"STIMULATION OF OVARIAN MATURATION IN THE INDIAN WHITE PRAWN, *PENAEUS INDICUS* (H.MILNE EDWARDS) BY METHYL FARNESOATE" (MF)

Dissertation submitted by

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in partial fulfilment for the Degree of

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CENTRAL MARINE FISHERIES RESEARCH INSTITUTE

P.B.No.1603, Cochin- 682 014.

INDIA.

DEDICATED TO MY PARENTS

C O N T E N T S

C E R T I F I C A T E

This is to certify that the dissertation entitled "Stimulation of ovarian maturation in the Indian White Prawn, *Penaeus indicus* (H.Milne Edwards) by Methyl Farnesoate" (MF) is a bonafide record of the research work done under my guidance and supervision under the Post Graduate Programme in Mariculture at Central Marine Fisheries Research Institute, Cochin and that no part thereof has been presented for the award of any other Degree, Diploma, Associateship, Fellowship or other similar titles or recognition.

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I hereby declare that this dissertation entitled "Stimulation of Ovarian maturation in the Indian White Prawn, *Penaeus indicus* (H. Milne Edwards) by Methyl Farnesoate" (MF) is based on my own research and has not previously formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar titles or recognition.

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सारांश

दशपाद कवच प्राणियों के किशोरों मे देखे जाने वाला होर्मोन मिश्रण है मीथायिल फारनेसोएट (एम.एफ) जो कीटों के किशोरों के होर्मोन (जे.एच) का अनएपोक्सिडेटड रूप है। भारतीय शवेत झींगा पेनिअस इंडिकस में 0.1 मि.लि. एथनोल के साथ 10-15µg की दर में दो बार एक एक का इन्जोक्शन लगाए जाने पर अंडाशाय परिपक्वन का उद्दीपन देख गया, 25 दिनों बाद अपरिपक्व मादा झींगों के अंडाशयों मे भी अनुकूल प्रतिक्रिया दिखाई पडी। परीक्षण किए गए वर्गा जिन्हें होर्मोन युक्त स्वाध और विना होर्मोन के स्वाध खिलाए जाने से अनुकूल प्रतिक्रिया नहीं दिखाई पडी। 25 दिनों कि अवधि के दुसरे परिक्षण में भी इसी प्रकार का परिणाम निकला। पहले और दुसरे परिक्षणों में होर्मोन इन्जेक्शन लगाए गए झींगों की परिपक्वता क्रमशः 33.3% और 50% था।

इस अध्ययन के परिणाम से यह स्थापित होता है कि पेनिआइड झींगों में एम.एफ परिपक्वता का उद्दीपन कर सकता हैं। इन परिणामों के नेत्रवृन्त अपक्षरण के वदले होर्मोन इनजेक्शन से प्रेरित परिपक्ववन की तकनोलजी विकसित की जा सकती हैं।

1. PREFACE

PREFACE

The Indian white prawn, *Penaeus Indicus* is the next preferred species to *Penaeus monodon* for shrimp farming in South East Asia . The availablity of quality spawners or gravid is a prerequisite for the rapid expansion of hatcheries to meet the quality seed demand. The total estimated area under shrimp cultivation is about 1,40,000 ha during 1998. According to an estimate the distribution of cultivable area under extensive (98,000 ha), modi-fied extensive (28,000 ha), and semi - intensive (14,000 ha) farming operations is 70%, 20% and 10% respectively. The estimated seed requirements will be reach is around 19 billion by 2,000 AD. Availability of seed from nature is seasonal, location specific and of a mixed quality, hence to ensure a steady supply of pure quality seed in large quantities, hatchery production under controlled conditions is the only alternative.

Shrimp hatchery operations rely on the availablity and abundance of spawners from the wild, which is seasonal and limited. In order to meet the increasing and growing demand for gravid females. It is high time and essential to develop techniques for induce them to mature under controlled conditions.

Possible techniques available so far are

A. Environmental Manipulation

B. Nutritional Manipulation

C. Hormonal Manipulation.

The basic principle of eyestalk ablation is based on approach of the neurohormones. These hormones are secreted from the X- organ - Sinus Gland Complex, brain and thoracic ganglia. Decapod peptide hormones effect on ovary, hepatopancreas and subdermic adipose tissue to synthesize the yolk protein (Vitellogenine), However, divergent views have been expressed regarding the reproductive control by recent upsurge in crustacean endocrinolgy. The endocrine manipulation of reproductive phenomena has new impetus all over the world. A new perspective of classical nonneural endocrine research in decapoda crustacean is the application of it to "Blue Revolution" in this respect, an interesting dichotomy is the functional and structural elucidation of terpenoids (Juvenoids) have been mostly done in spider crab, *Libinia emarginata* ; crayfish, *Procambarus clarkii* ; American lobster, *Homarus americanus*.

This certainly warrants an investigation of the terpenoid hormones of commercially important Penaeid species . Current study based on the terpenoid or juvenoids hormone (JH) will help for inducing them to maturity which may further lead to rapid expansion of hatchery technology.

