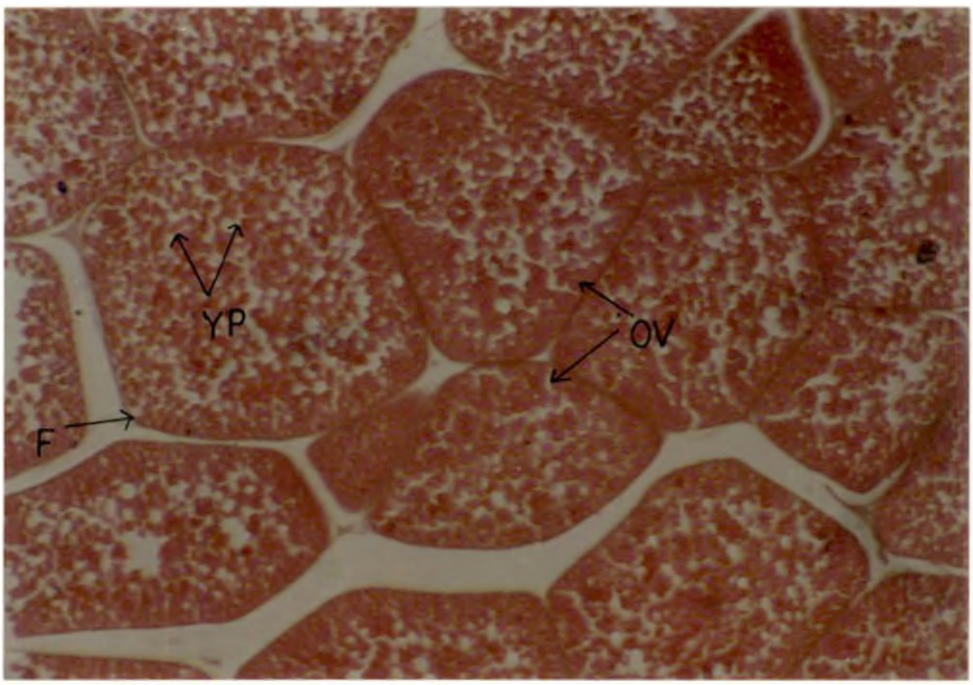

PVO
YP
VO
GV

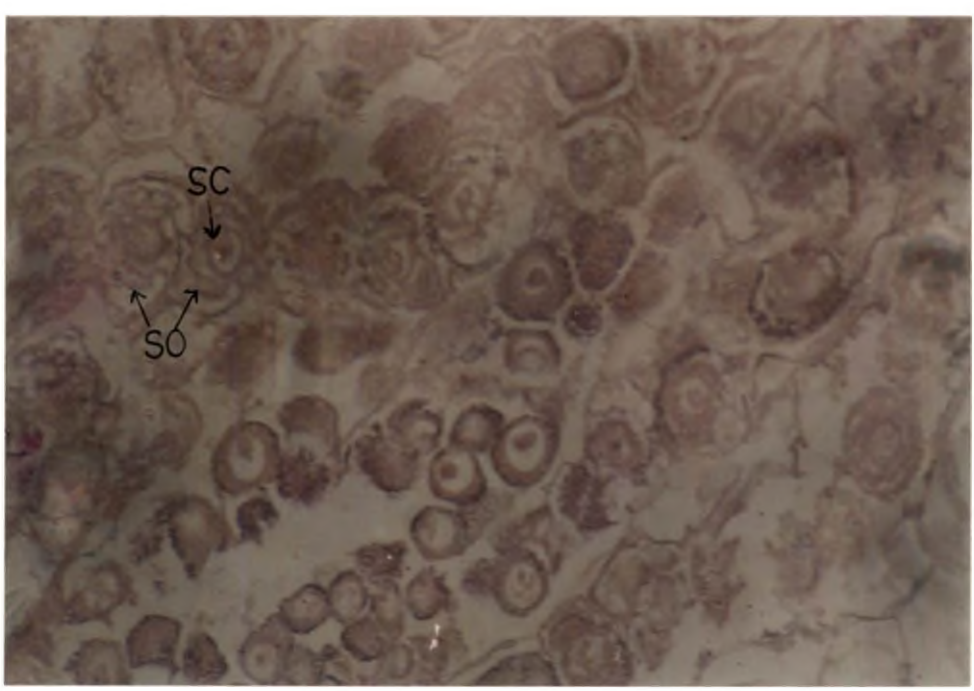
Plate 6

Photomicrograph of ovary in maturity stage VI (ripe), showing yolk platelets (YP), ovum (OV) and follicular layer (F). Haematoxylin and Eosin staining 6 – 8 μ sections ×150.

Plate 7

Photomicrograph of maturation arrested ovary, showing shrunken chromatin (SC) and spaces in ooplasm (SO). Haematoxylin and Eosin staining 6 – 8 μ sections ×300.


YP
OV
F


SC
SO

4.1.3. Ovarian index (OI):

The average Ovarian Index (OI) values for each stage of maturity is given in Table 1.

Generally, ovarian index was high for maturing and mature stages of ovary. It was lowest (0.30) for stage I and highest (4.11) for stage VI.

The progressive increase of OI from Stage I to VI is graphically presented in Fig.2.

It can be marked that there is clear differentiation in OI values between the vitellogenic stages starting from Stage III to Stage VI. Also it can be seen that there is a fall in OI value of stage VII (spent recovering ovary) (1.23).

The results of Anlaysis of variance for comparing the ovarian index values among stages are given in Table 2. Significant difference is seen among stages. However results of pairwise comparison shows that the stages I, II, III, IV and VII belongs to the same homogenous group.

4.1.4. Ova diameter measurement:

The results of ova diameter measurements are presented in Table 3. It can be seen that the range of ova diameter and Mean Ova Diameter (MOD) was highest for fully mature ovary (Stage VI) and lowest for immature Stage I and Stage II.

Table 1 : Average OI values for different stages of maturity

Stages	Average weight of ovary (gm)	Average OI values
I	0.004	0.30
II	0.016	0.61
III	0.023	0.67
IV	0.036	1.45
V	0.106	2.67
VI	0.177	4.11
VII(spent)	0.034	1.23

Table 2 : ANOVA for ovarian index value

Source of variation	Sum of squares	Degree of freedom	Mean sum of squares	F value
Between stages	55.62	6	9.27	
Error	6.66	28	0.24	38.63**
Total	62.28	34	-	

** Significant at 1% level

Critical difference 0.9439

Treatments (Stages)	T_1-I	T_2-II	T_3-III	T_7-VII	T_4-IV	T_5-V	T_6-VI
Mean Values (OI)	0.306	0.61	0.668	1.23	1.446	2.67	4.1

Underscored treatments are not significantly different.

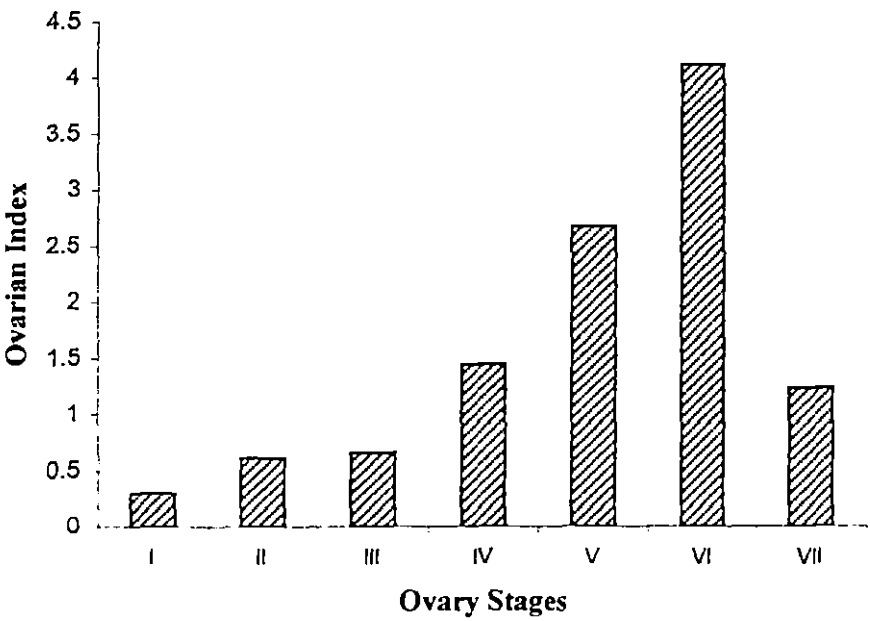


Fig.2 The Progressive increase of OI from stage I to stage VI

Table 3 : Ova diameter range with mean and standard deviation for different maturity stages

Ovary stages	Ova diameter range (μm) (Mean ± S.D.)
I	16 – 165 (71.88 ± 41.76)
II	19 – 160 (85.12 ± 30.57)
III	18 – 225 (89.83 ± 47.45)
IV	31 – 265 (108.91 ± 56.07)
V	36 – 348 (125.38 ± 78.20)
VI	46 – 403 (185.28 ± 105.03)
VII	26 – 206 (83.66 ± 41.85)

The ova diameter range for stage I was 16 – 165 μm (mean = 71.88 and S.D. = 41.76), while for stage VI, it was 46 - 403 μm (mean = 182.28 and S.D. = 105. 03).

Fig.3 shows the mean ova diameter for anterior, middle and posterior regions of the ovary in different stages of maturity.

Table 4 gives the mean ova diameter values with S.D. for the anterior, middle and posterior parts of the ovary. As is seen from Fig. 3, in the mature ovary, the middle and posterior regions had comparatively larger oocytes than the anterior region.