2. INTRODUCTION

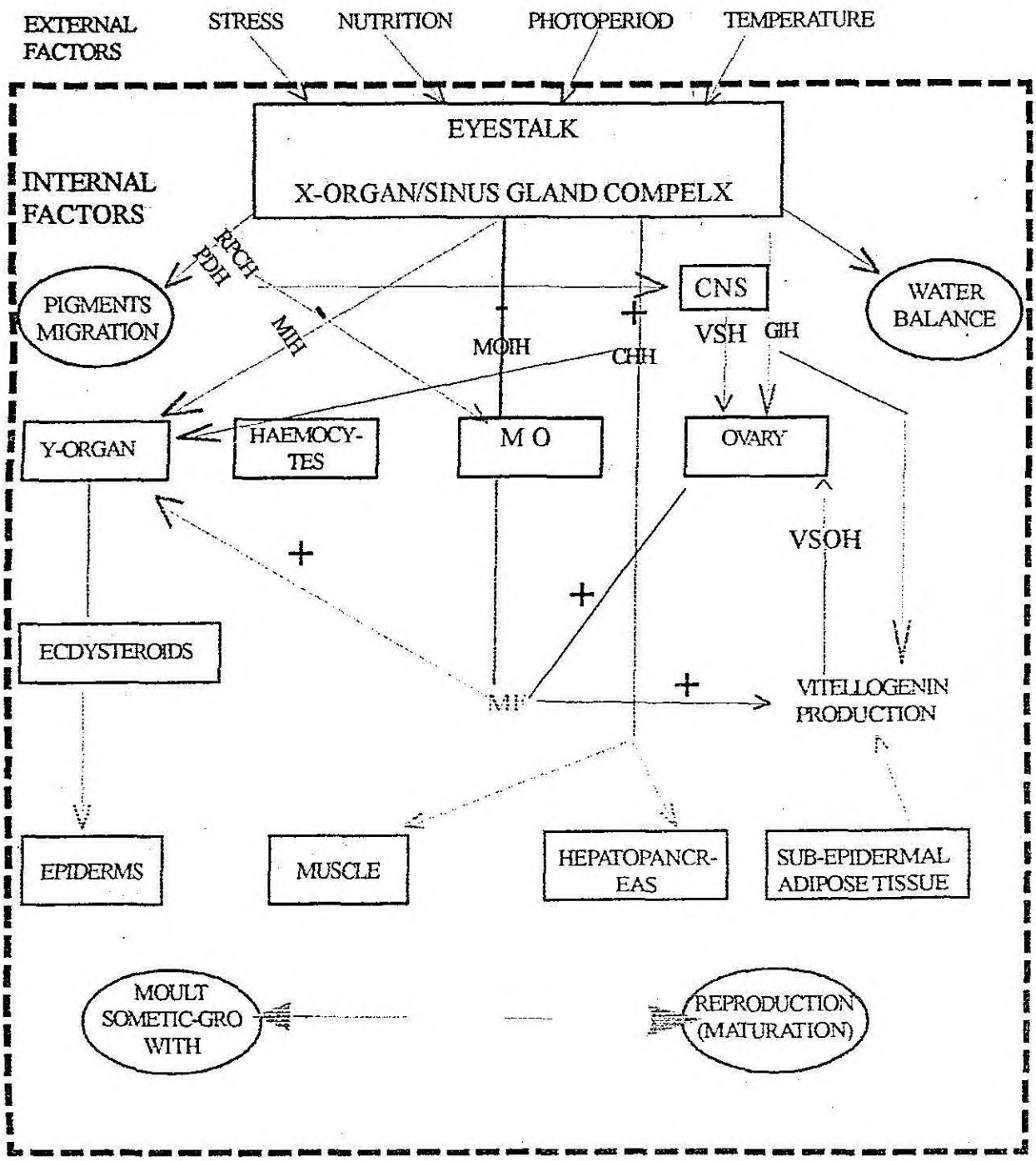
INTRODUCTION

Basic understanding of crustacean endocrinology has advanced rapidly in recent years since the pioneering classical endocrinological experiments involving glandular ablation and reimplantation approaches (Quackenbush, 1986 ; Fingerman,1987). Stimulation of ovarian maturation represents a remarkable influence on the synthesis of organic material . The process of ovarian maturation in penaeids like in other crustacean is a dynamic process mediated by several exogenous and endogenous factors . These factors effect on the development and formation of oocytes, their secondary oocyte maturation and subsequent release.

These exogenous factors includes stress , nutrition , photoperiod ,temperature etc. These factors act directly on neurosecretory sites and neurohaemal organs (X - organ/Sinus gland complex). Several neuropeptide hormones are synthesis and releasing into the haemolymph from these organs. These are implicated in almost every aspects of crustacean physiology including gonadal development, inhibition of moulting, pigment migration, limb regeneration , cardiac control, blood glucose, metabolism and respiratory control , ion and water balance, endogenous rhythmicity and locomotion.

The process of vitellogenesis in crustacean involves two phases , Primary and secondary vitellogenesis. The oogonial proliferation and primary proteic vitellus (vitellin - glycoprotein) is endogenous and continuous process. The secondary vitellogenesis takes place during the reproductive season, The prominent feature of this process is the uptake of vitellogenin (Vg) (Female specific protein) by oocytes.

Diagram to show complexity of controls in Endocrine system controlling growth and maturation in female crustacea.



CNS - CENTRAL NERVOUS SYSTEM
CHH - CRUSTACEAN HYPERGLYCAEMIC HORMONE
GIH - GONAD INHIBITING HORMONE
MOIH -MANDIBULAR ORGAN INHIHITING HORMONE
MIH - MOULT INHIBITING HORMONE
RPCH - RED PIGMENT CONCENTRATING HORMONE
PHD - PIGMENT DISPERSING HORMONE
MF - METHYL FARNESOATE
VSH - VITELLOGENESIS STIMULATING HORMONE

It is now well established that the origin of yolk is dual, intra ooplasmic (ovary) or extraooplasmic (Hepatopancreas or subdermic adipose tissues) . During the secondary vitellogenesis , incorporation of Vg (Lipoglycocaroteneprotein) into the oocytes through the process of endocytosis. This substance is immunologically not distinguishable from the major egg yolk protein (vitellin) (Meusy,1980).

One of the most challenging problem in obtaining mature females because most of the commercially important species are incapable of spontaneous maturation under artificial conditions. At present the most commonly employed method of stimulation of ovarian maturation in crustacean is the eyestalk ablation, eventhough it has been employed by aquaculturist to stimulate ovarian maturation in shrimp in all over the world, is a permanent alteration that ultimately interferes with the organisms capacity to regulate a no. of physiological process in addition to reproduction. It has also been noted that unilateral eyestalk ablation results in the production of more shrimp larvae, they are believed to be inferior in quality to those of that intact females (Choy,1987).