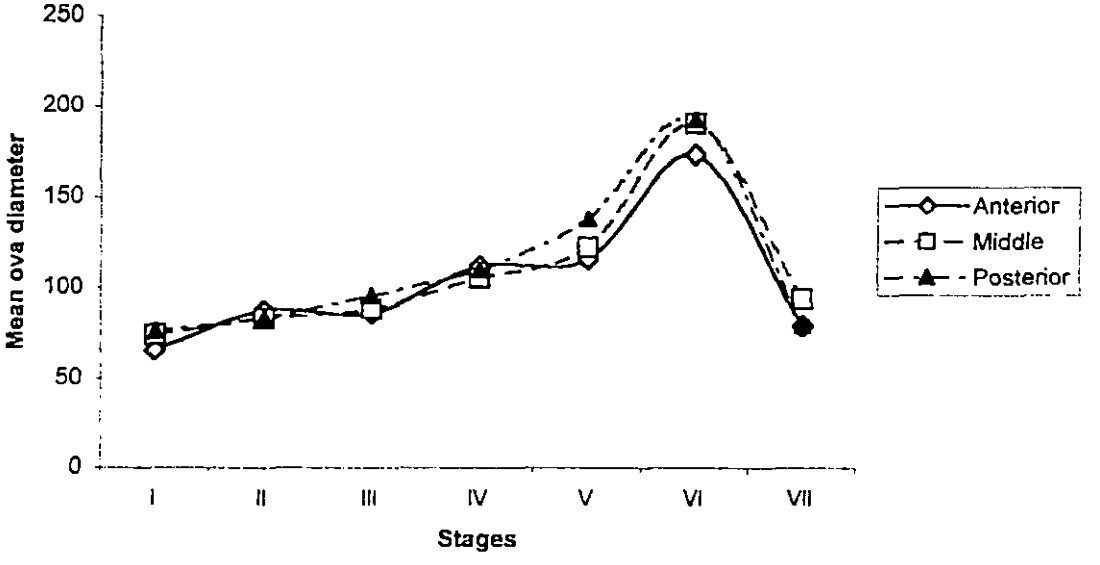
4.2 Experimental Observations

4.2.1. Regulatory role of visual / chemical stimuli on ovarian maturation (First Experiment)

In the Ist compartment, which contained isolated females with only visual contact with males kept in the IInd compartment, all prawns were found in the maturity stages between II & III. Not a single prawn was found with ovary in advanced maturity stage.

In the IInd compartment, which contained females in the company of males; 4 out of 6 prawns were found advanced to berried condition.

In the IIIrd compartment, which contained only females, which could receive chemical stimuli from males in the IInd compartment through a common water circulation, 4 out of 7 prawns were also found advanced to berried condition.



3 Mean Ova Diameter for anterior, middle and posterior regions of the ovary in different maturity stages

Table 4 : Mean ova diameter with standard deviation for the anterior, middle and posterior parts of the ovary

Ovary stages	Anterior portion Mean ± S.D.	Middle Mean ± S.D.	Posterior Mean ± S.D.
I	65.71 ± 43.29	74.06 ± 46.27	75.86 ± 34.92
II	87.34 ± 31.19	82.96 ± 31.77	83.06 ± 30.83
III	85.67 ± 51.01	88.16 ± 51.22	95.66 ± 48.10
IV	112.03 ± 60.28	104.97 ± 55.15	109.73 ± 55.91
V	115.72 ± 74.29	122.41 ± 84.48	138.02 ± 82.05
VI	173.06 ± 88.34	190.36 ± 112.83	192.41 ± 115.73
VII	78.66 ± 30.94	93.45 ± 52.61	78.86 ± 38.85

The comparison of the results of prawns kept in these compartments was done using Fisher's exact test.

When comparison was made between Ist and IInd compartments (Table 5), the calculated probability was less than 0.05, which implies that there is significant difference between these two at 5% level. So isolated females even if they had visual stimuli from males could not have ovarian maturation and consequent ovulation.

Similarly, when comparison was made between IInd and III rd compartments (Table 6), the calculated probability was found to be more than 0.05, showing that there is no significant difference between these two compartments at 5% level. This indicates that either actual presence of male or chemical stimuli from males through water circulation had the same effect on ovarian maturation and ovulation.

When comparison was made between Ist and IIIrd compartments (Table 7), the calculated probability was less than 0.05. Therefore, there is a significant difference between these two compartments at 5% level. This shows that chemical stimuli and not visual stimuli from males facilitates ovarian maturation and ovulation.

4.2.2. Source of pheromone (Second experiment)

When treated with testis extract, 5 out of 8 females showed advanced ovarian development and successfully ovulated to become berried. With vas deferens extract 6 out of 8 females became berried whereas in muscle extract (control) none of the females became berried.

Table 5 : The comparision between I^{st} and II^{nd} compartment

	Berried	Non – berried	Total
I^{st} Compartment	0	8	8
II^{nd} Compartment	4	2	6
Total	4	10	14

Probability (p) = 0.01, which is less than 0.05.Therefore there is significant difference between these two at 5% level.

Table 6 : The comparison between II and III compartment

		Berried	Non berried	Total
1	II compartment	4	2	6
	III Compartment	4	3	7
	Total	8	5	13

		Berried	Non berried	Total
2	II Compartment	5	1	6
	III Compartment	5	2	7
	Total	10	3	13

		Berried	Non-berried	Total
3	II Compartment	6	0	6
	III Compartment	6	1	7
	Total	12	1	13

P1 = 0.41

P2 = 0.44

P3 = 0.54

Therefore the required probability is,

P = P1 + P2 + P3

= 0.41 + 0.44 + 0.54

= 1.39

which is more than 0.05. There is no significant difference between these two compartments at 5% level.

Table 7 : The comparison between I and III compartment

	Berried	Non berried	Total
I Compartment	0	8	8
III Compartment	4	3	7
Total	4	11	15

The probability (p) =0.03, which is less than 0.05. Therefore there is significant difference between these two compartments at 5% level.

This result was statistically analysed using Fisher's exact test.

When comparison was made between testis extract and control, it was found that there is a significant difference between these two treatments at 5% level (Table 8). Therefore, the introduction of testis extract definitely facilitated ovarian maturation and the number of berry was increased compared to the control.

Similarly, when comparison was made between vas deferens extract and control, it was found that there is also significant difference between these two treatments at 5% level (Table 9). Therefore, vas deferens treatment also improved ovarian maturation as evidenced by the increase in number of berried animals.

4.2.3. Nature of pheromone (Third experiment)

When untreated extracts of testis and vas deferens (uTVDE) were added to medium, 5 females out of 8 were found berried. Similarly when boiled (treated) testis and vas deferens extract (bTVDE) was added, 4 females out of 7 became berried, whereas charcoal added testis and vas deferens extract (cTVDE) resulted in only 2 berried females out of 8. In the control, on adding muscle extract not a single female was found with berry.

This result was also statistically analysed using Fisher's exact test.

When the comparison was made between uTVDE treated group and control group (Table 10), it was found that there is significant difference between

Table 8 : The comparison between testis and muscle extract

	Berried	Non berried	Total
Testis	5	3	8
Muscle (control)	0	8	8
Total	5	11	16

Probability (p) = 0.01, is less than 0.05. Therefore there is significant difference between these two treatments at 5% level.

Table 9 : The comparison between vas deferens and muscle extract

	Berried	Non berried	Total
Vas deferens	6	2	8
Muscle (control)	0	8	8
Total	6	10	16

Probability, P = 0.03, is less than 0.05. Therefore, there is also significant difference between these two treatments at 5% level.

Table 10 : The comparison between untreated T and VD extract and muscle extract

	Berried	Non berried	Total
Untreated	5	3	8
Control	0	8	8
Total	5	11	16

Probability (p) = 0.01, which is less than 0.05. Therefore, there is a significant difference between these two treatments at 5% level.

these two treatments at 5% level showing that the uTVDE at the dose level used in this experiment had enough pheromonal biological activity.

A similar significant difference was obtained between bTVDE treated group and control group (Table 11). From this it is clear that, boiling of extracts (temperature) did not affect the biological activity of pheromone.

However, a comparison between cTVDE treated group and contol, revealed that there was no significant difference between these two treatments (Table 12). Thus it gives an idea that charcoal extraction affects the biological activity of the extract and so could not improve ovarian maturation and ovulation. The biological activity in T & VD extracts was affected by charcoal extraction.

Table 11 : The comparison between boiled T and VD extract and muscle extract

	Berried	Non berried	Total
Boiled	4	3	7
Control	0	8	8
Total	4	11	15

Probability (p) = 0.03, which is less than 0.05, hence there is a significant difference between these two treatments at 5% level.

Table 12 : The comparison between charcoal added T and VD extract and muscle extract

	Berried	Non berried	Total
Charcoal	2	6	8
Control	0	8	8
Total	2	14	16

Probability (p) = 0.23, which is more than 0.05, hence there is no significant difference between these two treatments at 5% level, which implies that neither of these are effective for berried condition.

Discussion

5. DISCUSSION

5.1. Biological observations:

5.1.1. Breeding biology of *M. idella*: -

M. idella exhibits clear sexual dimorphism. Sexes could be distinguished by : a) males are larger than the females, b) in males, second pair of pereiopods is excessively long and rather thick and genital aperture is situated on coxa of 5th pereiopod, while in females, second pair of pereiopods is shorter and slender and genital pore is situated on coxa of 3rd pereiopod (Jayachandran, 1984). Similar observations on sexual dimorphism have been reported in other important species like *M. rosenbergii* (Mary John, 1957); *M. malcolmsonii* (Philip and Subramoniam, 1992) and *M. gangeticum* (Singh and Roy, 1994).

The male and female reproductive systems of this species and histological changes associated with oogenesis have been well documented by Jayachandran and Joseph (1986; 1988).