It is high time that an alternative technique have to evolve for the acceleration of ovarian maturation by simply injecting microquantities of purified hormones. Based on this approach the following technique has evolved in some commercially important decapods by different workers. The crab,*Thalamitta crenata*(Oyama, 1968); the sand shrimp, *Crangon crangon* (Bomirski and Klek,1976); *Parapenaeopsis hardwickii* (kulakarni et al.,1979); the spider crab,*Libinia emarginata* (Hinsch,1980); *Penaeus setiferus* (Yudin et al.,1980); *Penaeus vannamei* (Yano and Wyban,1987a); the spider crab, *Libinia emarginata* (Sagi et al.,1991,1994 ;Laufer et al.,1994);Giant freshwater prawn,*Macrobrachium rosenbergii* (Wilder

et al., 1994,1995) *Penaeus japonicus* (Wilder et al., 1995); Freshwater crab, *Oziotelphusa senex senex* (Sreenuvasula Reddy and Ramamurthi, 1998).

3. REVIEV OF LITERATURE

Review of literature

Basic knowledge of female reproduction is of primary importance to crustacean aquaculture.Female reproduction in decapod crustacean is carefully regulated by several different endocrine factor(s). Three endocrine factors, each representing different chemical class of hormones, have been investigated so far : Ecdysteroids (the steroid moulting hormone of arthropods, predominatly 20- hydroxy ecdysone), Neuropeptides (Vitellogenesis or Gonad Inhibiting Hormone, Gonad Stimulating Hormone, Moult Inhibiting Hormone and Biogenic Amines) and Terpenoids or Juvenoids (Methyl Farnesoate- Laufer *et. al.*, 1987; Farnesylacetone- Ferezou *et.al.*, 1978).

Female reproduction, especially the egg maturation in the ovary of decapoda crustaceans, is negatively controlled by an inhibitory neuropeptide from X-Organ-Sinus gland complex. Recent structural studies reveal that its primary structure is related to that of other eyestalk peptides such as Crustacean Hyperglycaemic Hormone (CHH) and Moult Inhibiting Hormone (MIH). These peptides seem to inhibit yolk synthesize and uptake into the oocytes. Information on a gonad stimulatory hormone purportedly originating from the neurosecretory cells of brain and thoracic ganglia, is still very preliminary. However neuropeptide do not appears to be the sole regulator of female reproduction.

This work reviews the hormonal regulation of reproduction, focussing on the Mandibular Organ (MO) and, the target tissues affected by their secretion.

The role of terpenoid hormones, collectively known as the Juvenile Hormones (JHs) or Juvenoids has been well established in insect reproduction . Insect Juvenile hormone (JHs) ,0,I,II,III but in crustaceans, only Methyl Farnesoate (MF) appears to be a major product. Juvenile hormones a family of epoxidated sesquiterpenoids synthesize and release from the Corpus Allatum (CA) act as growth regulators in insects (Wigglesworth, 1970). The first report of the presence of such a compound in crustacean was that of Laufer *et al.*. (1987) who discovered MF, the unepoxidated form of JH III, in the spider crab, *Libinia emarginata*. Since then, it has been identified in the haemolymph of both males and females and in over 25 species of crustacea, including crabs, lobsters, crayfish (Borst *et.al.*.,1987; Laufer *et al.*., 1987; Laufer and Borst, 1988; Landau *et al.*, 1989; Tobe *et al.*., 1989; Homola and Chang, 1997) and fresh water prawn, *Macrobrachium rosenbergii* (Sagi *et al.*., 1991).

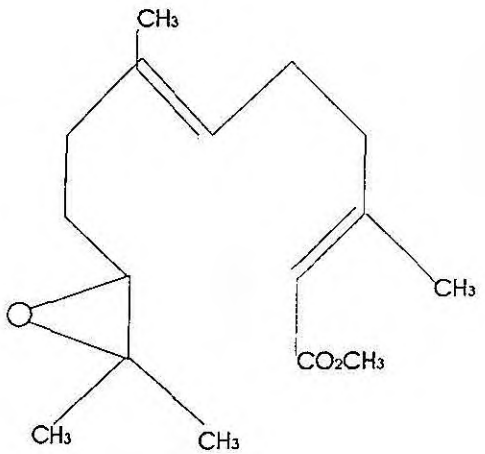
MF is a hydrophobic compound with solubility of about 10 µg in aque ous solutions (Hammock and Mumby, 1978; Tobe *et al.*., 1989) and a half lifeof about 30 min when injected into crustacean haemolymph (Takac *et al.*.,1997) In crustaceans, MF (Borst *et.al.*., 1987) and J III (Hertz and Chang, 1986) retarded larval development of *Homarus americanus* and juvenile hormone analogues, such as methoprene and hydroprene caused retardation of

larval development of the mud crab, *Rhithropanopeus harrisii* (Christiansen *et al*.., 1977 a,b) Methoprene also retarded growth in early larval stages and post larvae of the estuarine shrimp, *Palaemonates pugio*, although enhancement of growth was recorded in the premetamorphic stages (Mckenney and Celestial, 1993).

A single injection of juvenile hormone to larvae, *Homarus americanus* (Charmantier *et al*.., 1988), and is in accordance with administration of MF through dry food to adult crustaceans .

An enigmatic, possible endocrine gland is the paired Mandibular organs, first described by LeRoux (1968). These structures which lie close to the Y-organs have in fact been misidentified as Y-organs by some investigators. In contrast to the Y- Organs, Mandibular Organs (MO) been reported to consists of two cell types. Hinsch (1981a) stated that she observed two cell types only in these organs from female spider crab, *Libinia emarginata*; the organs from males having only one cell type. Recently, the mandibular organs in more than 25 species of crustacean including spider crab were found to produce (Borst *et al*.., 1985) in the circulatory system and haemolymph of this crab (Laufer *et al*.., 1985) and Ding and Tobe (1991) were found that production of Farnesoic Acid (FA) and MF by mandibular organs of the crayfish .Later, Tobe *et al* (1989) reported that FA is released by the MO of *Scylla serrata* and either rapidly metabolized or sequestered by the tissue . It is possible that FA may be a prohormone and converted into other isoprenoid products.

The chemical structure of MF is nearly identical to that of insect JH III, differing only by the absence of an epoxide group (Homola and Chang,1997))



METHYL FARNESOATE

Chang *et al.* (1989) synthesized a photoaffinity analog for the putative prawn reproductive hormone MF- a terpenoid , this analog, Farnesyl Diazomethyl Ketone (FDK), was used to demonstrate the presence of specific binding proteins to MF in shrimp haemolymph. Prestwich *et al.*. (1990) reported that the specific Methyl Farnesoate Binding Proteins (MFBP) in homogenates of tissue of the American lobster and this MFBP is thus functionally analogous to the haemolymph JH binding proteins of insects. Later, Li and Borst (1991) found that the binding component of MF was a protein, showed that binding activity had a molecular weight of about 650000 Da.

The fact that mandibular organs secrete MF, which is highly lipophilic and hydrophobic and may indeed in crustaceans be converted to JH (Laufer *et al* ., 1985; Borst *et al*.., 1985). Later, Albrecht and Laufer (1989) have reported that MF is not converted to JH III and raises the possibility

that FA is both a precursor and metabolite of MF. In such as JHs are gonadotrophic in many female insects, JH like compounds have been tested on female crustaceans, but did not promote vitellogenesis (Payen and Costlow,1977; Hinsch, 1981b). In *Rhithropanopeus harissii* these analogs produced the opposite effect, having a chemosterilizing action.

Tsukimura *et.al.*. (1991) suggested that several MOs compounds and their derivatives may be potent in stimulating oocyte growth such as 17α hydroxy progesterone; 17β estradiol; 17 α, 20β dihydroxyprogesterone and terpenoids, MF and JH III. MF was identified as the primary product of the Mandibular Organ *in vitro* (Laufer *et al* ., 1985), the isolated glands produce 5-20 ng MF/h, which has a JH activity in insects.

Secretion of MF was related to reproduction in females , its synthesis coincide with vitellogenesis in adult females (Laufer *et al*.., 1989). The regulation of reproduction in crustacean may be through direct action on the gonads or by indirect action of neuropeptides on the MO. The level of MF fluctuates with vitellgenin, the greatest amount of MF synthesis occurs Vg synthesis is highest (Laufer *et al*., 1989).

Divergent views expressed on functional elucidation of MF. Three roles of MF have emerged from a growing body of literature (Homola and Chang, 1997)

1. Stimulation of general protein synthesis.
2. Promotion of the moult cycle.

3. Reproduction in the males and females.

Homola *et al.*. (1991) suggested that MF may play a role in morphogenesis and reproduction in the male spider crab, *Libinia emarginata.* Laufer *et al.* (1994) reported that, MF may be involved in directing the differential reproductive behaviour in polymorphic male. The relationship between behaviour and hormone levels in blood suggest that MF may be involved in determining the type of mating behaviour displayed by different male morphotypes (Sagi *et al..*, 1994 ; Laufer and Ahl, 1995).

The primary ecdysteroid secreted by Y-Organ (Gabe, 1953; Hinsch et.al., 1980) and MF is shown to have its influence on moulting too (1980), Hormonal extracts of MO from the crayfish, *Procambarus clarkii*, accelerated moulting in the shrimp, *Caridina denticulata* (Taketomi *et al..*, 1989) by co -culturing the Y - Organs of the *Cancer magister* with the MO (Chang *et al..*, 1993) or with (2E,6E)-MF (Tamone and Chang, 1993) an increased secretion of ecdysone into the culture medium was noticed. obviously, the moulting gland is under the influence of a stimulatory neuropeptide (MIH) from the eyestalk. Interestingly, the MO is itself under the inhibitory control of an eyestalk neuropeptide. The presence of a dual controlling mechanisms on the Y-Organ activity is highly advantageous for a temporal spacing of moulting from reproduction. Later, found that its secretion is simulated by specific as trans Isomer of methyl farnesoate (2Z,6E)-MF) (Shernyl *et al..*, 1993; Chang *et al..*,1993). Later , Wilder and Aida (1995) are not clear opinion whether MF is involved in reproduction ,moulting and other physiological

processes. Wilder *et al*. (1995), suggested that, if MF has a physiological role, it may be more related to moulting than to reproductive processes.

In contrast to these views, Hinsch (1980) first reported that augmentation of ovarian development after the implantation of MOs in spider crab. Later, Sasikala and Subramoniam (1991) found that enhanced yolk deposition in the crab *Paratelphusa hydrodromus* after injection of JH III. Weixin *et al*.. (1997) were found that 1% Juvenile hormone analogue ZR515 (JHA-ZR515) 40 mul per individual to stimulate the ovarian maturation and spawning after 16-17 days treatment.

Recently ,Sreenuvasula Reddy and Ramamurthy (1998) strongly supported that MF act as an reproductive hormone in the freshwater crab, *Oziotelphusa senex senex*..