The observations made in present investigation on the sexual dimorphism, and reproductive organs in *M.idella* fully agree with the observations made by the above authors.

Seven maturity stages reported for the female of this species by Jayachandran (1984) have been distinguished in the present investigation also. The classifiation is based on ovary size in relation to carapace cavity, colour of ovary, microscopic structure and size of ova. However, Joshi and Diwan (1992) could recognize only 6 maturity stages in *M. idella*. In *M. rosenbergii*,

Damrongphol *et al.*, (1991) classified ovarian development into 4 stages, based on external examination of coloration and the relative size of the ovaries to the size of cephalothorax. In *M. malcolmsonii*, Philip and Subramoniam (1992) classified ovarian maturation into 6 stages and in *M. gangeticum*, Singh and Roy (1994) reported 5 maturity stages for females.

This species has been found to breed during June to December in Cochin backwaters, whereas it breeds from August to December/ January in Vellayani Lake (Jayachandran 1984). The variation observed may be due to geographical variation. Similarly Raman (1967) reported that the breeding season of *M. rosenbergii* extends from July to December in central Kerala while, Damronghol et al., (1991) reported that this species breeds throughout the year in Sri Lanka. Philip and Subramoniam (1992) observed that the breeding season of *M. malcolmsonii* extends from April to November and in *M. gangeticum* breeding season extends from May to September (Singh and Roy, 1994).

5.1.2. Histological examination of ovary in different maturity stages: -

The characteristic features of the oogonial stage, primary oocyte, previtellogenesis I and II, vitellogenesis I, II and III observed in the present investigation fully agree with the observations already made on the same species by Jayachandran (1984) and Jayachandran and Joseph (1988). The yolk granules are seen arranged at peripheral ooplasm. These granules are actually synthesized at the central portion (intra-oocytic) and then moved to the periphery, where they coalesce and form bigger granules (platelets) and get arranged as a layer. As the

ovarian maturation proceeds, the yolk platelets are seen filling up the entire ooplasm. In some decapods where only moderate yolk is present in ova, vitellogenesis is totally endogenous. In others, either subunits of yolk or lipid part may have extraovarian origin. Biochemical studies made by Quackenbush (1989) support the histological observations.

Sebastian (1993) reported 6 well marked histological stages in *M. equidens equidens* (= *M. equidens*) and *M. equidens pillaii* (= *M. sulcatus*) namely, Oogonial stage, Previtellogenesis I, Previtellogenesis II, Vitellogenesis I, Vitellogenesis II and Degenerating stage. Charles and Subramoniam (1982), identified only 5 histological stages for *M. malcolmsonii* and *M. lamarrei.* The present investigation revealed that sequential changes of oogenesis of *M. idella* follows very closely the pattern observed in the *M.equidens* and *M. sulcatus.*

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

In *Paratya compressa,* Takayanagi *et al.* (1986b) found that in isolated females ovary remains white at stage II (where only 4 maturity stages identified). Progress of maturation to stage III where the ovary is brown in colour and the ooplasm is with large number of oil droplets is dependent on the presence of male

(or male pheromone). The conclusion emerging from these studies is that in the absence of male, ovarian maturation is blocked at the stage of massive vitellogenesis. A similar observation has been made in *M. kistnensis* (Nagabhushanam *et al.*, 1989), and they arrived at similar conclusion based on the change of colour of ovary and mean oocyte diameter. Biologically, the advantage of not wasting energy in vitellogenesis when the male is not available nearby is very evident. In that respect *M. idella* is one species which is highly evolved and successful reproductively.

5.1.3. Ovarian index (OI)

The ovarian index progressively increases as maturity stage advances in *M. idella.* For stage I, OI is 0.30; while it is 0.61 for stage II; 0.67 in stage III; 1.45 in stage IV; 2.67 in stage V; 4.11 in stage VI and 1.23 in stage VII and is reported for first time in this species. It has been reported that in red swamp crayfish *Procambarus clarkii*, ovarian index was useful for classification of different maturity stages (Kulkarni *et al.*, 1991). In *M. idella*, it provides an additional parameter for establishing the late maturity stages. The rather high value of OI in stage VII may indicate partial spawning in laboratory conditions.

In *M. idella*, it is observed that there is clear differentiation in the size of ovary as the maturity progresses. In this species there is a special advantage as the ovary is visible externally through transparent carapace, so that the maturity stage can be determined without sacrificing them. The results of mean oocyte diameter and OI in *M. idella* indicate that it is a protracted multiple spawner. OI is a more reliable quantitative parameter than mean oocyte diameter for

differentiating the vitellogenic II maturity stages (stages IV, V and VI). These results are comparable to that in *P. clarkii.* Thus, though *M. idella* has multiple spawning habit within a breeding season, the maturation cycles are clearly demarcated and enormous increase in ovarian size and weight takes place especially in the vitellogenic phase. This condition is not seen in fishes.

5.1.4. Ova diameter measurement: -

Ova diameter measurements from the anterior, middle and posterior regions of the ovary were taken to study the progress of ova development within the ovary following the method adopted by Clark (1934); Prabhu (1956) and Jayachandran (1984). It has been found that the ova are evenly distributed in the anterior, middle and posterior regions of the maturing ovary. But in the mature stages (stage V and VI), larger ova are present in the posterior region of the ovary. Jayachandran (1984) made similar observations.

Growth of ova from immature to mature stages occurs by accumulation of yolk, because of this ova diameter increases. In this species, growth of ova showed a peculiar pattern. It was observed that the growth of ova from stages II to III and IV to V is faster than between stages I to II and III to IV. It is very clear that only a single batch of eggs passed through different stages of maturity. A second batch developed only after ovulation of the first batch into the brood chamber. The present observation is well in agreement with the observations made by Jayachandran (1984) mentioned above. In *M. rosenbergii* Damrongphol *et al.*, (1991) reported that more time was taken for maturation of ova from stage

1 to 2 i.e. from primary vitellogenesis to secondary vitellogenesis similar to the condition in *M. idella.*

In the case of annual spawners, mean oocyte diameter shows statistically significant variations between maturity stages and along with GSI help to characterize and differentiate maturity stages. But in *M. idella,* being a protracted multiple spawner, though the range of oocyte diameter increased with maturity stages, the difference between the mean oocyte diameter values were not significant which is due to the fact that only a single batch of ova underwent maturation at a time while a large number of immature ova remain close to the germogen. This type of development is quite visible in the histological sections.

5.2. Experimental Observations :

5.2.1. Regulatory role of visual/chemical stimuli on ovarian maturation :-

Takayanagi *et al.*, (1986b) for the first time reported that chemical stimuli from the males is essential for normal ovarian growth in female *Paratya compressa.* They proposed the existence of an ovary stimulating pheromone in that species. Takayanagi *et al.*, (1986b) allowed common water circulation in the 3 experimental chambers and found no difference in ovarian maturation between the chambers. i.e. the presence or absence of visual stimulus does not influence/ potentiate ovarian maturation if chemical stimulus is available.

The present experimental design was different from theirs. It was observed that the ovarian growth was normal in the females which were reared along with males (in second chamber) and also in the isolated females (in third chamber) which had common water circulation from the chamber which contained males

(but had no visual stimuli from males in second chamber). But in the tank, where females were reared in isolation, the ovarian growth was found to be arrested evethough they had visual stimuli from second chamber containing males. Therefore, it can be concluded that in *M. idella* the chemical stimulus from males is responsible for normal ovarian growth and that visual stimuli has no stimulatory effect. Therefore, the arrest of ovarian growth in isolated females is due to lack of male pheromone and not due to absence of visual signals.

Dunham (1978) reported that in the crab *Callinectis sapidus* both visual and chemical stimuli are needed for the full manifestation of the sexual behaviour. In sexual behaviour involving sexual display and courtship, visual signals also can be regarded as important along with sex pheromones. But in the present species for ovarian maturation visual signals have no effect by themselves. No evidence for synergistic effect of visual signals along with the pheromone was reported by Takayanagi, *et al.*, (1986b) in *P. compressa.*

5.2.2. Source of male pheromone :-

When the isolated females of *M. idella* with maturation arrested ovaries were exposed to the aqueous extracts of testis and vas deferens, complete ovarian maturation and ovulation were induced in the females. But when treated with muscle extract (control) there was no such stimulation of ovarian growth. Therefore, it is evident that the male pheromone is either produced both by testis and vas deferens or it is produced by testis and transported through or stored in the vas deferens.

Nagabhushanam *et al.*, (1989) observed that ovarian development in the fresh water shrimp, *Macrobrachium kistnensis* is delayed in the absence of the males. On the other hand if they are reared along with the males or male water (water in which males lived) normal ovarian development occurred. They used different parameters such as oocyte diameter, ratio of previtellogenic: vitellogenic oocytes and colour of the ovary (white to green) to understand the ovarian growth. A similar observation was also made by Takayanagi *et al.*, (1986b) in *Paratya compressa.* They observed arrested ovarian growth in the females isolated from males. When these females were exposed to homogenates of testis or vas deferens, their ovarian growth was found to be stimulated (colour of ovaries changed from white to brown), but in the muscle extract, there was no such stimulatory effect (ovarian colour remained as white). Therefore, they proposed that the ovary stimulating pheromone might be synthesized in the testis and transported to the vas deferens.

Jayachandran and Jose (1993) reported that in females of *M. idella* reared in isolation in the post breeding season, ovaries failed to develop beyond maturation stage IV. The females also suffered from severe hypophagia.