MECHANISMS OF METHYL FARNESOATE (MF) REGULATION

In an effort to understand the inhibitory action of the eyestalk on the MO. A peptide hormone extracted from the insect Corpora Cardiaca (CC) was reported by Applebaum and Moshitzky (1986) to inhibit yolk production in *Locusta migratoroides*; this peptide reacted with an antibody to Adipo Kinetic Hormone (AKH). Since AKH is very similar in structure to crustacean Red Pigment Concentrating Hormone (RPCH) (Fernlund and Josefsson 1968; Gade, 1990) and because inhibition of yolk protein synthesis might be the result of a substance that acts directly on the insect CA.

Histological and Ultrstructural studies (LeRoux,1968,1983; Demeusy, 1975; Bazin, 1976; Hinsch, 1977), suggested that the MO was regulated by the eyestalk neuropeptides. Furthermore, MF synthesis *in vitro* can be depressed with eyestalk extract (Laufer *et al.*, 1986).

Lambert and Fingerman (1979) had suggested that the RPCH might act as a Ca^{+2} seems to be involved in the regulation of JH synthesis by the CA . Landau *et al.*. (1989) reported that RPCH stimulates the MO of *Procambarus clarkii* to synthesis MF and hormonal stimulation can be mimicked calcium ionophore A23187 and synthesis could be inhibited by culturing the tissue in Ca^{+2} free media or including lanthanum. Further more, Landau *et al.* (1989) found that Pigment Dispersing Hormone (PDH) at 10^{-M} significantly inhibited MO synthesis of MF in *Procambarus clarkii.* The secretion of MF by the MO was inhibited by an eyestalk factor (MO-IH) (Laufer, 1989) and the action of GIH may be target tissue such as the hepatopancreas or ovary or also be an the MO (Laufer *et al.*, 1989) Later, Laufer (1989) were suggested that regulation of reproduction in crustacea may be through direct action of hormones on the gonads or by indirect action of neuropeptides on the MO.

Beltz (1988) reported that the biogenic amines (Serotonin and Octopamine) play a significant role in determining the mating behaviour in the American lobster, *Homarus americanus* and Octopamine stimulates JH synthesis from CA in *Locusta migratoria* (Lafon-Cazal and Baehr ,1988).Later, these views were strongly supported by Homola *et al.*. (1989)

that these biogenic amines may function as neuroregulator of MF. MO's activity seems to be under control of the eyestalk neuropeptides as evidenced by several eyestalk ablation studies.

Taggart *et al*.. (1991) reported that, the secretion of MF was regulated by eyestalk neuropeptides, suggesting Guanine Nucleotide Binding Protein (G-Protein) involvement. Later, Tsukimura and Borst (1991) found that cyclic GMP (cGMP) act as a second messenger for the sinus gland factor that inhibits MF synthesis in the MO. Tsukimura and Borst (1992) showed a distinctive elevation of MF level in haemolymph and MO after eyestalk ablation in *Homarus americanus*. Then they suggested that MO was negatively regulated by a fcator (s) from the Sinus gland extracts or neuropeptides. More recently , Liu and laufer (1996); Liu *et al*, (1997) have demonstrated that a sinus gland neuropeptide, with Crustacean Hyperglycaemic Hormone (CHH) activity , also inhibits MO's activity in the spider crab, *Libinia emarginata*..

Later, Wainwright *et al*.. (1996) reported that two neuropeptides, named Mandibular Organ - Inhibiting Hormone (MOIH -I & MOIH -II) repressed MF synthesis. The MOIH -I peptide consisted by 78 residues (M sub [r] 9235.6), with nonblocked termini and three intractio disulfide bridges. MOIH-II appeared to be almost identical to MOIH -I with the exception of glycin for lycine substitution at position 33.

4. MATERIALS AND METHODS

Plate No. 1: Adult Indian White Prawn, *Penaeus Indicus.*

MATERIALS AND METHODS

4.I.COLLECTION OF ANIMALS:

Fifty adult Indian white prawns *(Penaeus indicus*) with immature ovaries were collected from MASTYAFED (The Kerala State Federation for Development of Fisheries) Brackish water fish farm,Narakkal, Vypeen Island, Cochin, on 15 april, 1999. Size range was 120 *mm* - 150 *mm* in total length and 17- 27*gm* in body weight They were collected by using cast net between 0700 - 0900 hrs.

Selected prawns were immediately segregated from the catch and kept in a 60 lit plastic bucket with filtered seawater, they were carefully transported to the shrimp hatchery complex of Marine Product Export Development Authority (MPEDA), Vallarpadom , Cochin . Later they were stocked in plastic pool (10 tonne capacity) filled wih treated seawater and the plastic pool was provided with mild aeration from an Air blower.

4.2. STAGES OF OVARIAN DEVELOPMENT:

Prawns were dissected to identify the maturity stages . Dissection were generally carried out in crustacean saline (Smith and Ratcliff,1980). Five stages of ovarian development were recognised by Mohamed,K.S. (1989) in the Indian white prawn, *Penaeus indicus*..

4.2.1.The Immature Stage (Stage I) :

The ovary is thin , smooth, transparent and unpigmented with no distinguishable outline through the dorsal exoskeleton.

4,2,2.Early Maturing Stage (Stage II):

The ovary loses its transparency and now appears as opaque and is visible externally through the dorsal exoskeleton. The ovary is pale cream in colour and anterior and middle lobes of the ovary increase in size and the ovarian surface appears as granular.

4.2.3.Late Maturing Stage (Stage III) :

Development of a light green colour and increased granular appearance is the prominent characteristics . All lobes are seen to increasing in dimension and fill up the body cavity.

4.2.4.Mature or Ripe Stage (Stage IV) :

The ovarian lobes are so distended and fill all the available space and appeared to crowd. The ovary develops a dark green colour and the outline is distinct and a prominent characteristic of this stage is the traingular shape of the ovary as seen through first abdominal somite.

4.2.5.Spent stage (stage V) :

The spawned ovary designated as spent has a loose & flaccid appearance and creamy in colour. However, retained its ovacity and the outline distinct spawaning is imminent.

4.3. DISSECTION:

After externally identifed the prawns , their cephalothorax and abdo men was cut open to expose the ovary. The exposed ovary was then carefully excised out and weighed to the nearest milligram.The Gonado Somatic Index (GSI) was calculating as per the method described by Giese and Pearse, 1974.

$$GSI = \frac{\text{Wet Weight of ovary}}{\text{Wet weight of animal}} \times 100$$

4.4. HARMONE SOLUTION PREPARATION:

The hormone Methyl farnesoate (MF) (Methyl 3, 7, 11-tri methyl – 2,6,10-dodecatrienoate).The hormone was dissolved in absolute (95%) ethanol to desired level of concentration i.e 0.1ml of ethanol containing 10-15 µg Methyl Farnesoate.

4.5. HORMONE PELLET FEED PREPARATION:

Hormone Methyl farnesoate was dissolved in 95% ethanol, which was the solvent vehicle. This can be used were to prodvide 10- 15 µg dose per animal per day in the feed. The ethanol solution was mixed with the commerical pellets (Highashimuru feed), then air dried for 8 hrs at room temperature and control diet was prepared with 95% ethanol only.

4.6. SELECTION OF ANIMALS FOR INJECTION :

Female intermoult (C) prawns were selected based on morphological

Plate No. 4: Plate showing the injection of Methyl Farnesoate.

changes (rigid,hard, exoskeleton with firm rostrum, setal protoplasm retracts to well developed setal cones and cuticular nodes) mentioned by Vijayan, *et al.*(1997). The selected famales were divided equally into three groups. In one group, each prawn was injected twice (10 days interval) with 10 - 15 µg MF per prawn through the first and second lateral somites of the abdomen with glass Syringe & Hypodermic needle Number. 22 .In the third group (Control), each prawn was injected twice with 0.1 µl of pure elthanol per prawn.

4.7. EXPERIMENTAL DESIGN:

The Experimental design consisted of one 10 tonne plastic pool for acclimation and three FRP tanks (One tonne) and These tanks filled with filtered and treated sea water . These tanks were continously aerated. The water temperature was maintained at the range 25-32°c. The photoperiod was 12L:12D . Salinity , P^H and Dissolved Oxygen ranged from 31 -32‰, 7.8-8.0 and > 5 *ppm* respectively .However all other chemical parameters were considered as the negligible.

Water Exchange @ 100% thrice per week and daily siphoning of faecal matter from the bottom of all tanks. All experimental tanks were covered with black plastic sheets to reduce illumination and to prevent algal growth on the wall & at the bottom of maturation tanks.

The degree of ovarian maturation in the experimental prawns was checked daily morning hrs by external macroscopic examination of the colour and size of the developing ovaries through the exoskeleton.

Plate No. 2: Plastic Pool (10 Tonne) used for Acclimation.

Plate No. 3: Experimental Design (Three FRP Tanks).

Each treatment of experiment consisst of three groups. In each group there were 6 adult females (140 - 160 *mm* TL & 25 - 30 *gm* BW) in a one tone FRP tanks .

All groups of prawns were fed with squid meat and pelleted diets (Highashimuru Feed Ltd.,) thrice daily (0800 hrs; 1400 hrs; 1900 hrs) @ 8% and 3% of average body weight respectively. Total ration of squid meat were fed @ 70% (0800 hrs) , 30% (1400 hrs) per meal and total ration of pelleted feed were fed (1900 hrs) per single meal. In first group (Experimental tank No.1) each prawn was injected twice (10 days interval) with 10-15 µg MF in 0.1ml ethanol per prawn and fed with squid meat & without hormone pelleted diets, In the second group each prawn was fed approximately 10-15 µg MF with pelleted diets and In the third group (Control), each prawn was injected twice (10 days interval) with 0.1ml of ethanol and fed with Squid meat and with out hormone pelleted diets.

Each treatment was replicated two times. Firsr treatment of experiment was carried out during from 24 April - 18 May , 1999 (25 days) and Second treatment was carried out during from 18 May - 13 June,1999 (25 days).

5. RESULTS

RESULTS

The characteristics of ovaries from the control and trerment are Ilustrated in Fig5, Fig.6.and Fig.7.

Treatment trial one:

Methyl Farnesoate (MF) injected prawns become larger than those of the fed with hormone incorporated feed and control (injection with 0.1 ml ethanol only). The ovaries of control group prawns were thin, transparent and unpigmented and the average Gonado Somatic Index (GSI) was 1.75% for 25 days trial.

The ovaries from the Methyl farnesoate injected group (twice @ 10 -15μg MF/ 0.1ml ethanol / prawn) bocomes larger and showed positive response. After second injection (10 day) ovaries had light green colour and increased granular appearance to be the prominent characteristics; which were significantly larger than the day 0 and day 25 controls. Two female prawns had greatly enlarged lobes with light green colour that filled the entire body cavity and their GSI were 3.5% (Stage II) and 5.45% (Stage III).

Experiment trial two:

The ovaries from the initial (0 day) control group and fed with hormone incorporated feed group were thin , smooth, transparent and unpigmented with no distinguishable outline through the dorsal exoskeleton. The mean Gonado Somatic Index (GSI) was less than 2%. At the end of the 25 day trial, the ovaries were similar to initial day.

Plate No. 5: Dissected Prawn showing the Immature Ovary (*Stage I*).

Plate No. 6: Dissected Prawn showing the Mature Ovary (*Stage IV*).

Plate No. 7: Dissected and excised Prawn showing the late Maturing Ovary (*Stage III*).

The hormone (@ 10 - 15 µg MF/ 0.1ml ethanol/ prawn) treated group are significantly larger than the day 0 and day 25 day control group. After 20 day trial , three prawns from this group had significnatly enlarged lobes with pale cream in colour to a dark green colour, which further distended and filled all the available space appeared to crowd. Their GSI were 2.85% (Stage II), 5.64% (Stage III) and 7.39% (Stage IV) respectively.