Takayanagi *et al.*, (1986a) further reported that, in the female of *P. compressa* reared in the absence of males, the ovarian growth was found to be induced by extracts of brain or thoracic ganglion. On the basis of this they opined that the ovary stimulating pheromone may not act on ovarian development directly, but rather it may act indirectly through the release of the ovary

stimulating hormone from brain / thoracic ganglion. A similar observation was made by Nagabhushanam *et al.*, (1988) in *M. kistnensis*.

It is well known that the decapod eyestalk contains gonad inhibiting hormone (GIH) and when this is removed by eyestalk ablation ovarian development enhances. Bijulal (1994), observed that in eyestalk ablated females of *M. equidens* there was enhancement of frequency of spawns compared to control. Jayachandran and Jose (1993) observed that in the absence of males, ovarian growth was arrested at stage IV in both normal and unilaterally eyestalk ablated females. When males were reintroduced, both normal and eyestalk ablated females showed full ovarian maturation and ovulation. Unilateral eyestalk ablation (and probable decrease in GIH) was not able to overcome the block in ovarian maturation induced by pheromone deprivation. It can be construed that in the female *M. idella* similar to *P. compressa* and *M. kistnensis*, a gonad stimulating hormone is more important than GIH in regulating vitellogenesis and ovulation and the pheromone is probably acting through a gonad stimulating hormone (GSH). GSH is now shown to act directly on crustacean ovaries to stimulate maturation and ovulation similar to the effect of gonadotropin in vertebrate ovaries. Vertebrate type steroids were also reported to be present in the ovarian tissue of *Nephrops norvegicus* (See Fairs *et al.*, 1989). These steroids were found to activate maturation and spawning in many penaeid and non penaeid prawns (Kulkarni *et al.*, 1979, Nagabhushanam *et al.*, 1980; Yano, 1985).

5.2.3. Nature of pheromone :-

The exact chemical nature of ovary stimulating pheromone remains to be resolved. The female sex pheromone from the sternal gland of *M. kistnensis* is

reported to be a glycoprotein based on histochemical studies (Sarojini *et al.*, 1982). In fishes sex steroid hormones and /or steroid glucuronides are reported to act as sex pheromones (Hara, 1993). Testosterone is detected in the serum and testes of the American lobster *Homarus americanus* (Burns *et al.*, 1984). So the possibility is that the ovary stimulating pheromone may be either proteinaceous or steroidal in nature.

The present investigation tried to get clues regarding the chemical nature of this male pheromone. It was observed that both untreated and boiled extracts of testis and vas deferens stimulated ovarian growth, but charcoal extracted testis and vas deferens homogenate failed to do so. Most proteins are denatured by boiling. In the present study and also in the study by Takayanagi *et al.*, (1986b) boiling did not affect the pheromonal activity. So the pheromone might not be proteinaceous in nature. Charcoal extraction in the present study removed the active pheromonal components in the testis and vas deferens extract showing that it might be a steroidal component similar to that in fishes. Presence of male releaser pheromone has been identified in the fresh milt and mature testes extracts of the herring *Clupea harengus pallasi* (Sherwood *et al.*, 1991) and in the seminal vesicle fluid of catfish *Clarius gariepinus* (Lambert and Resink, 1991). Biochemical studies showed the presence of steroids and steroid glucuronides in these preparations. In the catfish *Clarius gareipinus*, 17 α- hydroxy progesterone glucuronide was shown to be the most potent odorant. So it can be proposed that in this species also, a steroid or steroid metabolite present in the testis and vas deferens is acting as the ovary stimulating pheromone.

Summary

6. SUMMARY

The present study was carried out to confirm the existence of a putative male pheromone in *Macrobrachium idella*. Experiments were also carried out to find the possible source and chemical nature of the male pheromone. Other reproductive biological parameters such as ovarian index (OI) and ova diameter were studied along with histological examinations of ovary in different stages of maturity. The methodology, important results and conclusions of the study are as follows.

1. The mature males and females of *M. idella* were collected from different localities in Cochin backwaters. The size range (total length) for experimental animals was 7.1-9.3 cm for males and 5.1-6.2 cm for females. The animals were fed with clam meat as well as pelleted feed.

2. Histological examination of the ovary in different maturity stages was carried out to observe the microscopic changes which takes place in different maturity stages. Stage I has previtellogenic oocytes. Yolk vesicles start appearing in the ooplasm from maturity stage II onwards, which is the beginning of vitellogenesis (vitellogenesis I). In stage IV, there is appearance of yolk platelets in between the yolk vesicles (vitellogenesis II). Accumulation of acidophilic yolk platelets continues through stage V and completely fills the cytoplasm in the ripe oocyte (stage VI). The ova having a diameter 80 μm (stageI) grows to 400 μm at stage VI.

3. The ovarian index (OI) was calculated for different maturity stages of the ovary, which helps to distinguish the maturity stages and also to follow the

progress of maturation. The OI value was smallest (0.30) for the stage I (immature) and highest (4.11) for the stage VI (mature or ripe). In stage VII (spent) there is again a fall in OI value (1.23).

4. Ova diameter measurements for each maturity stage were taken in different maturity stages of the ovary with mean and standard deviation. Being a protracted multiple spawner there is a large stock of immature ova around germogen and maturing ova in the ovary undergo maturation and ovulation as a single batch. Development of ova within ovary through the progressive maturity stages can be best understood from this. It was observed that in immature and maturing stages there is no difference in the distribution of ova in anterior, middle and posterior parts, but in mature stages i.e. stage V and VI, comparatively larger oocytes are seen in the posterior region of the ovary.

5. An experiment was carried out to gather information on the existence of male pheromone in this species. This experiment was carried out in aquaria, which had three compartments. In first water tight compartment, only females alone were kept which had visual stimuli (through glass partition) from the males in second compartment (i.e. middle compartment, which contained males and females). In the third compartment also only females were kept with no visual stimuli from the males in second compartment (due to dark screen) but had chemical stimuli through common water circulation from second compartment. At the end of experiment (48 days) the ovarian growth of the females in second (cohabitation with males) and third compartment (chemical stimuli from males) was found to be normal but ovarian growth of isolated

females in first compartment was found to be arrested at early stage IV, which suggest that a pheromone from male stimulates the ovarian maturation in females from stage IV. Visual stimuli has no role in this process.

6. An experiment was conducted to establish the possible source of this male pheromone. Isolated females with maturation arrested ovaries were exposed to the aqueous extracts of testis, vas deferens or muscle (control). The concentration of these extracts was 1 organ/animal/2.5 l. of water. The duration of this experiment was 24 days. Both testis and vas deferens extracts were found to stimulate the ovarian maturation and ovulation in these isolated females, while muscle extract was found to be ineffective in stimulating ovarian growth. Therefore, it is concluded that the male pheromone is probably produced by testis and vas deferens or it is produced by testis and transported to vas deferens.

7. Further experiment to find out the chemical nature of this male pheromone showed that charcoal extraction destroyed the pheromonal activity in testis and vas deferens extract while boiling had no such effects. This indicates that this male pheromone or ovary stimulating pheromone may be of steroidal nature as reported in some fishes. More knowledge on the role of pheromone in the reproductive biology of fresh water prawn will be needed for proper aquacultural practices.

References

7. REFERENCES

Adiyodi, K.G. (1980). Some Thought on the Evolution of "Reproductive" Hormones in Invertebrates. In : T. Subramonian and S. Varadarajan (eds.), *Progress in Invertebrate Reproduction and Aquaculture*, New Century Printers, Madras. pp. 89 – 93.

Adiyodi, K.G. and Adiyodi, R.G. (1970). Endocrine control of reproduction in crustacea. *Biol. Rev.*, **45** : 121 - 165.

*Adiyodi, R.G. (1969). Insect Hormones other than pheromones. *Vijin. Kair*, **1** : 57 – 65.

Adiyodi, R.G. (1985). Reproduction and its control. In : D.E. Bliss and L.H. Mantel (eds.). *The Biology of Crustacea*: 9. *Integument, Pigments and Hormonal Processes.* Academic Press, New York. p. 147 – 215.

Adiyodi, R.G. and Subramoniam, T. (1983). Arthropods – Crustacea. In : K.G. Adiyodi and R.G. Adiyodi (eds.), *Reproductive Biology of Invertebrates* 1. Academic Press, New York, pp. 443 - 495.

Ameyaw-Akumfi, C. and Hazlett, B.A. (1975). Sex recognition in crayfish *Procambarus clarkii. Science*, **190** :1225-1226.

Anilkumar, G. and Adiyodi, K.G. (1981). Ovarian growth induced by eyestalk ablation during the prebreeding season is not normal in the crab, *Paratelphusa hydrodromous* (Herbst). *Int. J. Invert. Reprod.*, **2** : 95 – 105.

*Arroya, M.G., Galaviz, I.J.R. and Gonzalez, L.D.G. (1982). Annual cycle of maturity and reproduction of the Chacal *Macrobrachium tenellum* Smith and its relationship with environmental parameters in the coastal lagoons

of Mitla and Tres Palos, Guerrero, Mexico, (Decapoda, Palemonidae) *An. Inst. Cienc. Del. Mar. Y. Limnol. Univ. Nac. Auton. Mexico*, **9**(1) :67– 80.

*Atema, J. and Gagosian, R.B. (1973). Behavioural responses of lobsters to ecdysones. *Mar. Behav. Physiol.*, **2** : 15-20.

Beltz, B.S. (1988). Crustacean neurohormones. In: H. Laufer and D. Downer (eds.), *Endocrinology of selected invertebrate types.* Alan R. Liss, New York, pp. 235 – 258.

*Berber, A.A.G. (1984). Reproductive aspects of *Macrobrachium acanthurus*, (Weigmann, 1836) from the Gonzales river basin Tubacco, Mexico (Crustacea, Decapoda, Palaemonidae). *An Inst. Cienc. Del. Mar. Y. Limnol. Univ. Nac. Auton. Mexico*, **11**(1) : 1 – 22.

Berreur – Bonnenfant, J. and Lawrence, F. (1984). Comparative effect of farnesylacetone on macromolecular synthesis in gonads of crustaceans. *Gen. Comp. Endocrinol.*, **54** : 462 – 468.

Bijulal, P.S. (1994). Effect of eyestalk ablation on growth and reproduction of *Macrobrachium equidens* (Dana). M.F.Sc. thesis, submitted to Kerala Agricultural University, Trichur, p. 121.

Bomirski, A. and Kelk, E. (1974). Action of eyestalk in the ovary of *Rhithopanopeus harrissi* and *Crangon crangon* (Crustacea : Decapoda). *Mar. Biol.*, **24** : 329 – 337.

Brown, F.A. Jr. and Jones, G.W. (1949). Ovarian inhibition by sinus – gland principle in fiddler crab. *Biol. Bull.*, **91** : 228 – 232.

Burns, B.G., Sangalang, G.B., Freeman, H.C. and Mc Maneniy, M. (1984). Isolation and identification of testosterone from the serum and testes of the

American lobster (*Homarus americanus*). *Gen. Comp. Endocrinol.*, **54** : 429 – 432.

*Byard, E.H., Shivers, R.K. and Aiken, D.E. (1975). The mandibular organ of the lobster, *Homarus americanus*. *Cell Tiss. Res.*, **162** : 13 – 22.

*Cabrera-Jimenez, J.A., Chavez, C. and Martinez, C. (1979). Fecundity and cultivation of *Macrobrachium tenellum* (Smith) in the laboratory. *An .Inst. Biol. Univ. Nac. Auton. Mex. Zool.*, **50**(1) : 127 – 152.

*Carlisle, D.B. (1953). Studies on *Lysmata seticaudata* Risso (Crustacea, Decapoda). V . The ovarian inhibiting hormone and the hormone inhibition of sex reversal. *Pubbl. Staz. Zool. Napoli*, **24** : 455 – 472.

*Carlisle, D.B. (1954). The X-organ-sinus gland complex, somatotropin, the ovarian inhibiting hormone and sex reversal in *Lysmata seticaudata* Natantia Hippolysidae) . *Pubbl. Staz. Zool. Napoli*, **24** : suppl. p. 79.

*Chang, E.S. (1984). Ecdysteroids in crustacea : Role in reproduction, moulting and larval development. In : W. Angles, W.H. Clarke, A. Fisher, P.J.W. Olive and D.F. Went (eds.), *Advances in Invertibrate Reprodcution* 3. Elsevier Science Publishers, Amsterdam, pp : 223 – 230.

*Chang, E.S. (1992). Endocrinology. In : W.A. Fast and L.J. Lester (eds.), *Marine shrimp culture: Principles and practices.* Elsevier Science Publishers, Amsterdam, pp. 53- 92.

Chang, E.S., Hertz, W.A. and Prestwich, G.D. (1992). Reprouctive Endocrinology of *Sycionia injentis* : Steroid, peptide and terpenoid hormones. *NOAA Tech. Rep.*, NMFS, **106** : 1-6.

Chang, E.S., Prestwich, G.D. and Bruce, M. (1990). Aminoacid sequence of a peptide with both molt and hyperglycemic activity in the lobster, *Homarus americanus*. *Biochem. Biophys. Res. Comm.*, **171** : 818 – 826.

Charles, P.M. and Subramoniam, T. (1982). A comparative study on the oogenesis of two freshwater prawns *Macrobrachium malcolmsonii* and *M. lamarrei*. *J. Reprod. Biol. Comp. Endocrinol.*, **2** (2) : 80-86.

*Chaudenneret, P.J. (1956). Le systeme nervaux dela region gnathale de l'ecrensse *Cambarus affinis* (say). *Ann. Sci. Nat. Zool. Biol. Anim.*, **18** : 33 – 61.

*Christofferson, J.P. (1970). An electrophysiological and chemical investigation of the female sex pheromone of the crab (*Portunus sanguinolentus*). Ph. D. thesis, University of Hawaii, p. 95.

Clark, F. N. (1934). Maturity of California sardine (*Sardoina caerelea*) determined by ova diameter measurements. *Calif. Fish. Bull.*, **41** : 1 - 49.

*Couch, E.F. and Hagino, N. (1983). Correlation of progesterone and estradiol production by the mandibular organ and other tissues with egg development in the American lobster. *J. Cell. Biol.*, **97** : 158 A (Abstr).

Couch, E.F., Hagino, N.and Lee, T.W. (1987). Changes in Estradiol and Progesterone Immunoreactivity in Tissues of the Lobster, *Homarus americanus* with developing and Immature ovaries. *Comp. Biochem. Physiol.*,**87A**: 765-770.

Damronghol, P., Eangchuan, N. and Poolsanguan, B. (1991). Spawning cycle and oocyte maturation in laboratory- maintained giant freshwater prawns (*Macrobrachium rosenbergii*). *Aquaculture*, **95** : 347-357.

*D'Croz, L., Wong, V.L., Justin, G. and Gupta, M. (1988). Prostaglandins and related compounds from the polychaete worm *Americanuphis ressei* Fauchald (Onuphidae) as possible inducers of gonad maturation in penaeid shrimps. *Rev. Biol. Trop.*, **36** : 331 – 332.

*De'meusy, N. and Veillet, A. (1952). De'clenchment de la punte chez le crabe, *Carcinus maenas* pennant par l'ablation dee pe'doncules ocularies. *C.R. Hebd. Se'anc. Acad. Sci. Paris*, **234** : 1224 – 1226.

Dobkin, S.(1969). Abbreviated larval development in caridean shrimps and its significance in the artificial cullture of these animals, *FAO Fish Rep.*, **57**(3) : 935 – 946.

*Donahue, J. K. (1957). Chromatographic identification of lobster estrogens. *State of Maine Dept. of Sea and Shore Fish Res. Bull.*, No. 28.

*Downer, R.G.H, and Laufer, H. (1983). *Invertebrate Endocrinology*: 1. *Endocrinology Insects* Alan R. Liss, New York, p. 707.

Dunham, P.J. (1978). Sex pheromones in crustacea. *Biol. Rev.*, **53** : 555 – 583.

*Eales, A.J. (1974). Sex pheromone in the shore crab *Carcinus maenas* and the site of its release from females. *Mar. Behav. Physiol.*, **2** : 345-355.

Eastman – Reks, S. and Figerman, M. (1984). Effects of Neuroendocrine Tissue and Cyclic AMP on Ovarian Growth *in vivo* and *in vitro* in the Fiddler Crab, *Uca pugilator. Comp. Biochem. Physiol.*, **79A** : 679 – 684.

Fairs, N.J., Evershed, R.P., Quinlan, P.T. and Goad, L.J. (1989). Detection of unconjugated and conjugated steroids in the ovary, eggs and hemolymph of the Decapod Crustacean, *Nephrops norvegicus. Gen. Comp. Endocrinol.*, **74** : 199 – 208.

Fairs, N.J., Quinlan, P.T. and Goad, L.J. (1990). Changes in ovarian conjugated and unconjugated steroid titers during vitelogenesis in *Penaeus monodon*. *Aquaculture*, **89** : 83 – 99.

*Ferzou, J.P., Barbier, M. and Berreur – Bonnenfant, J. (1978). Biosynthese de la farnesylacetone – (E,-E) par les glandes androgenes du crabe *Carcinus maenas*. *Helv. Chim. Acta.*, **61** : 669 – 674.

*Fingerman, M. (1985). The physiology and pharmacology of crustacean chromatophores. *Amer. Zool.*, **25** : 233 – 252.

Fingerman, M. (1987). The Endocrine Mechanisms of Crustacea. *J. Crust. Biol.*, **7** : 1-24.

*Gomez, R. (1965). Acceleration of gonads by implantation of brain in the crab *Paratelphusa hydrodromous*. *Naturwissenschaften*, **9** : 216.

*Goorah, D. and Parameswaran, S. (1983). Maturation and fecundity of the giant camaron, *Macrobrachium rosenbergii* (de Man) in ponds in Mauritius. *Rev. Agric. Sucr. Ile. Maurice*, **62** : 6 – 11.

*Hampshire, F. and Horn, D.H.S. (1966). Structure of crustecdysone, a crustacean molting hormone. *Chem. Comm.*, **2** : 37 – 38.

Hara, T.J. (1993). Role of olfaction in fish behaviour. In : T. J. Pitcher(ed.) *Behaviour of Teleost Fishes*. Chapman and Hall, London, pp. 171-199.

*Hazel, C.M. (1986). Steroidogenesis in the female crab *Carcinus maenas*. Ph.D. Thesis, University of Liverpool. p. 81 – 121.

*Hinsch, G.W. and Bennet, D.C. (1979). Vitellogenesis stimulated by thoracic glanglion implanted into destalked immature spidercrab, *Libinia emarginata*. *Tiss. Cell*, **11** : 345 – 351.

*Holthuis, L.B. (1950). The Palaemonidae collected in the Siboga and Snellies expeditions with remarks on the other species. The Decapoda of the Siboga Expedition. *Siboga Exped. Mon.*, **39A** (9) : 1-100.

*Holthuis, L.B. (1980). FAO species catalogue, Shrimps and prawns of the world. An annotated catalogue of species of interest to fisheries, *FAO Fish. Synop.*, **1** (125). p 261.

*Homola, E. Landau, M. and Laufer, H. (1989). An *in vitro* assay for the regulation of methyl farnysoate synthesis in desegregated organ cells from *Libinia emarginata* and the effect of serotin. *Biol. Bull.*, **176** : 69.