The above experiments were conducted at the room temperature . The ambient temperature varied from 25 to 32° c The dissolved oxygen level in the experimental tanks was maintained near air satuartion. The salinity variation in the acclimation and experimental tanks was 31 to 32 ppt the P^H variation was 7.8 to 8.0 . During the period of acclimation and ex periment, the tanks was covered with a black plastic sheet.

The female prawns fed with hormone (MFS) incorporated feed and those kept as control did not show any signs of maturity. Only the female prawns injected with hormone matured. The above experiments were conducted at the room temperature . The ambient temperature varied from 25 to 32° c.The dissolved oxygen level in the experimental tanks was maintained near air satuartion.

The female prawns fed with hormone (MF) incorporated feed and those kept as control did not show any signs of maturity. Only the female shrimps injected with hormone matured.

The experiments was continued for a period of 25 days. In the ex-

TREATEMENT ONE :

STIMULATION OF OVARIAN MATURATION IN THE INDIAN WHITE PRAWN, *PENAEUS INDICUS* BY METHYL FARNESOATE.

Treatement	No.Of Injections	Total Length (mm)	Body Weight (gm)	No.Of Prawns	No.Of female Showing Various Stages of Ovarian development																Moulting %	Mortality %
					Days					Days 10					Days 20					Days 25						
					I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V		
Group 1 (10 -15 µg MF/0.1ml Ethanol / Prawn)	Two	125-140	18-23	6	6	6	0	0	0	5	1	0	0	0	3	2	0	0	0	3	1	1	0	0	16.60	16.61
					MATURITY 33.3%																					
Group 2 (Fed with hormone incorporated feed)		130-148	19-24	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	16.60	00.00
Group3 (0.1 ml Ethanol per Prawn)	Two	130-145	18-22	6	6	6	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	33.30	16.60

Table No. 1

TREATEMENT NO : TWO

STIMULATION OF OVARIAN MATURATION IN THE INDIAN WHITE PRAWN, *PENAEUS INDICUS* BY METHYL FARNESOATE.

Treatement	No.Of Injections	Total Length (mm)	Body Weight (gm)	No.Of Prawns	No.Of female Showing Various Stages of Ovarian development																Moulting %	Mortality %
					Days					Days 10					Days 20					Days 25						
					I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V		
Group 1 (10 -15µg MF/0.1ml Ethanol / Prawn)	Two	118-141	18-24	6	6	0	0	0	0	6	0	0	0	0	3	2	1	0	0	3	1	1	1	0	16.60	00.00
					MATURITY 50%																					
Group 2 (fed with hormone incorporated feed)		120-140	18-22	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	50.00	00.00
Group 3 (0.1 ml Ethanol per prawn)	Two	120-139	16-23	6	6	0	0	0	0	6	0	0	0	0	5	0	0	0	0	5	0	0	0	0	16.60	16.60

Table No. 2

periment trial one, the mortality was 16.6% in the treated females and 16.6% in the control; one female and two female prawns were moulted in the treatment and control group respectively. However , the group of prawns fed with hormone incorporated feed did not showed any sign of mortality. The percentage of female matured hormone injection was 33.3.

In the experiment No.2 , the percentage of females matured after hormone injection was 50% . One prawn moulted during the experi mental period (25 days). No mortality was observed in hormone injected group of prawns. In control 5 prawns moulted . In the group fed with hormone incorporated feed, no mortality occurs. However, three prawns moulted.

GONADO SOMATIC INDEX:

EXPERIMENT ONE

Animal No:	Total Lenght (mm)	Body/ Weight (gm)	Gonado Weight (gm)	Gonado Somatic Index (%)	Ovarian Stage
1.	139	22	1.2	5.45	Stage III
2.	134	20	0.7	3.5	Stage II

Table No: 3

EXPERIMENT TWO

Animal No:	Total Lenght(mm)	Body/ Weight (gm)	Gonado Weight(gm)	Gonado Somatic Index (%)	Ovarian Stage
1.	138	21	0.6	2.85	Stage II
2.	134	19.5	1.1	5.64	Stage III

Table No : 4

6. DISCUSSION

DISCUSSION

The aim of this study was to find out whether there is any stimulatory effect of exogenous application of Methyl Farnesoate (MF) on ovarian maturation in females of the Indian white prawn, *Penaeus indicus*.. In the Indian white prawn, ovarian maturation takes place in five stages which can be identified on based on morphological characteristics and colouration of ovary (Mohamed, K.S, 1991). The oogonial proliferation and ovarian differentiation takes place when the ovary changes from transparent to opaque. During vitellegenesis, the colour of the ovary changes from pale cream to dark green colour and then it becomes the triangular in shape which can be seen through first abdominal somite prior to spawning. Maturation of the ovary also includes an increase in size as the oocytes proliferate and increase in diameter during yolk incorporation (Charniaux- Cotton and Payen,1988). Using these criteria the Gonado Somatic Index (GSI) and maturation was assessed.

In each of two experiments stimulation and enhancement of ovarian maturation was achieved by administration of MF through abdominal injection. This was assessed by observing macroscopic morphological characteristics and significantly greater Gonado Somatic Index from treateted females compared to those of the untreated controls when both groups were at the day 25 period.

The presence of Juvenile Hormone (JH) or MF which is synthesized and released from the classical epithelial endocrine gland,

Mandibular organ (MO) and their structural and functional elucidation's have been documented in many crustaceans. Several isoforms of juvenile hormones namely, JH O, JH I, JH II, JH III, and MF were detected in insects (Wilder and Aida, 1995) , but in crustacean only MF appears to be a major product. The chemical structure of MF is nearly identical to that of insect JH III, differing only by the absence of unepoxide group (Homola and Chang, 1997).