Ibrahim, K.H. (1962). Observation of the fishery and biology of the freshwater prawn *M. malcolmsonii* H. Milne Edwards of river Godavari. *Indian J. Fish.*, **9A**(2) : 433 – 467.

*Inyang, N.M. (1984). On the biology of *Macrobrachium fellicinum* Holthuis in lower Niger river of south – Eastern Nigeria (Crustacea, palaemonidae). *Rev. Zool. Afr.*, **98**(2) : 440 – 449.

Jayachandran, K.V. (1984). Studies on the biology of the palaemonid prawns of the south west coast of India. Ph.D. thesis, The university of Kerala, Trivandrum, p. 520.

Jayachandran, K.V. (2001). *Palaemonid prawns – Biodiversity, Taxonomy, Biology and Management.* Science Publishers, Inc., Plymouth, UK, p.640

Jayachandran, K.V. and Jose, T.M. (1993). Effects of unilateral eyestalk ablation and male deprivation on ovarian maturation and ovulation in *Macrobrachium idella idella* (Hilgendorf, 1898) during the post breeding season. *Indian J. Comp. Anim. Physiol.*, **11** : 35 – 38.

Jayachandran, K.V. and Joseph, N.I. (1986). Male reproductive system of the slender river prawn, *Macrobrachium idella* (Hilgendorf, 1898). *Ann.Zool. (Agra)*, **24**(2): 77 – 87.

Jayachandran, K. V. and Joseph, N. I. (1988). Studies on the ovaries of slender river prawn, *Macrobrachium idella* (Hilgendorf, 1898) (Decapoda, Palaemonidae). *Zoologica Orient.*, **5**(1&2) : 6-9.

Jayachandran, K. V. and Joseph, N. I. (1989). Resources, enbiology, taxonomy and distribution and proximate composition of the palaemonid prawns of S. W. coast of India. In: N.Balakrishnan Nair(ed.), *Proceedings of the First Kerala Science Congress,February 26-28,1989*, Cochin. State Committee on Science,Technology and Environment, Government of Kerala,Trivandrum, pp. 104-108.

Jayachandran, K.V. and Joseph, N.I. (1992). A key for the field identification of commercially important *Macrobrachium* spp. with a review on their bionomics. In : E.G Silas (ed.), *Fresh water Prawns* , Proceedings of the National Symposium on Freshwater Prawns (*Macrobrachium spp.*),1990. Kerala Agricultural University, Trichur, pp. 72-79.

Jeng, S.S., Wan, M.W.C.M. and Chang, C.F. (1987). Existence of an Estrogen like compound in the ovary of the shrimp *Parapenaeus fissurus*. *Gen. Comp. Endocrinol.*, **36** : 211 – 214.

Joshi, V.P. and Diwan, A.D. (1992). Artificial insemination studies in *Macrobrachium idella* (Hilgendorf 1898). In E.G Silas. (ed.), *Fresh water Prawns*, Proceedings of the National Symposium on Fresh water Prawns,1990. Kerala Agricultural University, Trichur, pp. 110 – 118.

*Juchault, P. and Legrand, J.J. (1967). Contribution a 'le' etude du controle neurohumarate de la glande androge'ne chez les onoschides. *C.R. Hebd. Se'anc. Acad. Sci. Paris*, **264**: 1644 – 1646.

Kallen, J. and Meusy, J.J.(1989). Do the hormones VIH and CHH in crusteaceans have a common precursor? Immunolocalization of VIH and CHH in the x-organ sinus gland complex of the lobster *Homarus americanus*. *J.Inv.Reprod.Dev.*, **16**:43-52.

*Kamaguchi, Y. (1972). Mating behaviour in the freshwater prawn, *Palaemon paucidens*. A study of the sex pheromone and its effects on males. *J. Fac. Sci. Hokaido Univ. (Ser. Zoology)* **18**:355-374 .

*Kanazawa, A. and Teshima, S. (1971). *In vitro* conversion of cholestrol to steroid hormones in the spiny lobster, *Panulirus japonicus*. *Bull. Jap. Soc. Sci. Fish.*, **37** : 891 – 898.

*Karlson, P. (1956). Biochemical studies on insect hormones.*Vit.Horm.*,14:227-266

Karlson, P. and Luscher, M. (1959). "Pheromones" : A new term for a class of biologically active substances. *Nature* , **183** : 55-56

Kelk – Kawinska, E. and Bomirski, A. (1975). Ovary inhibiting hormone in the shrimp (*Crangon crangon*) eyestalks during the reproductive cycle. *Gen. Comp. Endocrinol.*, **25** : 9 –13.

*Kittredge, J.S. and Takahashi, F.T. (1972). The evolution of sex pheromone communication in the Arthopoda. *J.Theor.Biol.*,**35**:467-471.

*Kittredge, J.S., Terry, M. and Takahashi, F.T. (1971). Sex pheromone activity of the moulting hormone, crustecdysone, on male crabs (*Pachygrapsus crassipes, Cancer antennarius, Cancer anthonyi). Fish.Bull (U.S.)*,**69**: 337-343.

*Klassen, F. (1975). Okologische and Ethologische under suchungen zur for tpflanzubiologie von *Gecarcinus lateralis* (Decapoda, Brachyura). *Formafumctiio*, **8** : 101 – 174.

Knowles, F.G.W. and Carlisle, D.B. (1956). Endocrine control in crustacea. *Biol. Rev.*, **31** : 396-473.

Koshy, M. (1969). On the sexual dimorphism in the freshwater prawn *Macrobrachium lamarrei* (H. Milne Edwards, 1837). (Decapoda, Caridea). *Crustaceana*, **6** (2) : 185 – 193.

Krishnakumar, R. (1985). Studies on spermatheca of some decapod crustaceans. Ph. D. Thesis, Calicut University, Calicut,p.321..

Kulkarni, G.K., Glade, L. and Fingerman, M. (1991). Oogenesis and effects of neuroendocrine tissues on *in vitro* synthesis of protein by the ovary of the red swamp crayfish *Procambarus clarkii* (Girard). *J. Crust. Biol.*, **11** : 513-522.

Kulkarni, G.K., Nagabhushanam, R. and Joshi, P.K. (1979). Effect of progesterone on ovarian maturation in a marine penaeid prawn *Parapenaeopsis hardwickii*. *Indian J. Exp. Biol.*, **17**: 986-987.

Kurup, K.N.P. and Adiyodi, R.G. (1980). Pattern of regeneration during the breeding in the crab, *Paratelphusa hydrodromous* (Herbst). In : T. Subramoniam and S. Varadarajan (eds.), *Progress in Invertedrate Reproduction and Aquaculture*, New Century Printers, Madras, pp. : 25 – 26.

*Lachaise, F. and Hoffman, J.A. (1977). Endysone et development chez un De'capode, *Carcinus maenas*. *C.R. Acad. Sci. Paris*, **285** : 701 – 704.

Lambert, J.G.D. and Resink, J.W. (1991). Steroid glucuronides as male pheromones in the reproduction of the African catfish *Clarius gariepinus* - a brief review. *J. Steroid Biochem. Mol. Biol.*, **40**(4-5) :549-556.

Landau, M. Laufer, H. and Homola, E. (1989). Control of methylfarnesoate synthesis in mandibular organ of the crayfish *Procambarus clarkii* : evidence for peptide neurohormone with dual function. *J. Inv. Reprod. Dev.*, **16** : 165 – 168.

Laufer, H. and Landau, M. (1991). Endocrine control of reproduction in shrimp and other crustaceae. In : P.F. DeLoach, W.J., Dougherty and M.A. Davidson (eds.). *Frontiers of Shrimp Research.* Elsevier Science Publishers, Amsterdam, pp. 65 – 82.

*Laufer, H., Borst, D.W. and Homola, E. (1986). The synthesis and regulation of methyl farnesoate, a new juvenile hormone for crustacean reproduction. In : M. Porchet, J.C. Andrien and A. Dhainaut (eds.), *Advances in Invertebrate Reproduction*, Elsevier Science Publishers, Amsterdam, pp. 135 – 143.

*Laufer, H., Homola, E. and Landau, M. (1987a). Control of methyl farnesoate synthesis in crustacean mandibular organs. *Amer. Zool.*, **27** : 69A (Abstr.).

Laufer H., Homola, E. and Landau, M. (1992). Hormonal regulation of reproduction in female crustacea. *NOAA Tech. Rep.* NMFS, **106** : 89 – 97.

Laufer, H., Borst, D., Baker, F.C., Carrasco, C., Sinkins, M., Renter, C.C., Tsai, L.W. and Schooley, P.A. (1987b). Identification of a juvenile hormone like compound in a crustacea. *Science*, **235** : 202 – 205.

*Le Roux, A. (1968). Description d'organes mandibularies on veaux chez les crustace's Decapodes. *C.R. Hebd. Acad. Sci. Paris*, **266 D** : 1414 – 1417.

*Lee, C.I. and Fielder, D.R. (1982). Maintanence and reproductive behavious in the fresh water prawn *Macrobrachium australience* (Crustacea : Decapoda, Palaemonidae). *Aust. J. Mar. Fresh Water Res.*, **33** : 629 – 646.

*Ling, S.W.(1962). studies on the rearing of larvae and juveniles and culturing of adults of *Macrobranchium rosenbergii* (De Man). *Indo-Pac. Fish. Coun. Curr. Affairs Bull.*, **35**: 1-11.

Ling, S.W.(1964). A general account on the biology of the gaint freshwater prawn, *Macrobrachium rosennbergii* and methods for its rearing and culturing. *Proc. Indo–Pac. Fish. Coun.*, **40** : 2 –21.

*Lisk, R.D. (1961). Estradiol – 17b in the eggs of American lobster, *Homarus americanus*. *Can. J. Biochem. Physiol.*, **39** : 659 – 662.

*Lockwood, A.P.M. (1968). Aspects of the physiology of crustacea. Olivier and Boyd, London. p. 328.

Mary John, C. (1957). Bionomics and life history of *Macrobrachium rosenbergii* (De Man). *Bull. Central Res. Inst., Univ. Travancore*, **5** (C, 1) : 93 – 102.

*Mattson, M.P. and Spaziani, E. (1985). 5 –tryptamine mediate release of the moult inhibiting hormone activity from isolated crab eyestalk ganglia. *Biol. Bull.*, **169** : 246 – 255.

Menon, M.K. (1938). The early larval stages of two species of Palaemon. *Proc. Indian Acad. Sci.*, **8B** : 288 – 294.

Middleditch, B.S., Missler, S.R., Ward, D.G., Mc Vey, J.P., Brown, A. and Lawrence, A.L. (1979). Maturation of penaeid shrimp : Dietary fattyacids. *Proc. Wld. Maricult. Soc.*, **10** : 472 – 476.

Nagabhushanam, R., Joshi, P.K. and Kulkarni, G.K. (1980). Induced spawning in prawn *Parapenaeopsis stylifera* (H. Milne Edwards) using a steroid hormone 17α-hydroxyprogesterone. *Indian J. Mar. Sci.*, **9** : 227.

Nagabhushanam, R., Machale, P.R. and Sarojini, R. (1988). Presence of ovarian stimulating factors in brain and thoracic ganglion of fresh water prawn, *Macrobrachium kistnensis*. *J. Zool. Res.*, **1** : 17 – 21.

Nagabhushanam,R., Machale, P.R. and Sarojini, R.(1989). Pheromonal and hormonal control of reproduction in the freshwater prawn, *Macrobrachium kistnensis*. *Poc. Indian Acad. Sci.(Anim. Sci.)*, **98** :95-98

Narayanan, S. and Adiyodi, K.G. (1992). The inter–relationship between moulting and ovarian growth during the breeding season in the freshwater shrimp *Macrobrachium idella*. In: E.G. Silas (ed.), *Fresh water prawns* (*Macrobrachium spp.*) Proceedings of the National Symposium on Fresh water Prawns, 1990. Kerala Agricultural University, Trichur, pp. 137 – 141.

Ollivier, F., De Clarcke, D., Diederik, K.H. and De Loof, A. (1986). Identification of non-ecdysteroid in the haemolymph of both male and female *Astacus leptodactylus* (Crustacea) by gas chromatographic – mass spectrometry. *Gen. Comp. Endocrinol.*, **61** : 214 – 228.

*Otsu, T. (1960). Precocious development of the ovaries in the crab, *Potamon dehaani*, following implantation of thoracic ganglion. *Annot. Zool. Japan*, **33** : 90 – 96.

*Otsu, T. (1963). Bihormonal control of sexual cycle in the freshwater crab, *Potamon dehaani*. *Embryologica*, **8** : 1- 20.

*Panouse, J.B., (1943). Influence de' l' ablation de pe'doncule oculaire sur la croissance de l' ovarie chez la crevette *Leander serratus*. *C.R. Acad. Sci. Paris*, **217** : 553 – 555.

*Panouse, J.B. (1944). L'action de la glande du sinus sur l ovarie chez la crevette *Leander*. *C.R. Acad. Sci. Paris*, **218** : 293 – 294.

*Panouse, J.B. (1946). Recherches sur les pheromones humoraux chez les crustace's l'adaptation chromatographique et la croissance – ovarienne chez la crevette *Leander serratus*. *Ann. Inst. Oceanogr. Monaco*, pp. 23 – 65.

Paulus, J.E. and Laufer, H. (1987). Vitellogenesis in the hepatopancreas of *Carcinus maenas* and *Libinia emarginata* (Decapoda, Brachyura). *J. Inv. Reprod. Dev.*, **11** : 29 – 44.

*Payen, G.G., Costlow, J.D. and Charniaux – Cotton, H. (1967). Recherches sur le de la neuroendocrine secretion dans la differentiation sexulle du crabe, *Callinectes sapidus* Rathbun. *C. R. Acad. Sci. Paris.*, **264** : 2148 – 2151.

Payen, G.G., Costlow, J.D. and Charniaux–Cotton, H. (1971). Etude comparitive del' ultrastructure des' glandes androge'ne's de crabe, nomaux et pe'doculectimised pendant lavia lawire on apre's la puberte chez les espece's : *Rhitropanopeus harrissi* (Glould) et *Callinectes sapidus* Rathbun. *Gen. Comp. Endocrinol*, **17** : 526-542.

Philip, T.J. and Subramoniam, T. (1992). Some aspects of reproductive biology of the freshwater prawn *Macrobrachium malcolmsonii*. In: E.G. Silas (ed.), *Freshwater Prawns*. Proceedings of the National Symposium on Freshwater Prawns (*Macrobrachium spp.*), 1990. Kerala Agricultural University, Trichur, pp. 103-105.

Pillai, N.N. and Mohamed, K.H. (1973). Larval history of *Macrobrachium idella* (Hilgendorf) reared in the laboratory. *J. Mar. Biol. Assoc. India.*, **15**(1) : 353 – 385.

Pillay, N. C. (1990). Observation on the breeding, larval development and taxonomic status of *Macrobrachium equidens* (Dana). *Indian J. Fish.*, **37**(2) :151-153.

Prabhu, M. S. (1956). Maturation of Intraovarian eggs and spawning periodicities in some fishes. *Indian J. Fish.*, **3** : 1-42.

Quackenbush, L.S. (1986). Crustacean endocrinology, A review. *Can. J. Fish Aquat. Sci.*, **43:** : 2271 – 2282.

Quackenbush, L.S. (1989). Yolk protein production in the marine shrimp *Penaeus vannamei. J. Crust. Biol.*, **9** : 509-516.

Quackenbush, L.S. (1991). Regulation of vitellogeneis in penaeid shrimp. In : P.F. DeLouch, W.J. Dougherty and M.A. Davidson (eds.), *Frontiers of shrimp research.* Elsevier science publishers, Amsterdam, pp. 71 – 81.

Quackenbush, L.S. and Herrnkind, W.F. (1981). Regulation of molt and gonadal development in the spiny lobster, *Panulirus argus* : effect of eyestalk ablation. *Comp. Biochem. Physiol.*, **69A** : 523-527.

Quackenbush, L.S. and Herrnkind, W.F. (1983). Partial characterization of eyestalk hormones controlling molt and gonad development in the spiny lobster, *Panulirus argus. J. Crust. Biol.*, **13** : 34 – 44.

*Quackenbush, L.S. and Keeley, L. L. (1987). Vitellogenesis in the shrimp *Penaeus vannamei. Amer. Zool.*, **27** : 810 A (Abstr.).

Quinitio, E.T., Yamouchi, K., Hara, A. and Fuji, A. (1991). Profiles of progesterone and estradiol like substances in the hemolymph of female *Pandalus kessleri* during an annual reproductive cycle. *Gen. Comp. Endocrinol.*, **81** : 343 – 348.

*Raabe, M. (1982). *Insect Neurohormones*. Plenum, New York p. 352.

Rajyalakshmi, T. (1961). Studies on the maturation and breeding in some estuarine palaemonid prawns. *Proc. nat. Inst. Sci. India.*, **27B**(4) : 179 – 188.

Rajyalakshmi, T. (1974). The freshwater prawn, *Macrobrachium malcolmsoni* for use in pond culture. *Seafood Exp. J.*, **6**(3) :25-31.

Rajyalakshmi, T. (1980). Comparitive study of the biology of the freshwater prawn *Macrobrachium malcolmsonii* of Godavari and Hooghly river systems. *Proc. Ind. nat. Sci. Acad.*, **46B** (1) : 72 – 89.

Raman, K. (1964). On the location of a nursery ground of the giant prawn *Macrobrachium rosenbergii* (de Man). *Curr. Sci.*, **33**(1) : 27 – 28.

Raman, K. (1967). Observations on the fishery and biology of the giant freshwater prawn *Macrobrachium rosenbergii* (deMan). In: *Symposium on Crustacea, Part 3.* Proceedings of the Symposium on Crustacea, 1965. Marine Biological Association of India, Mandapam Camp, pp. 649 – 669.

Rangneker, P.V. and Deshmukh, R.D. (1968). Effect of eyestalk ablation on the ovarian growth of the marine crab *Scylla serrata* (Forskal). *J. Anim. Morphol. Physiol.*, **15** : 116 – 126.

Rao, R.M. (1967). Studies on the biology of *Macrobrachium rosenbergii* (de Man) of the Hooghly estuary with notes on its fishery. *Proc. nat. Inst. Sci. India*, **33B** (5 &6) :252-279.

Richardson, H.G., Deecaraman, M. and Fingerman, M. (1991). The effect of Biogenic amines on ovarian development in the fiddler crab, *Uca pugilator*. *Comp. Biochem. Physiol.*, **99 C** : 53 – 56.

*Romero, M.E.D. (1982). Preliminary observations on potential of culture of *Macrobrachium amazonicum* in Venezuela. In : M.B. New (ed.), *Giant Prawn Farming*, Elsevier, Amsterdam, pp. 411 – 416.

Sankolli, K.N. and Shenoy, S. (1980). *Macrobrachium malcolmsonii*, A prospective competitor for the Jumbo prawn, *Macrobrachium rosenbergii* In : *Proceedings of the First National Symposium on Shrimp Farming, August 24 – 25, 1978.* Marine Products Export Development Authority, Cochin, pp. 151 – 153.

Sarojini, R., Mirajkar, M. S. and Nagabhushanam, R. (1982). The site of sex pheromone production in the freshwater prawn, *Macrobrachium kistnensis*. *Curr. Sci.*, **51**(20) : 975-978.