Evidence for a role played by MF in decapod crustacean female reproduction is accumulating and it appears to be related to vitellogenin production. In the spider carb, *Libinia emarginata* MO synthesis and secretion of MF is synchronous with the vitellogenic cycle. Laufer *et al.* (1987) reported that MF secretion *in vitro* was greatest with MOs excised from females with ovaries undergoing oocyte development and vitellogenesis and diminished secretory activity was observed with MOs from females with fully developed ovaries. Direct measurements of haemolymph levels of MF in the crab, *Cancer pagurus* showed an increase correlated with the onset of secondary vitellogenesis (Wainwright *et al..*,1996), MF has been reported to increased fecundity in cultured white prawn, *Penaeus vannamei* (Laufer,1992); Laufer *et al..*, 1997). The ovarian enhancement effect of MF, then, may be indirect by stimulating the Y- organs to synthesis and secrete ecdysteroids (Tamone and Chang, 1993). This subsequently accumulates in the ovaries (Wilder *et al..*, 1991; Chang ,1993; Young *et al..*, 1993) and stimulate ovarian development in *procambarus. clarkii* was also induced to mature with Injection of 5- hydroxytryptamine

(5-HT or Serotonin) (Kulakarni *et al.*, 1992) , which acts indirectly on the ovaries, by inducing the release of an Ovarian Stimulating Hormone (OSH), that MF may be such an OSH is unlikely because 5-HT administration *in vitro* inhibits MF synthesis by the MO of the spider crab, *Libinia emarginata* (Homola, 1989).

Controversial results have been reported in several insects species in which treatments with JH analogues, such as methoprene and hydroprene, caused retardation of larval development of mud crab, *Rhithropanopeus harrisii* (Christiansen *et al..*, 1977a) and treatment with juvenile hormone inhibited larval growth and ecdysis and no, or very limited, morphogenesis was recorded (Lohri-Kaelin and Masner, 1981). In crustaceans, JH III (Hertz and Chang, 1986) and MF (Borst *et al .*, 1987) retarded larval development of *Homarus americanus* . Later, Mc Kenney and Celestial (1993) supported that methoprene also retarded growth in early larval stages and postlarvae of the estuarine shrimp, *Palaemonetes pugio*, although enhancement of growth was recorded in the premetamorphic stage .

Later work done by various authors found that MF have stimulatory activiity on moulting (Yedin *et al .*, 1980 ; Taketomi *et al..*, 1989; Chang *et al.*, 1993; Tamone and Chang, 1993) and very recently Wilder and Aida (1995) are not clear in their opinion whether , MF is involved in reproduction , moulting and other physiological processes. Wilder *et al..* (1995) are of the view that , if MF has a physiological role. It may be more related to moulting than to reproductive processes.

In contrast to these putative views , the first evidence for MO's role in vitellogenesis was provided by Hinsch (1980), who recorded acceleration of ovarian development after the implantation of MO in spider crab, *Libinia emarginata* and Later, similar evidence was provided by Sasikala and Subramoniam (1991) who found enhanced yolk deposition in the crab, *Paratelphusa hydrodromus* by injection of JH III. MF may play a role in morphogenesis and reproduction in the male spider crab, *Libinia emarginata* (Homola *et al.*, 1991) and various workers found that MF may be involved in determining the type of mating behaviour in polymorphic males (Laufer *et al..*, 1994; Sagi *et al..*, 1994; Laufer and Ahl, 1995).

Weixin *et al..* (1997) suggested that juvenile hormone analogue has a stimulatory ctivity on maturation and spawning in giant freshwater prawn, *Macrobrachium rosenbergii.* Very recently , these views were strongly supported by Sreenuvasula Reddy and Ramamurthy (1998) who found that MF act as an reproductive hormone in the freshwater crab, *Oziotelphusa senex senex..*

The similarity between the results of previous studies and the current study suggest that MF may play a role in the regulation of ovarian maturation or reproductive activity. The major available technique at present for inducing maturation in penaeid prawns is eyestalk ablation . Researchers all over the world are on the new look out for an alternative technique of induced maturation. The results of the present study clearly indicate that the Penaeid prawns can be induced to mature by the administration of microquantities of

purified hormones like JH like compounds (MF), thus obviating the technique of eyestalk ablation. Thus results of this investigation are of immense value in induced maturation of Penaeid prawns.

7. SUMMARY

SUMMARY

1. In docapod crustaceans the only juvenile hormone like compounds found to date is Methyl Farnesoate (MF), the unepoxidated form of insect juvenile hormone. The Mandibular Organ (MO) synthesizes MF and it is under inhibitory control of neurosecretory factors from the X-organ/ Sinus Gland Complex and may be stimu lated by factors from the brain and or thoracic ganglion.

2. The possible involvement and the stimulatory pathway of MF in ovarian maturation of the Indian white prawn, *Penaeus indicus* were investigated. In one group of prawns were injected with twice @ 10 - 15 µg MF in 0.1 ml ethanol.

3. The second group of prawns were fed with hormone incorporated pelleted feed and the control group prawns injected only with 0.1 ml ethanol and fed without hormone incorporated pelleted feed.

4. After 25 days of experimental trial the ovaries of immature prawns showed the positive stimulatory response. The experimental groups fed with hor mone incorporated pelled feed and controls did not show any positive response. Simialr results were observed in second experimental trial of 25 days duration.

5. In the first and second experimental trials the maturity was 33.3% and 50 % respectively. The negative results like mortality and moulting was also re corded. The mortality may be due to temperary stress factrors.

6. This new technique will help thus to stimulate and enhance the ovarian maturation. It can also be used for development of induced maturation technique

by administration of purified hormones,thus obviating need for technique of eyestalk ablation. The results of this investigation are of immense value in induced maturation of Penaeid prawns.

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