*Schniederman, A. and Gilbert, L.I. (1958). Substances with juvenile hormone activity in crustacea and other invertebrates. *Biol. Bull.*, **115** : 530-535.

Sebastian, A. (1993). Comparative study on certain aspects of the biology of *Macrobrachium equidens equidens* (Dana, 1852) and *Macrobrachium equidens pillaii* Jayachandran, 1989. M.F.Sc. Thesis, Kerala Agricultural University, Trichur p. 182.

Sebastian, M.J. (1970). The clams and mussels of Kerala - prospects of a better and fuller utilization by means of canning. *Seafood Exp.J.* **2**(4) : 25-30.

Sherief, P.M. (1989). Clam meat as animal protein source in the diet of *M. roserbergii*. In : K. K. Balachandran, P. A., Perigreen, P. Madhavan and P.K. Surendran (eds.) *Recent Trends in Processing Low cost Fish.* Society of Fisheries Technologists (India), Cochin., pp. 213 – 217.

Sherwood, N.M., Kyle, A.L., Kreiberg, H., Warby, C.M., Magnus, T.H., Carolsfeld, J. and Price, W.S. (1991). Partial characterisation of a spawning pheromone in the herring *Clupea harengus pallasi. J. Can. Zool.*, **69**(1) : 91-103.

Singh, S.and Roy, D. (1994). Reproductive cycle of the freshwater prawn *Macrobrachium choprai* (Tiwari). *Asian Fish. Sci.*, **7** : 77-89.

Skinner, D.M. (1985). Molting and regeneration. In : D.E. Bliss and L.H. Mantel (eds.), *The Biology of Crustacea* 9., *Integument, Pigments and Hormonal Processes.* Academic. Press., New York, pp. 43 – 145.

*Soyez, D., Le caer, J.P., Noel, P.Y. and Rossier, J. (1991). Primary structure of two isoforms of vitellogenesis inhibiting hormone from the lobster, *Homarus americanus. Neuropeptides*, **20** : 25 – 32.

*Stephens, G.J. (1952). Mechansisms regulating reproductive cycle in the crayfish, *Cambanus*, I. The female cycle. *Physiol. Zool.*, **25** : 70 – 84.

Subramoniam, T. and Keller, R. (1993). A new look at the endocrine regulation of egg maturation in the decapod crustaceans. *Curr. Sci.*, **65** : 619 – 622.

Subramoniam, T. Sedlmeier, D.and Keller, R. (1999). Recent advances in the endrocrine regulation of reproduction and moulting in crustacea. In: K.P.Joy, A.Krishna and C.Haldar (eds.). *Comparative Endocrinology and Reproduction.* Narosa Publishing House, New Delhi, p. 462-475.

Subramonyam, M. (1975). Notes on some aspects of the biology of *Palaemon styliferus* Milne Edwards from the Godavari estuarine system. *J. Bombay Nat. Hist. Soc.*, **72**(3) : 683 – 691.

Sundara Rajulu, G., Santhanakrishnan, G. and Shyamalanath, S. (1973). Nature of the sex - attractant pheromone in a crab *Paratelphusa hydrodromus* (Crustacea). *Curr. Sci.*, **42** :467-468.

Takayanagi, H., Yamamoto, Y. and Takeda, N. C. (1986 a). An ovary stimulating factor in the freshwater shrimp, *Paratya compressa. J. Exp. Zool.*, **240**: 203-209.

Takayanagi, H., Yamamoto, Y. and Takeda, N.C. (1986 b). Ovary stimulating pheromone in the fresh water shrimp, *Paratya compressa. J.Exp. Zool.*, **240** : 397 – 400.

Tensen, C.P., Jannsen, K.P.C. and Van Herp, F. (1989). Isolation characterization and physiological significance of crustacean hyperglycemic factors from the sinus gland of the lobster, *Homarus americanus* (Milne Edwards). *J. Inv. Reprod. Dev.*, **16** : 155 – 164.

*Teshima, S. and Kanazawa, A. (1971). Production of ketotestosterone and other steroids by the sliced ovaries of *Portunus trituberculatus. Bull. Jap. Soc. Sci. Fish.*, **37** : 246 – 249.

Thampy, D.M. (1972). Studies on the endocrine physiology of sex and reproduction in a shrimp *Caridina natarajani* Tiwari and Pillay. Ph D. Thesis, University of Kerala, Trivandrum, p. 218.

*Tsukimura, B., Tanji, T.M. and Kamemoto, F.I. (1986). Sinus gland activation of cyclic GMP in the mandibular organ of *Homarus americanus. Amer. Zool.*, **26** : 91 A (Abstr.).

*Tsukimura, B., Markin, M., Frinsko, M. and Borst, D. W. (1989). Measurement of methyl farnesoate (MF) in crustacean haemolymph. *Amer. Zool.*, **29**: 49A (Abstr.).

Webster, S.G. and Keller, R. (1986). Purification, characterization and amino acid composition of the putative molt inhibiting hormone (MIH) of *Carcinus maenas* (Crustacea, Decapoda). *J. Comp. Physiol.*, **156B** : 617 – 624.

Wickins, J.F. and Beard, T.W. (1974). Observations on the breeding and growth of the giant freshwater prawn *Macrobrachium rosenbergii* (de Man) in the laboratory. *Aquaculture*, **3** : 159 – 174.

Yano, I. (1985). Induced ovarian maturation and spawning in greasback shrimp, *Matapenaeus ensis*, by progesterone. *Aquaculture*, **47** : 223-229.

Yano, I. (1992 a). Hormonal control of reproductive maturation in penaeid shrimp. *NOAA Tech. Rep.* NMFS , **106** : 7 – 14.

Yano, I. (1992 b). Effect of thoracic ganglion on vitellgenin secretion in kuruma prawn, *Penaeus japonicus. Bull. nat. Res. Inst. Aquacult.*, **21** : 9-14.

Yano, I. (1993). Ultraintensive culture and maturation in captivity of penaeid shrimp. In : J.P. McVey (ed.), *CRC Handbook of mariculture : Crustacean aquaculture*, CRC Press, Boca Raton, Florida, 2nd Ed. pp. 281-313.

Yano, I. and Wyban, J.A. (1992). Induced Ovarian Maturation of *Penaeus vannamei* by injection of Lobster Brain Extract. *Bull. nat. Res. Inst. Aquacult.*, **21** : 1 – 7.

Yano, I., Tsukimura, B., Sweeney, J.A. and Wyban, J. A. (1988). Induced ovarian maturation of *Penaeus vannamei* by implantation of lobster ganglion. *J. Wld. Aquacult. Soc.*, **19** : 204 – 209.

Young, N.J.,Quinlan, P.T. and Goad, L.T.(1992). Progresterone metabolism *in vitro* in the Decapod crustacean, *Penaeus monodon. Gen. Comp. Endocrinol.*, **87** : 300-311.

Young, N.J., Webster, S.G. and Rees, H.H.(1993). Ecdysteroid profiles and vitellogenesis in *Penaeus monodon* (Crustacea :Decapoda), *J.Inv. Reprod.Dev.*, **24**:107-118.

* Not consulted in original.

ROLE OF A PUTATIVE MALE PHEROMONE IN THE OVARIAN MATURATION OF THE FRESH WATER PRAWN *MACROBRACHIUM IDELLA* (HILGENDORF).

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Abstract

8. ABSTRACT

Sex pheromones are reported to be present in many crustaceans which mostly have releaser effect on sexual behaviour. The discovery of a male (primer) pheromone with stimulatory effect on ovarian maturation and ovulation has added an important external factor to the basic mechanisms regulating reproduction in fresh water prawns. But studies in these lines are very few in the economically important species of *Macrobrachium*. Knowledge about this male pheromone will be useful to help or potentiate reproduction and/or extend the breeding period of freshwater prawns, which are of great aquacultural importance.

The present study was carried out in the slender river prawn, *Macrobrachium idella*. Seven ovarian maturity stages have been distinguished here. Histological studies of the maturing oocytes helped to understand the changes taking place during oocyte growth and maturation through the progressive maturity stages. Yolk vesicles start appearing in the cytoplasm of oocyte in maturity stage II which is the beginning of vitellogeneis (vitellogenesis I). In stage IV, yolk platelet synthesis starts and a peripheral layer of yolk platelets appear in this stage. Later these yolk platelets completely fill the ooplasm through stages V and VI when the ova increase in size enormously. Gonado-somatic index (ovarian index) of different maturity stages was determined and was found useful to differentiate especially the late maturity stages. Ova diameter studies along with the other observations showed a clear picture of protracted multiple spawning in this species with batches of ova undergoing maturation and ovulation in successive maturation cycles.

When the females were reared in isolated condition, their ovarian growth was found to be arrested at early stage IV. This restriction of ovarian maturation was found mostly in the second maturation (gonadal) cycle after isolation. But those isolated females which had a common water circulation with males showed normal ovarian maturation and ovulation. Therefore, it can be concluded that a male pheromone exists in this species which is essential for normal ovarian growth and maturation. Another experiment was conducted to find out the tissue source of this putative male pheromone. When these isolated females were exposed to extracts of testis, vas deferens or muscle, both testis and vas deferens extracts were found to stimulate the ovarian maturation and ovulation in isolated females, while the muscle extract (control) was ineffective. This shows that in *M. idella*, the male pheromone is produced by testis and vas deferens or it is produced by testis and transported to vas deferens.

Further experiment showed that charcoal extraction destroys the pheromonal activity in testis and vas deferens extract while boiling has no such effect which indicates that the ovary stimulating pehromone in *M. idella* may be a steroidal molecule as reported in some fishes.